Worthington
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Tissue Dissociation Guide

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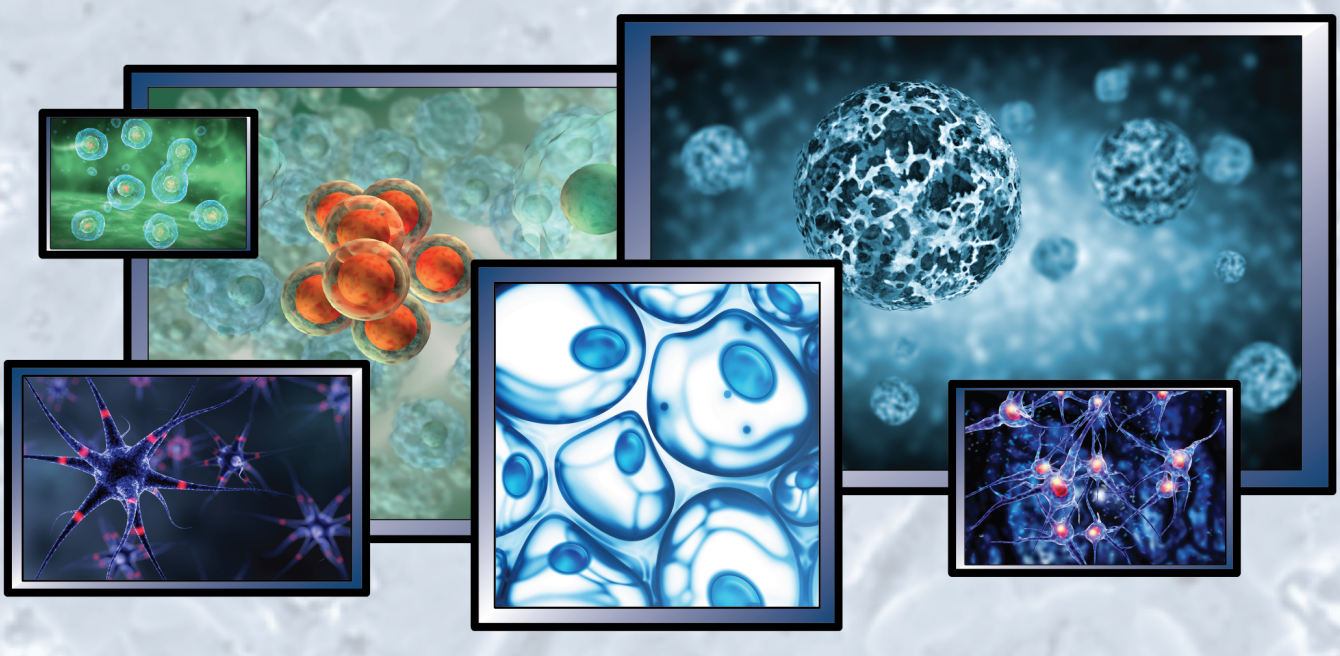

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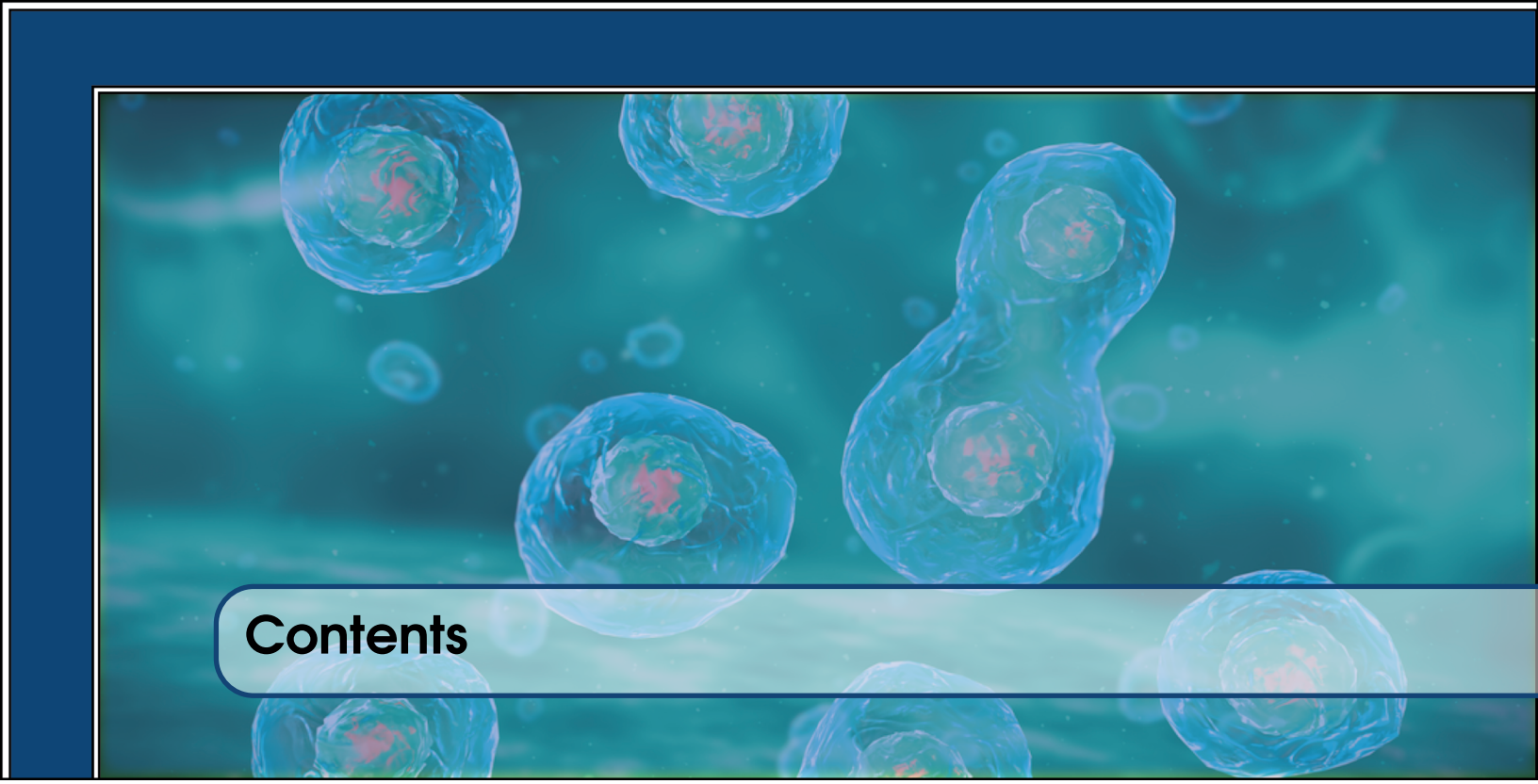

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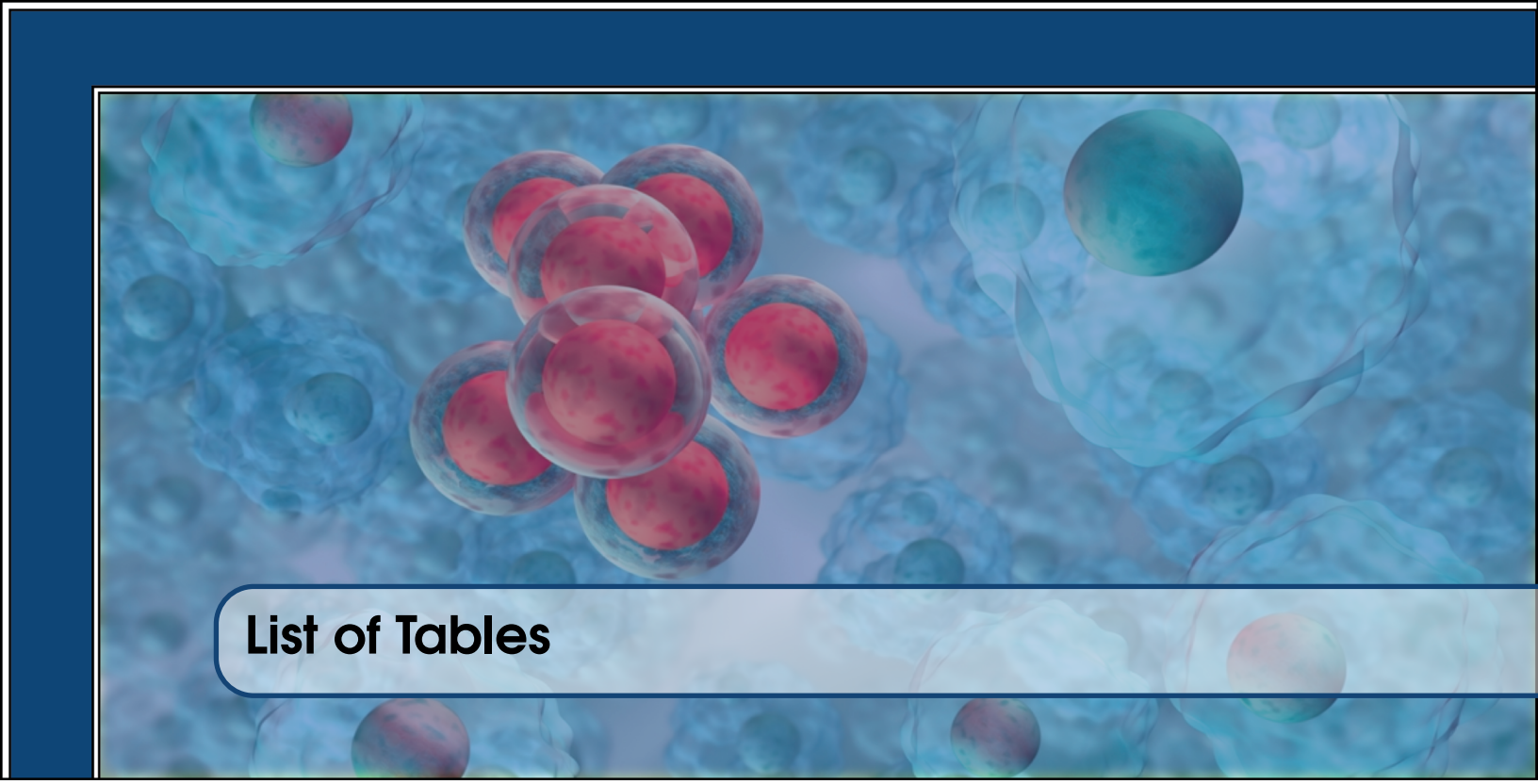

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1. Introduction

Introduction

Tissue dissociation/primary cell isolation and cell harvesting are principal applications for enzymes in tissue culture research and cell biology studies. Despite the widespread use of enzymes for these applications over the years, their mechanisms of action in dissociation and harvesting are not well understood. As a result, the choice of one technique over another is often arbitrary and based more on past experience than on an understanding of why the method works and what modifications could lead to even better results.

The goal of a cell isolation procedure is to maximize the yield of functionally viable, dissociated cells. There are many parameters which may affect the outcome of any particular procedure including but not limited to:

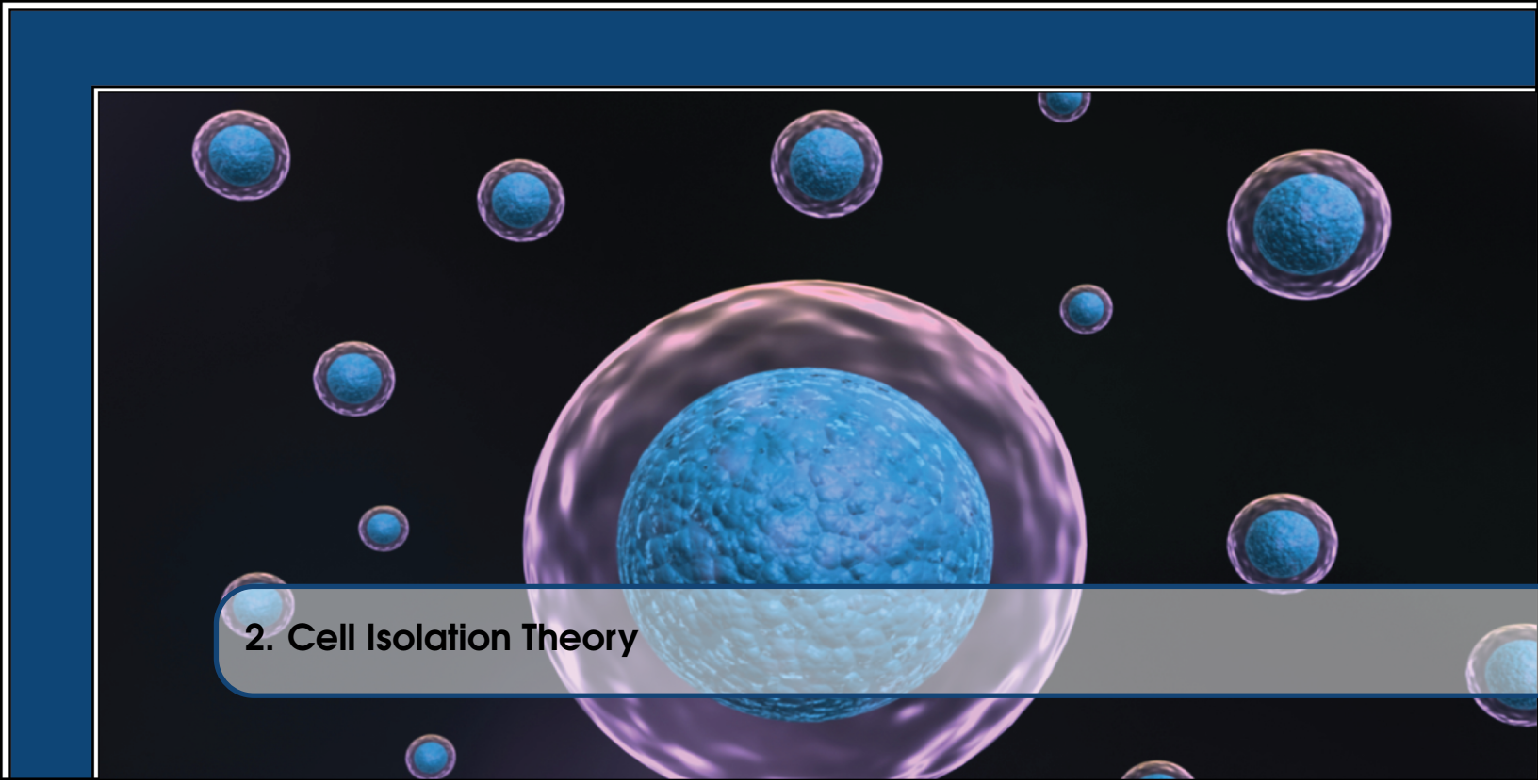
I. Type of tissue
II. Species of origin
III. Age of the animal
IV. Genetic modification(s) (knockouts, etc.)
V. Dissociation medium used
VI. Enzyme(s) used
VII. Impurities in any crude enzyme preparation used
VIII. Concentration(s) of enzyme(s) used
IX. Temperature
X. Incubation times

The first four items generally are not a matter of choice. To achieve suitable results the other variable

conditions are best defined empirically.

Researchers searching the scientific literature for information on the ideal enzymes and optimal conditions for tissue dissociation are often confronted with conflicting data. Much of the variation stems from the complex and dynamic nature of the extracellular matrix and from the historical use of relatively crude, undefined enzyme preparations for cell isolation applications. Also, the extracellular matrix is composed of a wide variety of proteins, glycoproteins, lipids and glycolipids, all of which can differ in abundance from species to species, tissue to tissue and with developmental age. Commonly used crude enzyme preparations such as Pronase, NF 1:250 and collagenase contain several proteases in variable concentrations, as well as a variety of polysaccharidases, nucleases and lipases.

This guide summarizes our knowledge of how these enzymes accomplish the "routine" operations of tissue dissociation and cell harvesting, describes standard lab procedures, offers a logical experimental approach for establishing a cell isolation protocol, and lists many tissue specific references.



2. Cell Isolation Theory

Tissue Types

This section summarizes the general characteristics of extracellular matrices associated with various types of tissue. Coupled with the descriptions of individual enzymes offered in the next section, this information will aid in choosing the enzyme(s) best suited for a particular tissue.

Epithelial Tissue

In the adult, epithelium forms tissues such as the epidermis, the glandular appendages of skin, the outer layer of the cornea, the lining of the alimentary and reproductive tracts, peritoneal and serous cavities, and blood and lymph vessels (where it is usually referred to as "endothelium"). Structures derived from outpouchings from the primitive gut, including portions of the liver, pancreas, pituitary, gastric and intestinal glands are also composed of epithelial tissue.

Epithelial cells are typically packed so closely together that there is very little intercellular material between them. An extremely tight bond exists between adjacent cells making dissociation of epithelium a difficult process.

On the lateral surfaces of adjacent epithelial cells there are four distinct types of intercellular bonds: the *zonula occludens*, *zonula adherens*, *macula adherens* and *nexus*. The former three are often closely associated to form a junctional complex. In the *zonula occludens*, or "tight junction," there are multiple sites of actual fusion of the adjacent unit membranes interspersed by short regions of unit membrane separation of approximately 100-150 . In a *zonula adherens*, or "intermediate junction," a fine network of cytoplasmic filaments radiates from the cell membrane into the cytoplasm. The space between unit membranes of adjacent cells is approximately 150-200 and is composed of an intercellular amorphous substance of unknown composition. In the *macula adherens*, or "desmosome," there is a somewhat

similar array of intracellular filaments. The adjacent unit membrane space is approximately 150-200 and consists of an extracellular protein and glycoprotein ground substance, often with an electron-dense bar visible within it. The integrity of the desmosome requires calcium, and it is broken down by EDTA and calcium-free media. The enzymes collagenase, trypsin and hyaluronidase can also dissociate the desmosome. The *nexus*, or "gap junction", covers most of the epithelial cell surface. In these areas, the unit membranes appear tightly attached and are separated by only 20 . The intercellular material consists of an amorphous, darkly-staining substance.

On the basal surface of the epithelium where it overlays connective tissue, there is an extracellular bonding layer or sheet called the basal lamina. The lamina is composed of a network of fine, collagen-like reticular fibers embedded in an amorphous matrix of high and low molecular weight glycoproteins.

Connective Tissue

Connective tissue develops from mesenchymal cells and forms the dermis of skin, the capsules and stroma of several organs, the sheaths of neural and muscular cells and bundles, mucous and serous membranes, cartilage, bone, tendons, ligaments and adipose tissue.

Connective tissue is composed of cells and extracellular fibers embedded in an amorphous ground substance and is classified as loose or dense, depending upon the relative abundance of the fibers. The cells, which may be either fixed or wandering, include fibroblasts, adipocytes, histiocytes, lymphocytes, monocytes, eosinophils, neutrophils, macrophages, mast cells and mesenchymal cells.

There are three types of fibers: *collagenous*, *reticular* and *elastic*, although there is evidence that the former two may simply be different morphological forms of the same basic protein. The proportion of cells, fibers and ground substance varies greatly in different tissues and changes markedly during the course of development.

Collagen fibers are present in varying concentrations in virtually all connective tissues. Measuring 1-10 μm in thickness, they are unbranched and often wavy, and contain repeating transverse bands at regular intervals. Biochemically, native collagen is a major fibrous component of animal extracellular connective tissue, skin, tendon, blood vessels, bone, etc. In brief, collagen consists of fibrils composed of laterally aggregated polarized tropocollagen molecules (M.W. 300,000). Each rod-like tropocollagen unit consists of three helical polypeptide a-chains wound around a single axis. The strands have repetitive glycine residues at every third position and an abundance of proline and hydroxyproline. The amino acid sequence is characteristic of the tissue of origin. Tropocollagen units combine uniformly in a lateral arrangement reflecting charged and uncharged amino acids along the molecule, thus creating an axially repeating periodicity. Fibroblasts and possibly other mesenchymal cells synthesize the tropocollagen subunits and release them into the extracellular matrix where they undergo enzymatic processing and aggregation into native collagen fibers. Interchain cross-linking of hydroxyprolyl residues stabilizes the collagen complex and makes it more insoluble and resistant to hydrolytic attack by most proteases. The abundance of collagen fibers and the degree of cross-linking tend to increase with advancing age, making cell isolation more difficult.

Reticular fibers form a delicate branching network in loose connective tissue. They exhibit a regular, repeating subunit structure similar to collagen and may be a morphological variant of the typical

collagen fibers described above. Reticular fibers tend to be more prevalent in tissues of younger animals.

Elastic fibers are less abundant than the collagen varieties. They are similar to reticular fibers in that they form branching networks in connective tissues. Individual fibers are usually less than 1 µm thick and exhibit no transverse periodicity. The fibers contain longitudinally-arranged bundles of microfibrils embedded in an amorphous substance called elastin. Like collagen, elastin contains high concentrations of glycine and proline, but in contrast has a high content of valine and two unusual amino acids, desmosine and isodesmosine. Fibroblasts and possibly other mesenchymal cells synthesize the elastin precursor, tropoelastin, and release it into the extracellular matrix where enzymes convert the lysine residues into the desmosines. Polymerization of elastin occurs during interchain cross-linking of the latter. In this state, elastin is very stable and also highly resistant to hydrolytic attack by most proteases.

The viscous extracellular ground substance in which connective tissue cells and fibers are embedded is a complex mixture of various glycoproteins, the most common being hyaluronic acid, chondroitin sulfate A, B, and C and keratin sulfate. Each of these glycoproteins is an unbranching polymer of two different alternating monosaccharides attached to a protein moiety. Hyaluronic acid, for example, contains acetyl glucosamine and glucuronate monomers and about 2% protein, while the chondroitin sulfates contain acetyl galactosamine and glucuronate or iduronate monomers and more than 15% protein. The relative abundance of these glycoproteins varies with the origin of the connective tissue.

Dissociating Enzymes

While many enzyme systems have been investigated by researchers performing cell isolations, the enzymes discussed here have been found satisfactory for a wide variety of tissues from many different species of various ages.

Collagenase

Bacterial collagenase is a crude complex containing a collagenase more accurately referred to as clostridiopeptidase A which is a protease with a specificity for the X-Gly bond in the sequence Pro-X-Gly-Pro, where X is most frequently a neutral amino acid. Such sequences are often found in collagen, but only rarely in other proteins. While many proteases can hydrolyze single-stranded, denatured collagen polypeptides, clostridiopeptidase A is unique among proteases in its ability to attack and degrade the triple-helical native collagen fibrils commonly found in connective tissue.

True collagenase may cleave simultaneously across all three chains or attack at a single strand. Mammalian collagenases split collagen in its native triple-helical conformation at a specific site yielding fragments, TC A and TC B, representing 3/4 and 1/4 lengths of the tropocollagen molecule. After fragmentation the pieces tend to uncoil into random polypeptides and are more susceptable to attack by other proteases.

Bacterial collagenases are usually extracted from host invasive strains. These enzymes differ from mammalian collagenases in that they attack many sites along the helix. Collagenases from Clostridium histolyticum, first prepared by Mandl, et al., have been most thoroughly studied. Commercially available collagenase has been limited primarily to that from Cl. histolyticum although other sources have recently become available. Clostridial collagenase also degrades the helical regions in native

collagen preferentially at the X-Gly bond in the sequence Pro-X-Gly-Pro where X is most frequently a neutral amino acid. This bond in synthetic peptide substrates may also be split.

Purified clostridiopeptidase A alone is usually inefficient in dissociating tissues due to incomplete hydrolysis of all collagenous polypeptides and its limited activity against the high concentrations of non-collagen proteins and other macromolecules found in the extracellular matrix. The collagenase most commonly used for tissue dissociation is a crude preparation containing clostridiopeptidase A in addition to a number of other proteases, polysaccharidases and lipases. Crude collagenase is well suited for tissue dissociation since it contains the enzyme required to attack native collagen and reticular fibers in addition to the enzymes which hydrolyze the other proteins, polysaccharides and lipids in the extracelluar matrix of connective and epithelial tissues.

The first commercially available collagenase was offered by Worthington in 1959. At that time we offered one type of crude enzyme which we tested only for collagenase activity. Eventually, with the cooperation of many in the research community, four basic profiles were identified:

Type 1 containing average amounts of assayed activities (collagenase, caseinase, clostripain, and tryptic activities). It is generally recommended for epithelial, liver, lung, fat, and adrenal tissue cell preparations.
Type 2 containing greater clostripain activity. It is generally used for heart, bone, muscle, thyroid and cartilage.
Type 3 selected because of low proteolytic activity. It is usually used for mammary cells.
Type 4 selected because of low tryptic activity.. It is commonly used for islets and other applications where receptor integrity is crucial.

Introduced in 2007, Animal Origin Free collagenase (code CLSAFA) is derived from cultures grown in medium completely devoid of animal based components and designed for bioprocessing applications where introduction of potential animal derived pathogens must be prevented. Levels of secondary proteases are similar to Types 1 and 2.

Correlations between type and effectiveness with different tissues have been good, but not perfect, due in part to variable parameters of use. Nevertheless most researchers consider the tissue-typing of crude collagenase lots to be a valuable service. A detailed description of the Worthington collagenase assay as well as our procedure for Clostridiopeptidase A testing can be found in the Worthington Enzyme Manual.

If you find one of the types of collagenases suitable for your cell isolation procedure, you may want to try Worthington's Collagenase Sampling Program. This cost-free program lets researchers pre-sample different lots of collagenase and evaluate them in their specific applications to achieve the best combination of cell yield and viability.

Trypsin

Trypsin is a pancreatic serine protease with a specificity for peptide bonds involving the carboxyl group of the basic amino acids, arginine and lysine. Trypsin is one of the most highly specific proteases known, although it also exhibits some esterase and amidase activity.

Purified trypsin alone is usually ineffective for tissue dissociation since it shows little selectivity for extracellular proteins. Combinations of purified trypsin and other enzymes such as elastase and/or collagenase have proven effective for dissociation.

"Trypsin" is also the name commercial suppliers have given to pancreatin, a crude mixture of proteases, polysaccharidases, nucleases and lipases extracted from porcine pancreas. NF 1:250, a commonly used "trypsin" preparation, has the potency to bring about the proteolytic digestion of 250 times its weight of casein under assay conditions specified by the National Formulary. It is important to realize that this assay procedure is not specific for trypsin, although pancreatin does contain this enzyme. Nomenclature notwithstanding, crude "trypsins" like NF 1:250 and 1:300 are widely used for dissociating tissues, perhaps because the tryptic and contaminating proteolytic and polysaccharidase activities do bring about a preferential attack of the extracellular matrix. It appears, however, that crude trypsin and crude collagenase dissociate tissues by different mechanisms, and difficulties are often encountered when using NF 1:250 preparations – the most common being incomplete solubility, lot-to-lot variability, cell toxicity, and cell surface protein/receptor damage.

In tissue culture laboratories, researchers use purified trypsin to release cells into suspension from monolayers growing on the interior surfaces of culture vessels. Most cells originating from normal tissues and not highly adapted to artificial culture conditions grow in monolayers, i.e., a layer of cells one cell thick adhering to the interior surface of the culture vessel. Because such cells are more like cells in normal tissues, many tissue culture researchers are studying cells that grow in monolayer culture.

Monolayer cultures are commonly grown in glass or polystyrene roller bottles, culture flasks, or Petri dishes. Plastic vessels used in tissue culture work are specially treated to ensure good adherence of cells to the vessel walls. For a detailed discussion of cell harvesting, see page xv of this guide.

Some of the most frequently used grades of purified trypsin for cell isolation procedures are the Worthington product Codes: TL, TRL, TRLS, and TRLVMF. These products are suitable for cell harvesting as well as tissue dissociation.

Elastase

Pancreatic elastase is a serine protease with a specificity for peptide bonds adjacent to neutral amino acids. It also exhibits esterase and amidase activity. While elastase will hydrolyze a wide variety of protein substrates, it is unique among proteases in its ability to hydrolyze native elastin, a substrate not attacked by trypsin, chymotrypsin or pepsin. It is produced in the pancreas as an inactive zymogen, proelastase, and activated in the duodenum by trypsin. Elastase is also found in blood components and bacteria.

Because elastin is found in highest concentrations in the elastic fibers of connective tissues, elastase

is frequently used to dissociate tissues which contain extensive intercellular fiber networks. For this purpose, it is usually used with other enzymes such as collagenase, trypsin, and chymotrypsin. Elastase is the enzyme of choice for the isolation of Type II cells from the lung.

Hyaluronidase

Hyaluronidase is a polysaccharidase with a specificity for endo-N-acetylhexosaminic bonds between 2-acetoamido-2-deoxy-beta-D-glucose and D-glucuronate. These bonds are common in hyaluronic acid and chondroitin sulfate A and C. Because these substances are found in high concentrations in the ground substance of virtually all connective tissues, hyaluronidase is often used for the dissociation of tissues, usually in combination with a crude protease such as collagenase.

Papain

Papain is a sulfhydryl protease from *Carica papaya* latex. Papain has wide specificity and it will degrade most protein substrates more extensively than the pancreatic proteases. It also exhibits esterase activity.

With some tissues papain has proved less damaging and more effective than other proteases. Huettner and Baughman (1986) describe a method using papain to obtain high yields of viable, morphologically intact cortical neurons from postnatal rats.

Chymotrypsin

Chymotrypsin is a protease which preferentially catalyzes the hydrolysis of peptide bonds involving the aromatic amino acids tyrosine, phenylalanine, and tryptophan. In addition it acts upon the peptide bonds of leucyl, methionyl, asparagenyl and glutamyl residues, and the amides and esters of susceptible amino acids.

Chymotrypsin is used to a limited extent in tissue dissociation, usually in combination with trypsin and elastase.

Deoxyribonuclease I

Often as a result of cell damage, deoxyribonucleic acid leaks into the dissociation medium increasing viscosity and causing handling problems. Purified deoxyribonuclease is sometimes included in cell isolation procedures to digest the nucleic acids without damaging the intact cells.

Neutral Protease (Dispase)

Neutral Protease (Dispase) is a bacterial enzyme produced by *Bacillus polymyxa* that hydrolyses N-terminal peptide bonds of non-polar amino acid residues and is classified as an amino-endopeptidase. Its mild proteolytic action makes the enzyme especially useful for the isolation of primary and secondary (subcultivation) cells since it maintains cell membrane integrity.

Neutral Protease (Dispase) is also frequently used as a secondary enzyme in conjunction with collagenase and/or other proteases in many primary cell isolation and tissue dissociation applications. Neutral Protease (Dispase) dissociates fibroblast-like cells more efficiently than epithelial-like cells so it has also been used for differential isolation and culture applications. Other advantages are its non-mammalian (bacterial) source and its ability to be inhibited by EDTA.

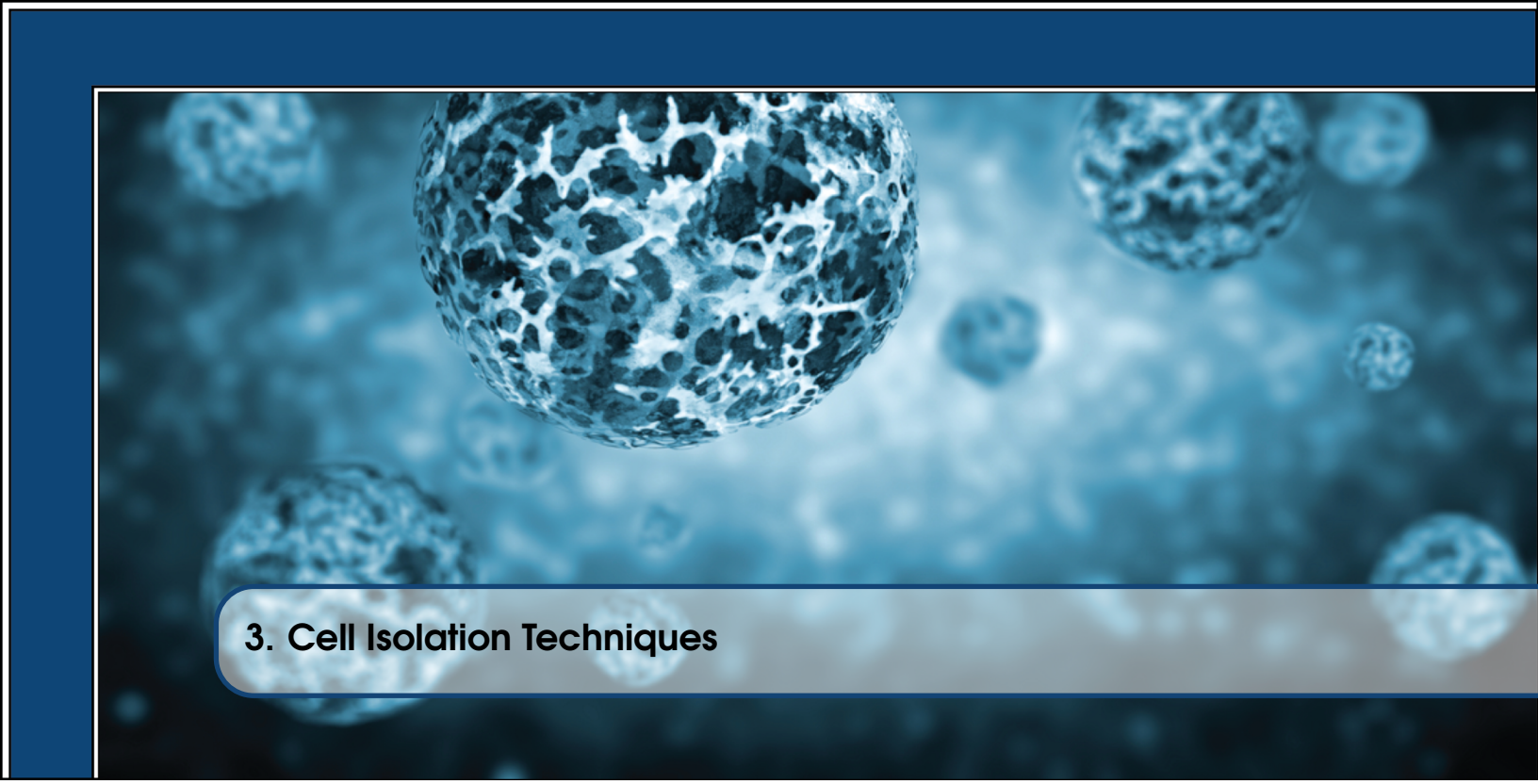
Trypsin Inhibitor (Soybean)

The trypsin inhibitor from soybean inactivates trypsin on an equimolar basis; however it exhibits no effects on the esterolytic, proteolytic or elastolytic activities of porcine elastase. Cell isolation procedures occasionally call for a trypsin inhibitor, usually the inhibitor from soybean (Worthington code SIC).

Dissociating Enzymes: Animal Origin Free (AOF) Enzymes

General interest in Animal Origin Free (AOF) tissue dissociation enzymes has dramatically increased to avoid potential contamination with mammalian agents such as prions and viruses. Worthington produces several AOF collagenases, proteases and nucleases for those requiring AOF enzymes; please check our current catalog for our these products.

Note: Application specific cell isolation systems have been developed by Worthington to eliminate the need for experimenting with various enzyme combinations and use testing several lots of collagenase. Descriptions for these systems can be found in our current catalog.



3. Cell Isolation Techniques

Working With Enzymes

All of the enzymes Worthington offers for tissue dissociation applications are available as lyophilized powders for convenience, versatility, and stability. As such they may be stored at 2 - 8°, and they can be shipped without special handling. While lyophilization makes shipping and storing the enzymes easier, special care is required when opening any of the vials.

Lyophilized proteins tend to be very hygroscopic so they should not be opened in humid areas. Be sure that any vial has been brought to room temperature before opening. Ideally, the vials should be taken from the refrigerator at least a half hour before opening, and they should be left in a desiccator. Before opening any of the vials, be sure it is not at all cool to the touch. All of the cell isolation enzymes cited in this section can be repeatedly warmed to room temperature and then returned to the refrigerator as long as these precautions are followed.

Once diluted with media or buffer, proteolytic enzymes can undergo autolysis. Dissolve enzymes immediately before use.

Special care must be taken with deoxyribonuclease (DNASE). This product is very prone to shear denaturation. Mix gently.

Reconstituted enzymes should not be stored at 2 - 8°C. If necessary they can be aliquoted and frozen at -20°C. Avoid repeated freeze-thaw cycles.

All enzymes, upon reconstitution, can be sterile filtered through a 0.22 μm pore size membrane.

Generally most of the enzymes used in cell isolation procedures (except trypsin) can be directly dissolved in a balanced salt solution or buffer of choice. Stock solutions of trypsin should be made initially by reconstituting the enzyme in 0.001 N HCl. This solution can be diluted into the digestion medium or buffer immediately prior to use.

The compilation of standard balanced salt solutions with their references found in the following table can be helpful in selecting an appropriate dissociation solution.

Table 3.1: Standard Solution Table - Composition of Selected Balanced Salt Solutions [a,b]

	Ringer[c]	Tyrode[de]	Gey[f]	Earle[g]	Puck[h]	Hank's[l]	Dulbecco (PBS)[j,k]
NaCl	9.00	8.00	7.00	6.80	8.00	8.00	8.00
KCl	0.42	0.20	0.37	0.40	0.40	0.40	0.20
$CaCl_2$	0.25	0.20	0.17	0.20	0.012	0.14	0.40
$MgCl_2 \cdot 6H_2O$		0.10	0.21			0.10	0.10
$MgSO_4 \cdot 7H_2O$			0.07	0.10	0.154	0.10	
$Na_2HPO_4 \cdot 12H_2O$			3.00		0.39	0.12	2.31
$NaH_2PO_4 \cdot H_2O$		0.05		0.125			
KH_2PO_4			0.03		0.15	0.06	0.20
$NaHCO_3$		1.00	2.27	2.20		0.35	
Glucose		1.00	1.00	1.00	1.10	1.00	
Phenol Red				0.05	0.005	0.02	
Atmosphere	Air	Air	95% Air 5% CO_2	95% Air 5% CO_2	Air	Air	Air

a Amounts are given as grams per liter of solution
b In some instances the values given represent calculations from data presented by the authors to account for the use of hydrated or anhydrous salts
c S. Ringer, *J. Physiol. (London)* 18, 425 (1895)
d M.V. Tyrode, *Arch. Int. Pharmacodyn. Ther.*, 20, 2025 (1910)
e R.C. Parker, *Methods of Tissue Culture*, 3rd ed., p. 57, Harper, New York, 1961
f G.O. Gey and M.K. Hey, *Am J. Cancer*, 27, 55 (1936)
g W.R. Earle, *J. Natl. Cancer Inst*, 4, 165 (1943)
h T.T. Puck, S.J. Cieciura, and A. Robinson, *J. Exp. Med.* 108, 945 (1958)
i J.H. Hanks and R.E. Wallace, *Proc. Soc. Exp. Biol. Med.*, 71, 196 (1949)
j PBS, phosphate-buffered saline
k R. Dulbecco and M. Vogt, *J. Exp. Med.*, 99, 167 (1954)

Basic Primary Cell Isolation

(Refer to references for application specific parameters)

- For non-perfusion, mince or cut the isolated piece of tissue into 2-4 millimeter pieces with sterile scissors or scalpel.
- Add the tissue pieces to the appropriate buffer or balanced salt solution on ice and wash 2-3 times.
- Add appropriate amount of enzyme(s) and incubate at optimum temperature (usually 37°C) for appropriate time, mixing intermittently.
- Gently disperse the cells by pipetting (trituration).
- Filter the cell suspension through fine mesh.
- Allow the cells to settle and decant excess liquid containing enzymes. Wash and repeat 2-3 times.
- Re-suspend cells in appropriate medium or buffer.
- Quantitate cell yield and viability.
- Seed cells for culture, if required.
- Perfusion procedures require special equipment and techniques for recirculating the buffers, media and enzymes. Please refer to referenced texts for additional information and guidance.

Equilibration with 95% O_2:5% CO_2

In many cell isolation procedures it is important to the survival of the tissue during dissociation that the incubation medium be both well oxygenated and buffered at physiological pH. Both requirements are satisfied when the medium is equilibrated with 95%O_2:5%CO_2. Several balanced salt solutions contain the pH sensitive indicator dye, phenol red. When it is red or purple in color, the medium is too alkaline. This sometimes occurs when the tissue is placed in the dissociation enzyme solution. Re-equilibration with O_2:CO_2 is usually necessary prior to incubation.

Gas should not be bubbled directly into any solution containing protein. This can result in frothing and denaturation of the protein with loss of biological activity. Gas can be sterilized by passage through a 0.22 μm membrane filter or through a sterile fiber plug such as the cotton plug in a sterile Pasteur or volumetric pipette. While mixing the solution, pass O_2:CO_2 continuously through the space above the liquid until color indicates pH 7.2-7.4. The balanced salt solution is often pre-gassed but should be equilibrated with sterile O_2:CO_2 each time the bottle is opened.

Buffered balanced salt solutions will usually maintain constant pH regardless of the degree of oxygenation/carbonation and as a result can be easier to work with. Certain cell types may be sensitive to particular buffer salts. The reference tables can be useful in selecting an appropriate balanced salt solution, buffer, or dissociation media for a specific application.

Enzymatic Cell Harvesting

Most non-malignant cells growing in vitro move about and divide until they form a monolayer one cell thick completely covering the surfaces of the culture vessel. Movement and proliferation normally cease when confluence is reached. Harvesting cells for study, processing or subculture requires dissociation and detachment of the monolayer. Limited treatment of the cell layer with the enzyme trypsin is the method most frequently applied.

It was formerly thought that trypsin preparations simply hydrolyzed a proteinaceous adhesive bonding

substance responsible for the tenacious attachment of cells to their substratum with the resultant detachment of the cells from the culture vessel. It is now felt that the mechanism of action of trypsin in cell harvesting is more complex. This section summarizes recent information on this subject.

Cell Adhesion and Harvesting

During interphase, fibroblast-like cells in culture are spread out on the substratum in a characteristic, spindle-shaped configuration. There are differences of opinion as to whether the actual areas of cell adhesion are distributed over most of the undersurface of the cell or are localized in relatively narrow patches near the cell margins, principally in the vicinity of ruffling activity. In either case, these areas of adhesion appear to be composed of clusters of attachment points, each about 1 μm in diameter. The individual attachment points are apparently the distal portions of a cell cytoskeleton structure bound to the substratum.

Within minutes after subjecting cultured cells to cold temperatures, chelating agents or trypsin solutions, they change shape drastically by rounding up and blebbing. Electron micrographs show many long retraction fibers with a diameter of 0.25 - 0.5 μm running from the surface of the rounded cell body to enlarged, terminal bulb attachment points previously located on the flattened cell's undersurface.

The cells remain attached to the substratum until the fibers are broken, either mechanically by tapping or shaking the culture vessel, or chemically by the continued action of chelators and/or trypsin. (Cold temperatures alone are sufficient for rounding up but not for detachment. These conditions also greatly diminish the entry of trypsin into the cell.) Soon after cell detachment from the surface of the culture vessel, and subculture into new vessels containing trypsin-free medium, cytoplasm flows into the broken retraction fibers and refills them. Within an hour the rounded cells begin to take on their characteristic shape.

Trypsin for Cell Harvesting

In 1916, Rous and Jones used "the trypsin powders of Merck, Brubler and Kahlbaum" to digest the plasma clots in which living cells were growing in order to obtain a cell suspension for subculturing. Vogelaar and Erlichman in 1934 were the next researchers to utilize the digestive enzymes in a crude trypsin preparation to liquify the coagulated plasma in which human fibroblasts were growing prior to subculturing. Techniques using trypsin similar to those used today were introduced by Scherer, Syverton and Gey in 1953 to harvest the then newly cultivated HeLa cell strain for subculturing and biochemical analysis. These workers tested both recrystallized trypsin and NF 1:250 trypsin for cell harvesting and found that the purified trypsin was more potent and less toxic to cells. Nevertheless the NF 1:250 preparation was employed for routine harvesting simply because it was less expensive.

Relatively crude pancreatic preparations like NF 1:250 trypsin are still used today for cell harvesting in spite of the fact that they exhibit considerable lot-to-lot variability and contain extraneous substances and other enzymatic activities. Impurities in crude trypsin can cause unnecessary damage to cells and a reduction of cloning efficiency. Use of higher purity crystalline trypsin can eliminate many of these difficulties.

None of the contaminants present in the NF 1:250 materials appears to be essential for cell harvesting activity since purified trypsin is very effective for monolayer dissociation, and since crude NF 1:250

trypsin plus soybean trypsin inhibitor is ineffective.

McKeehan and Ham report markedly improved viability and multiplication potential to single cells in low serum medium when harvesting with crystalline trypsin at reduced temperatures, i.e., at 4°C.

Cell Release Procedure

In order to transfer or pass cells in monolayer culture from one culture vessel to another it is necessary to release cells from the monolayer into suspension so that they can be easily handled by pipetting and diluting.

Releasing cells from the monolayer is almost always accomplished with purified trypsin by a procedure known as trypsinization. A usual trypsinization procedure is detailed in the inset below.

Trypsinization Procedure

I. Remove culture medium from cells.
II. Add sterile trypsin solution (in BSS-balanced-salt solution, normally calcium-free Hanks.
III. Allow trypsin solution to act on monolayer for several minutes at room temperature or 37°C (or longer at 4°C).
IV. Remove trypsin solution gently so as not to disturb cells.
V. Add BSS or media (often with serum or trypsin inhibitor to inactivate residual trypsin) and agitate vessel to disrupt monolayer and suspend cells.

Some researchers have found that procedures using crystalline trypsin can provide increased viability in cells after they are released. Viability is usually determined by measuring cloning efficiency, i.e., the ability of a single cell to attach to the wall of a culture vessel and divide to produce a colony of cells which is visible to the naked eye after staining.

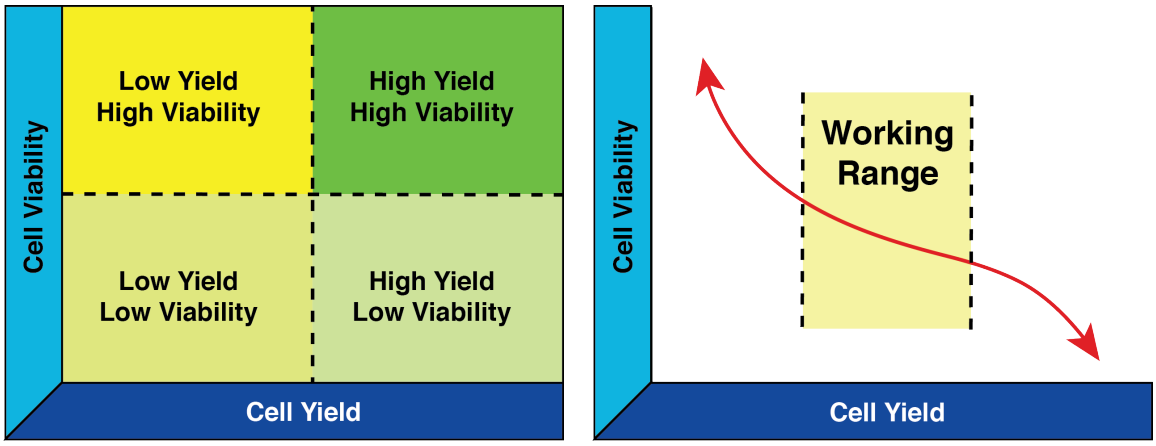
4. Optimization Techniques

General Guidelines

Although optimization of a cell isolation procedure for a particular cell type is dependent upon the adequate recovery of cells having various required characteristics, some guidelines can be established. The information in this guide regarding cell isolation and the enzymes used, when combined with logic and suitable experimental design, should lead to the development of a satisfactory cell isolation method. (See Freshney 1987 for a detailed discussion.) The complex relationship between cell yield and viability can be represented by the simplified illustrations shown in Figure 4.1b. In general there is an area of optimized recovery balanced between yield and viability; working near the middle of this range will reduce variability in the results of the cell isolation procedure. Understanding this relationship and how it can vary with a particular cell type and application, can make the optimization process easier.

For troubleshooting purposes various possible results, along with suggested corrective actions are listed below. Keep in mind that there are no clear lines between the quadrants but rather converging zones with variable areas of overlap.

Low Yield/Low Viability - Over/under dissociation, cellular damage. Change to less digestive type enzyme and/or decrease working concentration. (e.g. from trypsin to collagenase/ from Type 2 collagenase to Type 1).
Low Yield/High Viability - Under dissociation. Increase enzyme concentration and/or incubation time and monitor both yield and viability response. If yield remains poor, evaluate a more digestive type enzyme and/or the addition of secondary enzyme(s).
Low Yield/High Viability - Good dissociation, cellular damage. Enzyme overly digestive and/or at too high a working concentration. Reduce concentration and/or incubation time and monitor yield and



(a) Cell Yield vs. Cell Viability (b) Working Range

Figure 4.1: The Complex Relationship Between Cell Yield and Viability.

viability response.
Try diluting the proteolytic action by adding bovine serum albumin (BSA) (0.1 - 0.5% w/v) or soybean trypsin inhibitor (0.01 - 0.1% w/v) to the dissociation.
Try using less proteolytic enzyme although yield may be affected and should be monitored.
High Yield/High Viability - The place to be. Consider evaluating the effect of dissociation parameters to learn their limitations for future reference.

A scale (Figure 4.2) showing the relative digestive power of the enzymes commonly used follows for reference. Refer to this scale when troubleshooting a dissociation and planning isolation strategy.

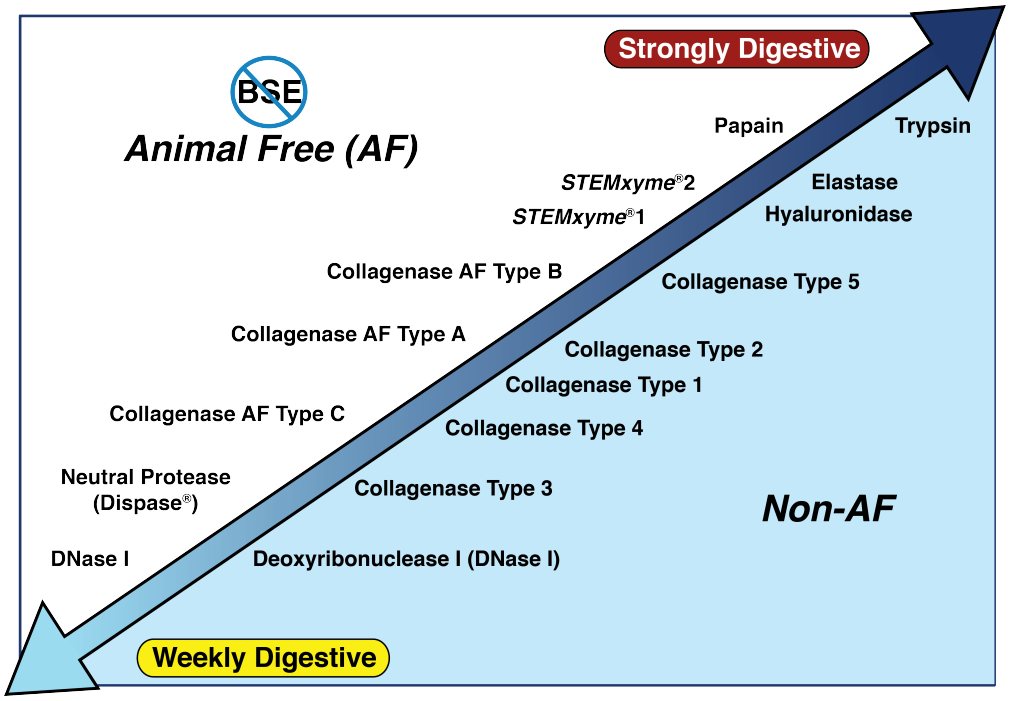


Figure 4.2: Primary Cell Isolation Enzyme Digestion Scale

Optimization Strategy

Review the References of the *Worthington Tissue Dissociation Guide* for the particular tissue and cell type of interest, and then apply this information to the practical application of tissue dissociation. An example of a basic optimization strategy follows:

Based upon the enzyme(s) cited, working concentrations and the buffer or media system used, set up proposed preliminary dissociation conditions similar to the closest available reference(s) listed in the tables.

If a majority of the most similar referenced procedures cite the use of more than one enzyme, optimize the concentration of the primary enzyme (the one at the highest relative concentration) before adding the secondary enzyme(s). For example, if the two most similar references cite collagenase 0.1% with DNase 0.01% and collagenase 0.075% with hyaluronidase 0.025%, optimize the collagenase concentration empirically before evaluating the effects of either the hyaluronidase or the deoxyribonuclease.

After optimizing the primary enzyme's concentration and incubation conditions evaluate any secondary enzyme(s).

Initially vary the concentration of the primary enzyme approximately 50% relative to the referenced procedure(s). The above example of collagenase concentrations 0.1% and 0.075% suggests an evaluation of enzyme concentrations between 0.025% and 0.15%. The concentration increments should be evenly distributed to cover this entire range. As a result incremental concentrations of 0.025%, 0.05%, 0.075%, 0.10%, 0.125% and 0.15% would be indicated. To simplify the initial screening the middle of the range can be selected and, after evaluation of yield and viability results, a decision can be made

regarding the need for further studies. In this case initial collagenase concentrations evaluated may be 0.05%, 0.075%, 0.10% and 0.125%.

Historically, most tissue dissociation and cell isolation protocols have cited the enzyme concentration used in terms of weight per unit volume (w/v). More recently, however, some researchers have begun to use the enzymes on an activity basis, that is, units per milliliter (u/ml). Use either method but consider the advantages and disadvantages of each:

a The traditional weight per unit volume method most likely resulted from the use of cruder, partially purified mixtures of enzymes and is used independently of any specific or contaminating activities which may be present. With some of these crude preparations the lot-to-lot variation can be significant resulting in up to a two-fold difference in the amount of enzymatic activity added on a weight basis.

b Adding by activity can result in a possible two-fold difference in the amount of weight added to a dissociation; however, normalizes the potency used based upon the primary activity for each lot.

Both methods ignore the relative contaminant activity levels. Upon establishing a basic method, consider pre-sampling different lots of enzyme(s) to evaluate these factors and to select a lot of enzyme which has minimal effect upon the critical parameters of a specific application.

Important: For accurate evaluation of a particular procedure's performance, cell yield and viability should be quantitated and compared. After optimizing basic dissociation and isolation conditions, the specific application parameters such as metabolic function(s) or receptor binding capability should also be evaluated. Based upon these results the method may be judged suitable for use or re-optimized for higher retention of native cellular characteristics.

Cell Quantitation

It is important to quantitate the results of each dissociation step in order to effectively evaluate each procedure. The use of a cell counting chamber (hemocytometer) for yield quantitation and the use of trypan blue for viability quantitation are recommended. The use of a hemocytometer for cell yield quantitation is outlined; however, newcomers to this procedure can refer to more detailed discussions (see Freshney, Culture of Animal Cells, page 227).

Required Supplies:

- Improved Neubauer Hemocytometer
- Cell Compatible Media or BSS
- Pasteur Pipet or Micropipettor
- Microscope (10x)
- Counter

Procedure:

I. Carefully clean the counting chamber surface and the coverslip of the hemocytometer with 70% isopropanol and allow to air dry. Be careful not to scratch these surfaces.

II. Wet the sides of the coverslip with reagent grade water and align the coverslip over the counting chamber.

III. Take a well mixed 20-50 μl aliquot of the dissociated cell suspension using either a Pasteur pipet or a micropipettor only drawing the cells into the tip. Immediately transfer the cell suspension to

the counting chamber by placing the tip of the pipet at the edge of the chamber and allowing the chamber to fill completely via capillary action. Do not over- or under - fill the chamber.

IV. Repeat this procedure using another aliquot sample for the second chamber on the opposite side of the hemocytometer.

V. Place the hemocytometer on the microscope stage and, using the counting illustration 10x objective, focus on the counting chamber grid lines. Adjust the contrast as needed to clearly see both the grid and the dispersed cells.

VI. Adjust the field area by slowly moving the slide to obtain a central grid bounded by three lines on all sides (see Figure 4.3). Count the total number of cells present in this 1 mm^2 area including those cells which are on the top and left borders and excluding those on the right and bottom borders.

VII. For accuracy count at least 100 - 500 cells. Depending upon yield and density more or fewer areas may be counted.

VIII. Repeat the count for the second chamber. If no second chamber exists, the slide should be cleaned and the process repeated.

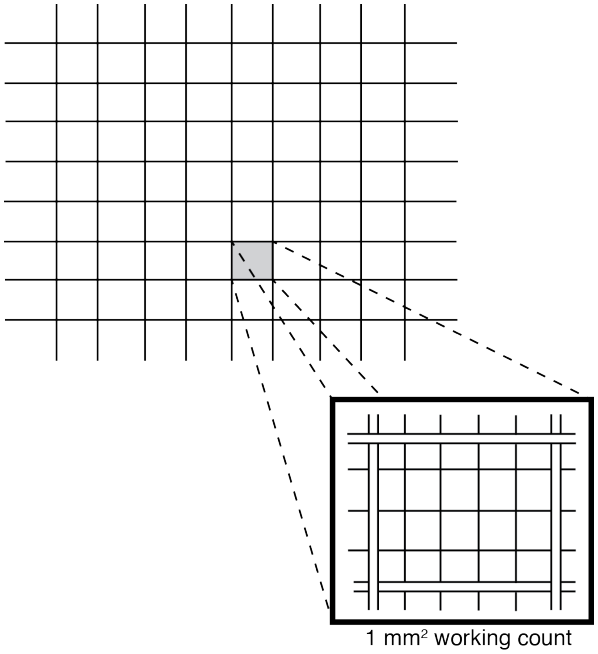


Figure 4.3: Counting Chamber Grid Lines

Calculation:

$C = \bar{N}$ x 10^4

where C = cell per milliliter

$\bar{N}$ = average number of cells counted

10^4 = volume conversion factor for 1 mm^2 (4.1)

Total Yield = C x V

where V = total value of cells (ml)

Example:

$Count_1 = 183$ cells/mm^2

$Count_2 = 175$ cells/mm^2

Volume of Cells = 55 ml

$$\text{Average Cells Counted} = \frac{Count_1 + Count_2}{2} \quad (4.2)$$

$$= \frac{183 + 175}{2}$$

$$= 179$$

$C = 179 \times 10^4 = 1{,}790{,}000$ cells/ml

Total Yield = C x V = 1,790,000 x 55 = 98,450,000 cells

Note: For best results the cell density should be at least 105 cells per milliliter. Common errors occur by improper mixing of the cell suspension prior to sampling and/or by allowing the cells to settle in the pipet prior to loading the hemocytometer counting chamber. Avoid the counting of multiple cell aggregates; the presence of aggregates indicates incomplete dissociation which may require further optimization of the isolation parameters. A single cell suspension provides the best results.

Measure of Viability

One of the simplest methods to approximate cell viability is the dye exclusion technique. This method utilizes an indicator dye to demonstrate cell membrane damage. Cells which absorb the dye become stained and are considered non-viable. Dyes such as trypan blue, erythrosin, and nigrosin are commonly used with trypan blue being the most common in preliminary cell isolation procedures.

This procedure can be performed along with the cell counting procedure but cell density may require adjustment in order to obtain approximately 106 cells per milliliter.

Procedure:

I. Mix 1 drop of trypan blue with one drop of the cell suspension and allow 1 - 2 minutes for absorption
II. Prepare hemocytometer and load chambers as described in "Cell Quantitation".
III. Count both the total number of cells and the number of stained (dark) cells.

Calculation:

$$\text{Percent Viability} = \frac{\text{Total Cells Counted - Stained Cells}}{\text{Total Cells Counted}} \times 100 \quad (4.3)$$

Example:

$$\frac{\text{Total Cells}}{1\ \text{mm}^2} = 182$$

$$\text{Stained Cells} = 24 \tag{4.4}$$

$$\text{Percent Viability} = \frac{182 - 24}{182} \times 100 = \frac{158}{182} \times 100$$

$$= 86.8\%\ \text{Viability}$$

Note: Dye exclusion viability procedures tend to give high estimates of cell viability when compared to cell attachment or metabolic assays, but for optimization of cell isolation procedures trypan blue does provide a rapid estimate of dissociation performance in conjunction with yield quantitation.

5. Use-Tested Cell Isolation Systems

Worthington currently offers Cell Isolation Systems which are kits containing enzymes and other required reagents for performing tissue dissociations without having to purchase individual packages of one or more enzymes and pretesting various lots of some enzymes. Some are designed for working with specific tissues, and one kit is a general purpose procedure development system. In all cases, the enzymes which are included in the kits are regular Worthington products which can be purchased independently.

Hepatocyte Isolation System (HIS)

Introduction

Most traditional methods published for isolating hepatocytes use crude and partially purified enzyme preparations including various types of collagenase and other proteases. More recently the use of better characterized preparations of collagenase such as Worthington Types 1 and 4 (CLS-1, 4) have provided better results. All crude collagenase preparations can contain lot-variable contaminating proteases, esterases and other enzymes requiring researchers to pre-screen several lots of enzyme and/or continually modify isolation parameters and protocols.

The Worthington Hepatocyte Isolation System has been developed to provide researchers with a reliable, convenient, and consistent hepatocyte cell isolation system. By using the pre-optimized combination of enzymes contained in this kit, it is possible to minimize the lot-to-lot variation and improve the quality of the isolated hepatocytes. In addition, Worthington use-tests each lot by isolating hepatocytes from adult rat to assure performance, reliability, and consistent yield of viable cells.

The method is based on that described by Berry, M.N., modified by Seglen, P.O. (Methods in Cell Biology, vol XIII, David M. Prescott ed., Academic Press, 1976; Chapter 4, "Preparation of Isolated Rat Liver Cells", pp 29-83), and further optimized in conjunction with several researchers.

Description and Package Contents:

Stability/Storage: The reagents are stable at ambient temperatures for the periods of time expected in normal shipping procedures, but the package should be refrigerated upon arrival. Contents may be stored at 2-8°C for 4-6 months before use. Store at 2-8°C.

Package Contents: The package contains sufficient materials for five separate adult rat liver perfusions. For larger or smaller tissue applications, prepare proportionate volumes of reagents at each step and combine them in the same ratio as described in the protocol.

Vial #1: 10x CMF-HBSS Concentrate, 1 bottle, 500 ml Sterile calcium- and magnesium-free Hank's Balanced Salt Solution (CMF-HBSS). The solution is used for washing and perfusing the liver prior to the addition of the dissociating enzyme solution.

Vial #2: Collagenase-Elastase Enzyme Vial, 5 Vials Worthington collagenase (Code: CLS-1) and elastase (Code: ESL), filtered through 0.22 μm pore size membrane, and lyophilized. Before use, reconstitute with the L-15/MOPS solution and swirl gently to dissolve contents as directed in the following procedure. Store unreconstituted vials at 2–8°C.

Vial #3: 1,000 Units DNase I each, 5 Vials Worthington DNase I (Code: D), filtered through 0.22 μm pore size membrane, and lyophilized. Before use, reconstitute with L-15/MOPS solution and swirl gently to dissolve contents as directed in the following procedure. Store unreconstituted vials at 2–8°C.

Vial #4: 0.15M MOPS, pH 7.5, 1 bottle, 75ml 0.15M MOPS, pH 7.5 buffer concentrate, used to buffer the reconstituted Leibovitz L-15 media.

Vial #5: 7.5% Sodium Bicarbonate ($NaHCO_3$), 1 bottle, 100ml 7.5% Sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS.

Pouch, containing Leibovitz L-15 Media Powder, 1 x 1L: Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing approximately 800 ml of cell culture grade water. Rinse pouch 2 - 3 times with an additional 100 ml water. Bring total volume to 1000 ml and filter through a 0.22 μm pore size membrane.

Required for Perfusion Isolation but not Included:

- Equipment and tools for animal anesthesia and surgery
- A perfusion apparatus with a bubble trap suitable for liver perfusion at 10-30ml/min, 37°C. The tubing to be inserted into the portal vein is thin-walled with an inner diameter of 0.35-0.45mm. Note: Measure the dead volume of the perfusion circuit
- A low-speed centrifuge suitable for sedimentation of hepatocytes
- Labware for cell sedimentation, and culture or incubation including sterile 150 x 25 mm culture plates
- A means to count or estimate the yield of cells
- A means to sterile-filter solutions, if desired
- Cell culture media and supplies, if needed
- Sterile cell culture grade water

- Concentrated antibiotics: penicillin, streptomycin, Fungazone, etc. for culture, if needed.
- Surgical thread, silk, size 000
- Heparin (optional)

For Cell Quantitation and Viability Assessment:

- Improved Neubauer hemocytometer
- Counter
- Pasteur pipette or micropipettor
- Microscope (10x), preferably inverted phase-contrast
- Standard 10 ml serological pipettes

Note: The following procedure presumes previous experience in liver digestion and cell isolation. For those not experienced, refer to the publication by Seglen referenced above, or to Alpini et al. entitled "Recent Advances in the Isolation of Liver Cells" published in Hepatology (1994) 20:494-514. Perfusion of the liver while still in the peritoneal cavity is described in "Isolated Hepatocytes Preparation, Properties and Application", by Berry, M.N., Edwards, A.M. and Barritt, GJ; RH Burdon and PH Van Knippenberg, eds., Elsevier, Amsterdam, New York, Oxford, Chapter 2, (1991).

I. Preliminary Steps for Digestion of 1 Liver The volumes specified in the following protocol are suitable for perfusion volumes of approximately 80-100 ml. Proportional adjustments may be necessary for different perfusion systems.

Note: Sterile techniques, glassware and plasticware should be used. The use of a sterile hood is also recommended to avoid culture contamination.

Prepare:

- **Vial #1, 10x CMF-HBSS:** Dilute 100 ml of the 10x CMF-HBSS with 850 ml of sterile water and add 4.7 ml of 7.5% Sodium Bicarbonate (Vial #5, $NaHCO_3$) in a sterile 1L bottle. Adjust pH if necessary to 7.4. Bring (QS) to a total volume of 1 L with sterile water. If sterile water is not available, mix ingredients and sterile (0.22 μm) filter. Makes a total of 5 L.
- **Leibovitz L-15 Media, 1 x 1L:** Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing 800ml of cell culture grade water. Rinse pouch 2 - 3 times with an additional 100ml water. Bring total volume to 1000 ml and filter through a 0.22 μm pore size membrane.
- **Enzyme Buffer Solution:** Combine 13.3 ml of MOPS concentrate with 10ml sterile water and 76.7ml of L-15 in a sterile 100ml bottle. Transfer sufficient L-15/MOPS into one each of Vial #2 and into one Vial #3 to dissolve the contents, mix gently to completely dissolve and transfer the enzymes back to the 100ml bottle. The collagenase, elastase and DNase concentrations will be approximately 225 U/ml, 0.3 U/ml and 10 U/ml, respectively.
- Flush the sterile perfusion apparatus with CMF-HBSS, eliminating all air from the system except that in a bubble trap.
- Place the 150 x 25 mm or equivalent Petri dish close to the perfusion apparatus to receive the perfused liver.

II. Perfusion and Digestion of Adult Rat Liver The following steps should be performed in a laminar flow hood or safety cabinet. In particular, the digested liver should be processed under sterile conditions

unless acute incubations will terminate the procedures.

I. Pretreatment of the rat with heparin is helpful. Inject i.p. about 20 minutes before perfusion, or into a vein (Seglen suggests the iliolumbar vein) after opening the abdomen. Use from 100-200 U/100g body weight.

II. Anesthetize a rat, 200-400 g weight, and position it for dissection. Install sufficient padding under the rat to hold the blood and initial perfusate. Place the rat on its back, tape down the legs, sterilize the abdomen with an iodine solution or 70% ethanol, and open the abdomen to expose the liver. Move the intestines to the left side of the abdomen (to the right as you look down with the rat's head away from you) exposing the hepatic portal vein.

III. Using a pair of fine, curved forceps, place a segment of 000 surgical thread underneath and around the portal vein just above (toward the head) the intersection of the portal vein and the final mesenteric vein close to the liver. Tie a loose half-square or equivalent knot around the vein. Locate the vena cava so it can be opened for drainage just before the portal vein (vena porta) is cannulated.

IV. Turn on the perfusion pump containing plain CMF-HBSS with a flow rate 10-15 ml/min so that the tubing or cannula can be inserted into the portal vein. The bath temperature is adjusted so that the perfusate temperature is 37°C. Cut a nick in the vena cava near the right kidney to lower the blood pressure, and then with fine surgical scissors cut a nick in the portal vein (partially through) about 5mm below (towards the tail) the knotted thread. Insert the tubing into the portal vein towards the liver and only several millimeters past the loose knot. The liver should clear of blood. Tie the surgical threads tightly around the portal vein and tubing. Cut the vena cava through and increase the perfusate flow rate to 20-25 ml/min. Note: Establishment of an effective perfusion that flushes the entire vasculature is essential to the success of the digestion.

V. Remove the liver from the animal with great care; do not rush. Place the liver onto a mesh stage in such a manner that it can be perfused in a recirculating fashion. The initial CMF-HBSS perfusate, however, goes to waste.

VI. After 7-10 min of CMF-HBSS perfusion, switch to perfusion with the Enzyme Buffer Solution (L-15 digestion medium containing the enzymes). Start recirculation after one system-dead-volume of the remaining CMF-HBSS has gone to waste.

VII. Perfuse the liver with the digestion mixture until it swells fully (but not prematurely) and the liver is fully digested, about 20-30 minutes. Note: Halt the perfusion immediately by stopping the pump and removing the liver if the portal vein breaks or if the surface of the liver shows signs of disintegration when touched with forceps or a blunt object.

VIII. At the end of the perfusion, stop the pump, gently place the liver in the 150 ml or equivalent culture dish and remove the perfusion tube. Transfer the culture dish to a sterile hood if not already in one, and add approximately 150 ml of fresh CMF-HBSS to the dish.

IX. In the culture dish, gently pull off the lobular capsule membranes with forceps or dog comb (recommended by Seglen), and rake out the cells. Remove the large central tree of connective and vascular tissue, and any undigested tissue or connective tissue.

X. Gently agitate the dish to disperse the cells. Place the dish at an angle by propping one side on the lid. Allow clumps or connective tissue to settle for a minute or so, then remove the dispersed cells from the top of the buffer at the deepest part of the plate, i.e. close to the lower edge, and transfer the cell suspension to 50 ml sterile tubes.

XI. Centrifuge for three minutes at low speed (just rapidly enough for loose cell pellets, e.g. 100 x g) at room temperature.

XII. Add more CMF-HBSS to the culture dish and repeat the process to increase the yield of cells.

Repeat as long as clean cells can be removed.

XIII. As soon as cells are sedimented, add fresh CMF-HBSS, suspend the cells by inverting the capped tubes, and re-centrifuge as above. Repeat process once more to remove traces of the digestive enzymes from the cells. Discard the supernatant(s) and transfer cells to culture medium or buffered medium in a second 100 mm or 150 mm culture dish. The yield of cells from a good digestion of a liver of a 300 gm rat is approximately 4-5 ml of packed volume after gentle sedimentation in a centrifuge.

Culture of Hepatocytes (Optional) Although application specific, hepatocytes have been cultured in a number of media including DMEM, Leibovitz's L15, modified Chee's medium, Williams E medium, RPMI and Waymouth's MB 752/1. In general, media are supplemented with numerous factors in order to maintain a differentiated state. Among these are EGF, insulin, glucagon, dexamethasone, selenite, nicotinamide and hepatocyte growth factor (Chen et al., 1998). Specialized media for hepatocytes may be purchased commercially from other suppliers. In order to successfully plate hepatocytes, cultureware is generally coated with a matrix such as collagen, laminin, or some type of commercial matrix.

Advances in the culture of hepatocytes include the use of threedimensional matrices (gels) of collagen or Matrigel^{T}M. Chen et al. (1998) plated hepatocytes on Matrigel^{T}M (^{T}MBecton Dickinson, Bedford MA) and after several weeks removed the cells and replated them on a collagen gel. After 24hr, a second layer of collagen gel was added. Alternatively, cells may be directly plated in a collagen gel and maintained as a three-dimensional culture.

A review discusses the effects of culture variables on human hepatocytes (Le Cluyse, 2001), and is likely applicable to culture of hepatocytes from other species. The same author has reviewed optimal conditions for the culture of rat hepatocytes (Le Cluyse et al., 1996).

Neonatal Cardiomyocyte Isolation System

Introduction:

Most traditional methods published for isolating neonatal cardiomyocytes utilize crude and partially purified enzyme preparations such as trypsin NF 1:250 (Gross, et al. 1968) and collagenase. More recently the use of better characterized preparations of collagenase such as Worthington type 2 collagenase (CLS2) has provided better, more reproducible results. Like all crude collagenase preparations, though there can be significant lot-to-lot variations so many researchers make use of the Worthington Sampling Program to pre-screen several lots of enzyme before selecting a lot to purchase. Pre-sampling assures that the enzyme preparation ultimately purchased will be satisfactory; however, it is time consuming.

The Worthington Neonatal Cardiomyocyte Isolation System has been introduced to provide researchers with a reliable, convenient, and consistent cell isolation system. By utilizing purified rather than crude enzyme preparations, it has been possible to minimize the lot-to-lot variation. In addition, Worthington use-tests the kits by isolating cardiomyocytes from neonatal rat hearts to assure performance, reliability, and consistent yield of viable cells.

The kit has been formulated in conjunction with Dr. Ronal MacGregor of the University of Kansas Medical Center. The method is based on that described by Toraason, et al. (1988) in which the minced

tissue is incubated overnight with trypsin in the cold. As pointed out by Toraason, this step reduces the hands on time required to harvest cells compared to the time involved in sequential incubations in warm trypsin or collagenase.

Description and Package Contents:

The package contains sufficient materials for five separate tissue dissociations, each containing up to twelve hearts. For larger or smaller tissue samples prepare proportionate volumes of reagents at each step and combine them in the same ratio as described in the protocol.

Package Contents:

Vial #1: 1 bottle, 500 ml, Sterile calcium- and magnesium-free Hank's Balanced Salt Solution (CMF HBSS), pH 7.4. The solution is used for reconstituting the contents of Vials #2 and #3 in addition to serving as the medium for the dissociation.

Vial #2: 5 vials, 1000 μg each, Worthington Trypsin (Code: TRLS), 3x crystallized, dialyzed against 1 mM HCl, filtered through 0.22 μm pore size membrane, and lyophilized. Before use, reconstitute with 2 ml CMF HBSS (Vial #1) and swirl gently to dissolve contents. Store at 2–8°C.

Vial #3: 5 vials, 2000 μg each,Worthington Soybean Trypsin Inhibitor (Code: SIC), a 0.22 micron pore size membrane filtered, lyophilized powder. Before use, reconstitute with 1ml CMF HBSS (Vial #1) and swirl gently to dissolve contents. Store at 2–8°C. **Vial #4:** 5 vials, 1500 Units each, Worthington Purified Collagenase (Code: CLSPA), a 0.22 μm pore size membrane filtered, lyophilized powder which has been chromatographically purified. It contains less than 50 caseinase units per milligram and is composed of two separable but very similar collagenases. Before use, reconstitute with 5 ml Leibovitz L-15 Media (prepared as described below) and swirl gently to dissolve contents. Store at 2–8°C.

Vial #5: 7.5% Sodium Bicarbonate ($NaHCO_3$), 1 bottle, 100ml 7.5% Sodium bicarbonate concentrate, used to buffer the diluted CMF-HBSS.

Pouch, containing Leibovitz L-15 Media Powder, 1 x 1L: Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing approximately 800 ml of cell culture grade water. Rinse pouch 2 - 3 times with an additional 100 ml water. Bring total volume to 1000 ml and filter through a 0.22 μm pore size membrane.

The kit also includes 5 Cell Strainers (Falcon), and a card correlating phenol red color with pH for checking the pH of balanced salt solution and culture medium.

Needed but not supplied:

- Sterile 50 ml centrifuge tubes
- 10 cm Petri dishes, one per dispersion
- Standard 10 ml plastic serological pipette
- Rotating or shaking instrument for incubating at 37°C
- Oxygen supply
- Centrifuge (capable of 50-100 x g)
- Cultureware
- Sterile cell culture grade water
- Fetal Bovine Serum (FBS) for cell culture

For Cell Quantitation and Viability Assessment:

- Improved Neubauer Hemocytometer
- Counter
- Pasteur Pipet or Micropipettor
- Microscope (10x), preferably inverted phase-contrast
- Standard 10 ml serological pipettes

Procedure

The volumes specified in the following protocol are suitable for hearts from 10 rat pups, 1 to 7 days old: Adjust proportionally for different numbers of hearts.

Note: Do not process more than one litter or about 15 hearts in one Petri dish or 50 ml tube. The procedure works best for 5-15 hearts per preparation.

Note: All glass and plasticware is sterile. Steps #1-4 may be performed in a cold room without ice, but a sterile hood is preferable.

Day 1: Perform the following in the afternoon

Prepare:

- Reagent #1, CMF HBSS: 50-60 ml from Vial #1, ice cold.
- Reagent #2, Trypsin: reconstitute one of Vial #2 with 2 ml Reagent #1, ice cold.
- One sterile 50 ml centrifuge tube, in ice.
- 10 cm Petri dish, sterile, on ice.

Procedure:

I. Transfer 30 - 40 ml of Reagent #1 to the centrifuge tube.

II. Anesthetize each rat pup, sterilize the abdomen with an antiseptic solution, and surgically remove the beating heart; immediately place the heart in the centrifuge tube to chill and rinse. Repeat for remaining rat pups. Swirl the tube to rinse hearts, then pour off most of the liquid. Rinse the hearts with 10 ml of Reagent #1, pour off the liquid as before, then transfer the hearts to the Petri dish. Mince the tissue with small scissors or a razor blade to less than 1 mm3 pieces keeping tissue at 0°C.

III. Add Reagent #1 to Petri dish to a final volume of approximately 9 ml.

IV. Transfer 1 ml of the contents of the trypsin vial (Vial #2) into the Petri dish and mix completely by swirling. Final trypsin concentration is 50 μg/ml.

V. Place the lid on the petri dish and immediately place in refrigerator overnight (16 - 20 hours) at 2-8°C.

Note: If animals are 4 days old or older, increase the trypsin concentration up to a maximum of 100 μg/ml.

Day 2: Begin the following in the morning:

Prepare:

- Reagent #1, CMF HBSS: 30 ml. ice cold.
- Reagent #3, Trypsin Inhibitor: reconstitute one of Vial #3 with 1 ml Reagent #1. Room temperature.

- Reagent #4 Collagenase: reconstitute one of Vial #4 with 5 ml prepared Leibovitz L-15. Room temperature.
- Enough culture medium containing calcium and magnesium for digestion, centrifugations, and plating in cultureware. (approximately 100 ml for 10 hearts). Room temperature.
- Wide-mouth 10 ml serological pipet, sterile (opening about 3 mm diameter)
- Standard 10 ml plastic serological pipet

Procedure:

I. Remove Petri dish from refrigerator and bring to sterile hood on ice. Transfer tissue and buffer to 50 ml centrifuge tube on ice using wide-mouth pipet.

II. Transfer contents of Vial #3 into tube and mix.

III. Oxygenate tissue for 30 seconds to 1 minute if O_2 is available by passing oxygen over the surface of the liquid.

IV. Warm tissue and buffer to 30 - 37°C in water bath, maintaining sterility (i.e. cap if needed). Do not add calcium-containing medium until tissue fragments are warm.

V. Slowly transfer the contents of Vial #4 into tube and mix. Cap tube tightly.

VI. Place tube in/on slowly rotating (tumbling) or shaking instrument (2 - 4 rpm) at 37°C and incubate for 30 to 45 minutes.
All subsequent steps at room temperature.

VII. Remove tube from incubator and return to sterile hood. With standard 10 ml plastic serological pipet, triturate about 10 times to release cells. (Trituration is discussed in the following inset.) Pipet as gently as possible consistent with successful tissue dispersion.

VIII. Rinse a Cell Strainer with 1 ml of the L-15 culture medium. Allow tissue residue to settle 3 - 4 minutes, then (with same pipette) filter the supernatant through the Cell Strainer into a fresh 50 ml centrifuge tube.

IX. Add 5 ml additional L-15 culture medium to tissue residue, repeat trituration step. Allow tissue residue to settle as before, then filter cells through the same Cell Strainer. Rinse mesh gently with 2 ml culture medium, oxygenate cells 1 minute, then allow filtered cells to remain undisturbed for about 20 minutes at room temperature. This allows complete digestion of the partially degraded collagen. (Cells can be held up to 1 hour at this point.)

X. Swirl cells gently; if no clumps have formed and appearance is uniform, sediment cells at 50 - 100 x g for 5 minutes (enough to settle the myocytes and some but not all red cells.) Suspend cells in additional portions of L-15 culture medium and repeat sedimentation as desired. If no sedimentation is desired, cells can be plated directly from the initial filtrate. Serum is generally required for plating cells in cultureware.

XI. Suspend final cell pellet in suitable culture medium. Pipet gently to disperse. No clumps or connective tissue strands should be visible. Count the cells using a hemocytometer or other method, adjust cell concentration and add serum as desired, then dispense to tissue cultureware. (Some brands of uncoated cultureware do not encourage high plating efficiencies. Use Falcon or equivalant for best results.) Routine cell yields are 2 - 3 x 10^6 cardiomyocytes per heart digested. Good (fairly heavy) seeding levels of cells should be obtained at 125,000 cardiomyocytes per cm^2 of culture wells or flasks. Adhesion may be improved by collagen or fibronectin coating of the plastic. Cell Quantitation and Estimation of Viability are discussed in the following sections.

XII. Place each plate or flask in a 37°C incubator as soon as it is plated. Do not touch or otherwise disturb the cells for at least 24 hours.

Papain Dissociation System

Introduction:

Proteolytic enzymes are widely used in cell dissociation. With some tissues papain has proved less damaging and more effective than other proteases. Lam found that of the enzymes used for dissociating turtle retina, papain produced the least trauma. Intact single photoreceptor cells have been isolated from adult salamander retina with papain. Huettner and Baughman described a method using papain to obtain high yields of viable, morphologically intact cortical neurons from postnatal rats. Finkbeiner and Stevens applied the Huettner and Baughman method to the dissociation of postnatal rat hippocampus. Papain is used with fetal as well as postnatal brain regions to provide maximal dissociation and viability of neurons.

The Worthington Papain Dissociation System is a set of reagents intended for use in the tissue dissociation method of Huettner and Baughman. The materials are designed for convenience and simplicity and are useful to the occasional user as well as the more experienced and frequent user. Each lot is use tested for performance in tissue dissociation and provides freshly prepared enzyme solutions for each dissociation.

The reagents are stable at ambient temperatures for the periods of time expected in normal shipping procedures, but the package should be refrigerated upon arrival and can be stored at 4-8°C for up to four months before use.

Description and Package Contents:

The package contains sufficient materials for dissociation of five separate tissue aliquots of up to 0.3 - 0.4 cm^3 each. For larger tissue samples prepare proportionately larger volumes of reagents at each step and combine them in the same ratio as described in the protocol.

Package Contents:

Vial #1: Sterile Earle's Balanced Salt Solution (EBSS) with bicarbonate and phenol red, one vial per package. Aliquots of this vial are used to reconstitute other vials and to prepare dilute inhibitor solution. Refrigerate between uses and equilibrate with sterile O_2:CO_2 before each use.

Vial #2: Papain containing L-cysteine and EDTA, five single use vials per package. This material is 0.22 μm membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with five ml of EBSS (vial 1) yields a solution at 20 units of papain per ml in one millimolar L-cysteine with 0.5 millimolar EDTA. Brief incubation is needed to insure full solubility and activity.

Vial #3: Deoxyribonuclease I (DNase), five single use vials per package.This material is 0.22 μm membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 0.5 ml of EBSS (vial 1) yields a solution at 2000 units of deoxyribonuclease per ml. Avoid vigorous mixing.

Vial #4: Ovomucoid protease inhibitor with bovine serum albumin, one vial per package. This material is 0.22 μm membrane filtered and lyophilized in autoclaved vials. A vial reconstituted with 32 ml of EBSS (vial 1) yields a solution at an effective concentration of 10 mg of ovomucoid inhibitor and 10 mg of albumin per ml. The inner rubber stopper can be discarded after reconstitution. Aliquots of this vial are used for each dissociation. Refrigerate between uses and equilibrate with sterile O_2:CO_2 before each use. Stable after reconstitution when stored at 4°C.

Pouch, containing Leibovitz L-15 Media Powder, 1 x 1L: Reconstitute entire contents of pouch by cutting open top of envelope and pouring contents into beaker containing approximately 800 ml of cell

culture grade water. Rinse pouch 2 - 3 times with an additional 100 ml water. Bring total volume to 1000 ml and filter through a 0.22 μm pore size membrane.

Also included is a card correlating color with pH for use as a guide in O_2:CO_2 equilibration.

Needed but not supplied:

- Sterile pipettes
- Sterile centrifuge tubes
- Centrifuge to operate at 70 g and 300 g
- Device for sterile 95% O_2:5%CO_2 equilibration of solutions
- Water bath at 37°C
- Device for incubation at 37°C with agitation.

Procedure

Briefly the procedure is as follows: Components of the dissociation medium are reconstituted as described previously; minced tissue is added and the mixture is equilibrated with O_2:CO_2. Tissue is dissociated by incubation with activated papain at 37°C, followed by trituration. Dissociated cells are pelleted then resuspended in medium containing ovomucoid, a papain inhibitor. Intact cells are separated from cell membranes by centrifugation through a single step discontinuous density gradient and the pellet finally resuspended in medium appropriate for cell culture or flow cytometric analysis.

For those unfamiliar with tissue dissociation and cell culture techniques, two operations deserve additional explanation.

1. Equilibration with 95% O_2:5%CO_2 It is important for the survival of the tissue during dissociation that the incubation medium be both well oxygenated and buffered at physiological pH. Both requirements are satisfied when the medium is equilibrated with 95% O_2:5%CO_2. The Earle's Balanced Salt Solution contains a pH sensitive indicator dye. When it is red or purple in color, the medium is too alkaline. This is likely to be the case when the tissue is placed in the papain solution (Step #4), and re-equilibration with O_2:CO_2 is usually necessary prior to incubation at 37°C.

Gas should not be bubbled directly into any solution containing protein. This can result in frothing and denaturation of the protein with loss of biological activity. Gas can be sterilized by passage through a sterile fiber plug such as the cotton plug in a sterile Pasteur or volumetric pipette. While mixing the solution, pass O_2:CO_2 continuously through the space above the liquid until the color indicates pH 7.2-7.4 according to the color chart included in the kit. The Earle's Balanced Salt Solution is pregassed but should be equilibrated with sterile O_2:CO_2 each time the bottle is opened. The reconstituted inhibitor should also be equilibrated with sterile O_2:CO_2 before each use.

2. Trituration (cell dispersion through mild pumping action) This is a crucial procedure. It serves to break up the tissue fragments following incubation in the dissociation mix. If done too vigorously, cells will be destroyed; too weakly and tissue fragments will be left intact. In the context of neuronal tissue, gentle trituration, using a 10 ml pipette, constitutes filling and emptying the barrel at a rate of about 5 ml per second. Avoid bubbling the cell suspension.

Dissociation Protocol

(Sterile procedures should be used throughout)

I. Add 32 ml of EBSS (vial 1) to the albumin ovomucoid inhibitor mixture (vial 4) and allow the contents to dissolve while preparing the other components. Mix before using and equilibrate with O_2:CO_2. Reconstitute for the first use, then store and reuse.

II. Add 5 ml of EBSS (vial 1) to a papain vial (vial 2). Place vial 2 in a 37°C water bath for ten minutes or until the papain is completely dissolved and the solution appears clear. If solution appears alkaline (red or purple) equilibrate the solution with 95% O_2:5%CO_2. The solution should be used promptly but can be held at room temperature during the dissection. A separate papain vial is provided for each dissociation. (If desired the papain can be transferred to a centrifuge tube or other container before proceeding.)

III. Add 500 μl of EBSS to a DNase vial (vial 3). Mix gently – DNase is sensitive to shear denaturation. Add 250 μls of this solution to the vial containing the papain. This preparation contains a final concentration of approximately 20 units/ml papain and 0.005% DNase. Save the balance of the DNase vial to use in step #VII. A separate DNase vial is provided for each dissociation..

IV. Place tissue in the papain solution. Tissue should be slightly minced or cut into small pieces (this can be done separately or on the side of the tube containing the papain). Displace air in vial with sterile O_2:CO_2. Do not bubble gas through the solution. Immediately cap vial.

V. Incubate the vial containing the tissue at 37°C with constant agitation (a rocker platform is ideal) for 30 min to 1 1/2 hrs. The amount of time must be determined empirically; however, embryonic tissue generally requires less time than postnatal tissue.

VI. Triturate the mixture with 10 ml pipette. Allow any pieces of undissociated tissue remaining after trituration to settle to the bottom of the tube. Vigorous trituration of neuronal tissue results in a high yield of cells, most of which are spherical and devoid of processes. Gentle trituration results in more undissociated tissue fragments and a lower yield of cells although many of these now retain their proximal processes.

VII. Carefully remove the cloudy cell suspension, place in sterile screwcapped tube and centrifuge at 300 g for 5 minutes at room temperature. Be careful to avoid including any pieces of undissociated tissue during this time – prepare medium to resuspend the pelleted cells. Mix 2.7 ml EBSS (vial 1) with 300 μl reconstituted albumin-ovomucoid inhibitor solution (vial 4) in a sterile tube. Add 150 μl of DNase solution (vial 3) saved at step #III.

VIII. Discard supernatant and immediately resuspend cell pellet in DNase dilute albumin-inhibitor solution prepared in step #VII.

IX. Prepare discontinuous density gradient. Add 5.0 ml of albumin-inhibitor solution (vial 4) to centrifuge tube, carefully layer cell suspension on top, then centrifuge at 70 g for 6 minutes at room temperature. The interface between the two layers of the gradient should be clearly visible although minimal mixing at this boundary does not affect the result. Dissociated cells pellet at the bottom of the tube, membrane fragments remain at the interface.

X. Discard the supernatant and immediately resuspend the pelleted cells in medium for cell culture or for flow cytometric analysis.

Cell Isolation Optimizing System

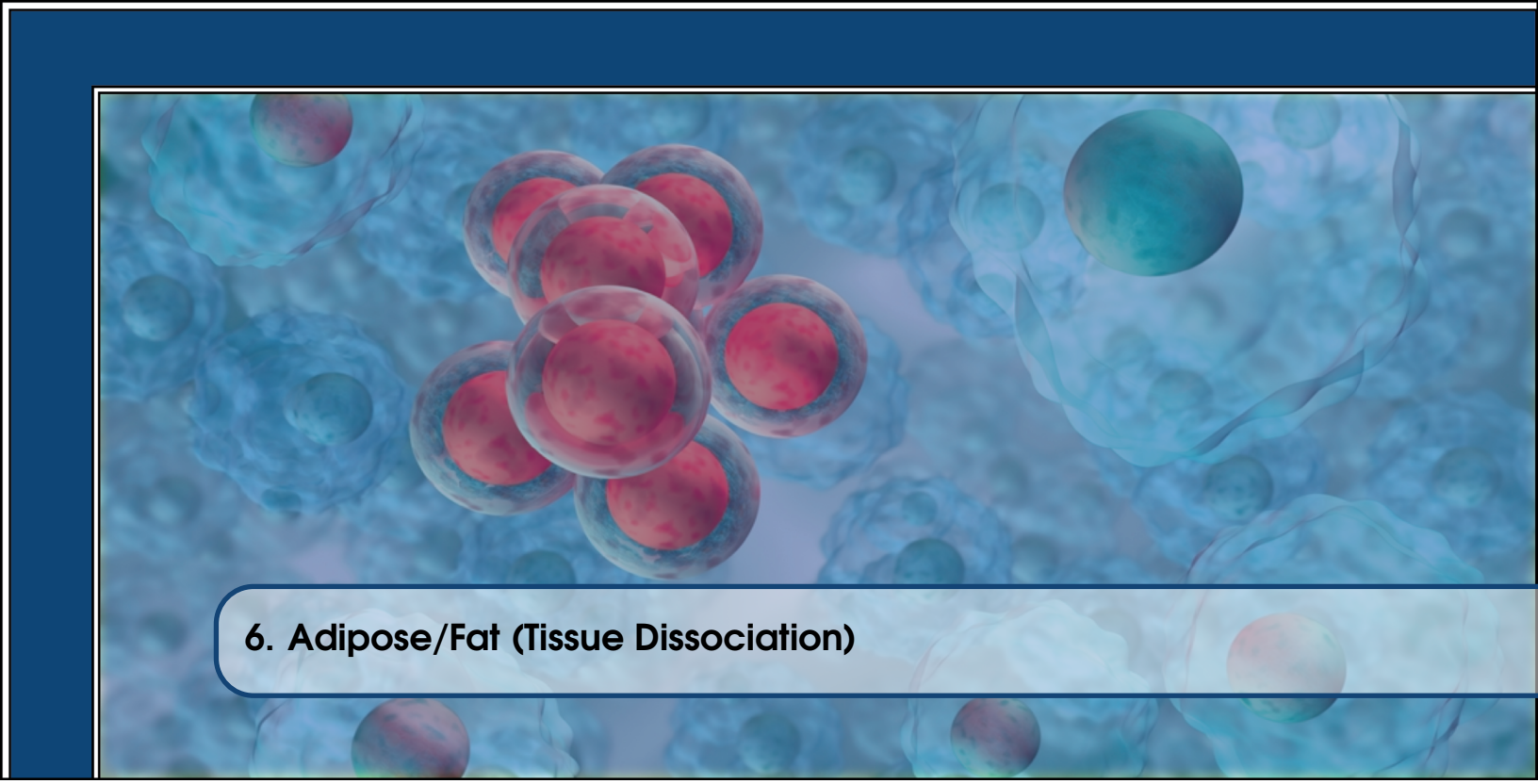
Worthington Biochemical Corporation offers a complete method development kit containing an assortment of enzymes most frequently used in tissue dissociation and cell isolation procedures. The "Cell

Isolation Optimizing System" includes instructions, references and strategies for the handling, use and optimization of enzymatic cell isolation methods to achieve maximum yield of viable cells.

The "System" contains all of the enzymes commonly referenced in tissue dissociation and cell isolation procedures along with the Cell Isolation Guide detailing the tissue types commonly used, the mode of action of the various enzymes, tissue culture techniques, and protocol optimization guidelines. In addition the guide lists hundreds of cell and tissue specific isolation references for getting started in enzymatic cell isolation.

Table 5.1: Cell Isolation Optimizing System Kit Contents

Enzyme	Product Code	Quantity per Vial
Collagenase Type 1	CLS1	500 mg dw
Collagenase Type 2	CLS2	500 mg dw
Collagenase Type 3	CLS3	500 mg dw
Collagenase Type 4	CLS4	500 mg dw
Trypsin	TL	Quantity per Vial
Hyaluronidase	HSE	50,000 Units
Elastase	ESL	100 mg P
Papain	PAPL	100 mg P
Deoxyribonuclease I	DP	25 mg dw
Trypsin Inhibitor	SIC	100 mg dw
Neutral Protease (Dispase)	NPRO	10 mg dw



6. Adipose/Fat (Tissue Dissociation)

Species: Bovine

Table 6.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Yang	Adipocytes	Collagenase Type 1: 40 u/ml	Krebs-Ringer bicarbonate

Species: Canine

Table 6.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Basu	Renal adipose derived cells	Collagenase Type 1: 0.3%	DMEM
3	DiGirolamo	White fat	Collagenase: 0.05%	Kreb's Ringer bicarbonate buffer
4	Fischer	Adipose stem cells	Collagenase: See Reference	Media-199

Species: Equine

Table 6.3: **Equine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
5	Vidal	Adipose derived stem cells	Collagenase Type 1: 0.1%	PBS
6	Vidal	Adipose derived stem cells	Collagenase Type 1: 0.1%	PBS

Species: Fish

Table 6.4: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Todorcevic	Preadipocytes	Collagenase Type 1: 0.1%	HBSS

Species: Gerbil

Table 6.5: **Gerbil**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Nedergaard	Brown fat	Collagenase Type 1: 0.10%	Bicarbonate buffer

Species: Guinea-Pig

Table 6.6: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Nedergaard	Brown fat	Collagenase Type 1: 0.10%	Bicarbonate buffer

Species: Hamster

Table 6.7: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	DiGirolamo	White fat	Collagenase: 0.05%	Kreb's Ringer bicarbonate buffer
8	Nedergaard	Brown fat	Collagenase Type 1: 0.10%	Bicarbonate buffer

Species: Human

Table 6.8: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
9	Choudhery	Mesenchymal Stromal Cells	Collagenase Type 4: 0.2%	PBS
10	Satish	Adipose derived stem cells	Collagenase Type 2: 0.1%	HBSS
11	Najar	Mesenchymal Stromal Cells	Collagenase Type 1: 0.1%	PBS
12	Doi	Stromal Vascular Fraction	Collagenase Type 1: 0.075%	PBS
13	Carvalho	Adipose stromal stem	Collagenase animal free: 200 u/ml	DMEM/Hams F-12
14	Wu	Adipose derived stem	Collagenase Type 1: 0.1%	DMEM/F-12
15	James	Perivascular	Collagenase Type 2: 0.1%	DMEM
16	Gentile	Adipose derived stromal vascular	Collagenase Type 1: 0.1%	PBS
17	Cervelli	Adipose-derived stem cells	Collagenase Type 1: 0.1%	PBS
18	Gentile	Stromal vascular, adipocytes	Collagenase Type 1: 0.1%	DMEM
19	Naaijkens	Adipose derived stromal	Collagenase Type 1: 0.1%	PBS
20	Nazarov	Chorionic mesenchymal	Collagenase Type 1: 0.1% Neutral Protease: 2.5 u/ml Trypsin: 0.25%	MEM
21	Yang	Adipose derived mesenchymal	Collagenase Type 1: 0.1%	L-DMEM
22	Yu	Adipose derived stem	Collagenase Type 1: 0.1%	DMEM/Ham's F-12
23	Basu	Adipocytes	Collagenase Type 1: 0.1%	RPMI
2	Basu	Renal adipose derived cells	Collagenase Type 1: 0.3%	DMEM
24	Tan	Adipose tissue-derived stem	Collagenase Type 2: 1.0%	DMEM/F12
25	Zimmerlin	Stromal vascular	Collagenase Type 2: 0.1%	HBSS
26	Peters	Adipocytes	Collagenase Type 1: 0.1%	KRB
27	Tan	Adipose tissue-derived stem	Collagenase Type 2: 1.0%	DMEM/F12
28	Suga	Adipocytes	Collagenase: 0.075%	DMEM
29	Cai	Adipose Stromal Cell	Collagenase Type 1: 0.1%	DMEM
30	Tandon	Adipose derived stem cells	Collagenase Type 1: 0.1%	DMEM
31	Pilgaard	Stem Cells	Collagenase: 280 u/ml	D-PBS
32	Traktuev	Adipose derived stromal cells	Collagenase Type 1: See Reference	DMEM
33	Minana	Stromal	Collagenase Type 1: 0.2%	PBS
34	Nie	Stromal	Collagenase Type 1: 0.1% Neutral Protease:	DMEM
35	Kilroy	Adipose derived stem cells	Collagenase Type 1: 0.1%	DMEM/F12
36	Bujalska Iwona J	Adipocytes	Collagenase Type 1: 0.2%	HBSS
37	Schaffler A	Adipose derived stromal cells	Collagenase Type 1: 0.15%	DMEM

38	Jeon Eun Su	Mesenchymal stem	Collagenase Type 1: 0.1%	HBSS
39	Koellensperger	Preadipocytes	Collagenase Type 1: 196 u/ml	M199
40	Devireddy	Adult stem cells	Collagenase Type 1: 0.1%	PBS
41	Boquest Andrew C	Adipocytes, stromal vascular	Collagenase: 0.2%	HBSS
42	Rodriguez Anne-Marie	Multipotent adipose derived stem	Collagenase: 0.2%	DMEM
43	Planat-Benard Valerie	Stromal vascular, adipocytes	Collagenase: 0.2%	DMEM/F12
44	Miranville A	Stromal vascular, adipocytes, stem	Collagenase: 300 u/ml	PBS
45	Patwardhan	Adipocytes	Collagenase Type 1: See Reference	Saline
46	Zuk Patricia A	Processed lipoaspirate cells	Collagenase Type 1: 0.075%	PBS
47	McTernan PG	Adipocytes	Collagenase Type 1: 0.2%	HBSS
48	Al-Saqi	Adipose derived mesenchymal stem	Collagenase Type 2: 0.1%	See Reference
49	Koellensperger	Adipose derived stem cells	Collagenase Type 1: 0.15%	DMEM
50	Hagman	Stromovascular	Collagenase Type 1: 0.1%	PBS with BSA
51	Fain JN	Adipocytes	Collagenase Type 1: 0.13%	See Reference
52	Quickler	Preadipocytes	Collagenase Type 1: 0.2%	DMEM/F-12
53	Gesta	Adipocytes	Collagenase Type 2: 0.05%	DMEM
54	McTernan	Adipocytes	Collagenase Type 1: 0.2%	DMEM/F12
55	Gottschling-Zelle	Adipocytes	Collagenase Type 1: 0.1%	DMEM/Ham's F-12
56	Zhang	Adipocytes	Collagenase Type 1: 0.1%	DMEM/F12
57	Seboek	Adipocytes	Collagenase Type 2: 0.1%	DMEM/F12
58	Blasi	Adipose derived stem cells	Collagenase: 0.25% Deoxyribonuclease I: 0.002%	PBS
59	Anderson	Adipocytes	Collagenase Type 1: 0.05%	Kreb's Ringer bicarbonate buffer

Species: Mouse

Table 6.9: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
60	Jang	Adipocytes	Collagenase: 0.1%	Krebs
61	Takahashi	Adipose derived stem	Collagenase Type 2: 0.1%	DMEM
62	Han	Stem and progenitor	Collagenase Type 2: 0.2%	HBSS
63	Wong	White adipocytes	Collagenase Type 2: 0.1%	DMEM
64	Thangarajah	Adipose Stromal Cell	Collagenase Type 2: 0.075%	DMEM
65	Cho	Adipose tissue-derived stem	Collagenase Type 1: 0.075%	Modified Eagles
43	Planat-Benard Valerie	Stromal vascular, adipocytes	Collagenase: 0.2%	DMEM/F12
66	Kondo	Stromal Vascular	Collagenase Type 2: 0.2%	RPMI-1640
67	De Matteis	Adipocytes	Collagenase Type 1: 0.2%	HBSS
68	Cowan Catherine M	Adipose-derived stromal cells	Collagenase Type 2: 0.075%	PBS
69	Launder	Vascular endothelial	Collagenase: 0.2%	PBS
70	Nadler	Adipocytes	Collagenase: 0.05%	Krebs-Ringer Phosphate HEPES (KRPH)
71	Aoyagi T	Adipocytes	Collagenase Type 1: 0.15%	DMEM/F12
72	Ruan	Adipocytes	Collagenase: See Reference	DMEM

Species: Porcine

Table 6.10: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
73	Ding	Adipose	Collagenase Type 1: 300 u/ml	HEPES
74	Liang	Adipocytes	Collagenase Type 1: 0.067%	Krebs-Ringer
75	Suryawan	Adipose, Stromal-vascular	Collagenase Type 1: 0.2%	DMEM/F12
76	Ramsay	Adipocytes	Collagenase Type 1: 0.2%	DMEM/F12
77	Williams	Adipose mesenchymal stem	Collagenase Type 1: 0.1%	DMEM
78	Wang Y	Adipocytes	Collagenase Type 1: 0.3%	Krebs-Ringer bicarbonate albumin

Species: Rat

Table 6.11: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
79	Emre	Mesenchymal Stem Cells	Collagenase Type 1: 0.075%	DMEM
80	Ogasawara	Adipocytes	Collagenase Type 1: 0.3%	Krebs-Ringer
81	Wei	Adipose Derived Stem	Collagenase Type 1: 0.1%	DMEM
82	Tomiyama	Adipose tissue-derived stem	Collagenase Type 2: 0.075%	DMEM
83	Serrero	Adipocytes	Collagenase Type 2: 0.2%	DMEM /F-12
84	Fain	Brown fat	Collagenase Type 1: 0.10%	Bicarbonate buffer
85	Gaben-Cogneville	Preadipocytes	Collagenase Type 3: 0.10%	Parker Medium 199
86	Leblanc	Stromal vascular	Collagenase Type 1: 0.2%	PBS
87	Pessin	Adipocytes Epididymal fat pads	Collagenase: 0.1%	Krebs Ringer bicarbonate buffer
88	Giacomello	Neurons	Papain: 0.05%	Neurobasal
89	Stiles	White fat	Collagenase: 0.3%	Kreb's Ringer bicarbonate buffer
2	Basu	Renal adipose derived cells	Collagenase Type 1: 0.3%	DMEM
90	Thompson	Adipocytes	Collagenase Type 2: 0.33%	RPMI 1640
91	Mora	Adipocytes	Collagenase Type 1: 0.2%	KRHB
92	Veronesi	Mesenchymal Stromal Cells	Collagenase Type 2: 0.075%	DMEM
93	Charron	Adipose Epididymal fat pads	Collagenase: 0.3%	Kreb's-Ringer bicarbonate buffer modified
94	Rodbell	Fat	Collagenase: 0.3%	Albumin-bicarbonate buffer
95	Omatsu-Kanbe	Brown adipocytes	Deoxyribonuclease I: 0.5%	DMEM
96	Liu	Brown adipocytes	Collagenase Type 4: 0.1% Neutral Protease: 0.1% Trypsin: 0.05%	PBS
97	Woodward	Brown adipocytes Interscapular & cervical depots	Collagenase: 0.2% Soybean Trypsin Inhibitor: 0.3%	Krebs Ringer bicarbonate buffer
98	Aoki	Adipocytes	Collagenase: 0.2%	Ham's F12
99	Green	Adipocytes Epididymal-fat pads	Collagenase: 0.3%	Kreb's Ringer
3	DiGirolamo	White fat	Collagenase: 0.05%	Kreb's Ringer bicarbonate buffer

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7. Adrenal (Tissue Dissociation)

Species: Bovine

Table 7.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Dominguez	Chromaffin	Collagenase Type 1: 0.1-0.2% Deoxyribonuclease I: 0.003-0.015% Hyaluronidase: 0.015%	DMEM/F12
2	Hahm	Chromaffin cells	Collagenase: 0.1% Deoxyribonuclease I: 30 u/ml	DMEM
3	Ortega	Chromaffin	Collagenase: 0.2%	Locke's solution
4	Zhu	Chromaffin	Deoxyribonuclease I: 30 u/mg	HEPES
5	Dahmer	Chromaffin	Collagenase Type 1: 0.25 %	DMEM
6	Higgins	Chromaffin	Collagenase: 0.2% Deoxyribonuclease I: 0.015%	Locke's solution
7	Aunis	Chromaffin	Collagenase: 0.05%	Locke's solution, CMF
8	Almazan	Chromaffin	Collagenase: 0.15%	Kreb's, CMF
9	Pollard	Chromaffin	Collagenase: 0.1%	See Reference
10	Cena	Chromaffin	Collagenase Type 1: 0.05%	CF Kreb's
11	Waymire	Chromaffin	Collagenase Type 1: 0.025%	HBSS, modified
12	Knight	Medulla	Hyaluronidase: 0.2%	Saline w/BSA 0.5%
13	Greenberg	Medulla	Collagenase: 0.2%	Krebs-Ringer bicarbonate buffer, CMF
14	Baker	Medulla	Protease: 0.2%	Saline
15	Wilson	Chromaffin	Deoxyribonuclease I: 15 μg/ml	Medium A See Reference
16	Trifaro	Chromaffin	Collagenase: 0.05%	Locke's solution, CMF
17	Kilpatrick	Medulla	Collagenase: 0.05%	Locke's solution, CF
18	Aunis	Chromaffin	Collagenase: 0.05%	DMEM
19	Kumakura	Chromaffin	Collagenase: 0.25%	F-12 medium
20	Hersey	Medullary	Collagenase Type 2: 0.2%	HEPES
21	Folkman	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum
22	Fenwick	Medulla	Collagenase Type 1: 0.05%	Kreb's, CF
23	Brooks	Chromaffin	Hyaluronidase: 0.2%	HEPES, CF

24	Moustafa T	Chromaffin	Collagenase Type 1: 0.125%	Locke's solution
25	Unsicker	Medulla	Collagenase Type 1: 0.5%	HBSS
26	Unsicker	Chromaffin	Collagenase: 0.5%	HBSS
27	Schneider	Chromaffin	Collagenase Type 1: 0.2%	Kreb's Ringer bicarbonate buffer, CF
28	Trifaro	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF

Species: Guinea-Pig

Table 7.2: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
29	Hochman	Adrenal Chromaffin	Collagenase: 0.05%-0.20%	Kreb's-Ringer bicarb glucose buffer, CF
26	Unsicker	Chromaffin	Collagenase: 0.5%	HBSS
30	Role	Chromaffin Medulla	Collagenase:	BSS See Reference

Species: Hamster

Table 7.3: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
31	Liang	Adrenal Chromaffin	Hyaluronidase: 0.20%	Kreb's Ringer bicarbonate buffer

Species: Human

Table 7.4: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
32	Santana	Adrenal medulla progenitor	Collagenase: 0.2%	DMEM/F-12
33	Jeon	Chromaffin cells	Collagenase: 0.2%	Locke's solution
34	Caroccia	Adrenocortical	Collagenase Type 1: 0.2% Deoxyribonuclease I: 0.01%	Krebs Ringer
35	Tischler	Chromaffin	Trypsin: 0.25%	Eagle's MEM
21	Folkman	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum

Species: Mouse

Table 7.5: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Dominguez	Chromaffin	Collagenase Type 1: 0.1-0.2% Deoxyribonuclease I: 0.003-0.015% Hyaluronidase: 0.015%	DMEM/F12
36	Kolski-Andreaco	Chromaffin	Papain: 40 u/ml	DMEM
37	Tian Jin-Hua	Chromafin cells	Papain: 20-25 u/ml	DMEM

Species: Ovine

Table 7.6: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
38	Canny B J	Anterior pituitary	Trypsin: 2.5% Deoxyribonuclease I: 0.004%	DMEM
39	Keating	Chromaffin cells	Collagenase Type 2: 0.2% Deoxyribonuclease I: 100 u/ml	Locke's solution
40	Valego	Adrenocortical	Collagenase Type 1: 0.4%	DMEM/Ham's F12
41	Valego	Adrenocortical	Collagenase Type 1: 0.4%	DMEM/Ham's F12

Species: Rat

Table 7.7: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Dominguez	Chromaffin	Collagenase Type 1: 0.1-0.2% Deoxyribonuclease I: 0.003-0.015% Hyaluronidase: 0.015%	DMEM/F12
42	Gilabert	Chromaffin	Collagenase Type 1: 0.26% Deoxyribonuclease I: 0.015% Hyaluronidase: 0.015%	HBSS
43	Englert	Chromaffin	Trypsin: 0.10%	Ham's F-12 w/HEPES
21	Folkman	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum
44	Braley	Glomerulosa	Deoxyribonuclease I: 0.05%	Kreb's
45	Leonard	Adrenocortical	Deoxyribonuclease I: 0.005%	BSS
46	Roskelley	Adrenocortical	Deoxyribonuclease I: 0.005%	BSS
47	Unsicker	Chromaffin	Collagenase: 0.5%	HBSS
48	Kloppenborg	Adrenal	Collagenase Type 1: 0.5%	Kreb's Ringer bicarbonate buffer
49	Payet	Glomerulosa	Collagenase: 0.2%	MEM-d-Val
50	Ng	Leydig Adrenal	Collagenase Type 2: 0.03% (adrenal)	Krebs Ringer bicarbonate buffer
51	Gilabert	Chromaffin cells	Collagenase Type 1: 0.26% Deoxyribonuclease I: 0.015% Hyaluronidase: 0.015%	HBSS
28	Trifaro	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF
52	Bruder Eric D	Zona fasciculata/reticularis	Collagenase: 0.4%	Krebs-HEPES
53	Sayed	ZG ZFR	Collagenase Type 1: 0.2%	Kreb's
54	Barofsky	Cortical	Trypsin: 0.25%	Kreb's Ringer bicarbonate buffer
55	Li	Decapular Capsular Glomerulosa	Deoxyribonuclease I: 0.01%	Medium 199
56	Zhang L	Chromaffin cells	Collagenase Type 1: 0.025% Deoxyribonuclease I: 0.0015%	DMEM
57	Unsicker	Medullary	Trypsin: 0.125%	HBSS

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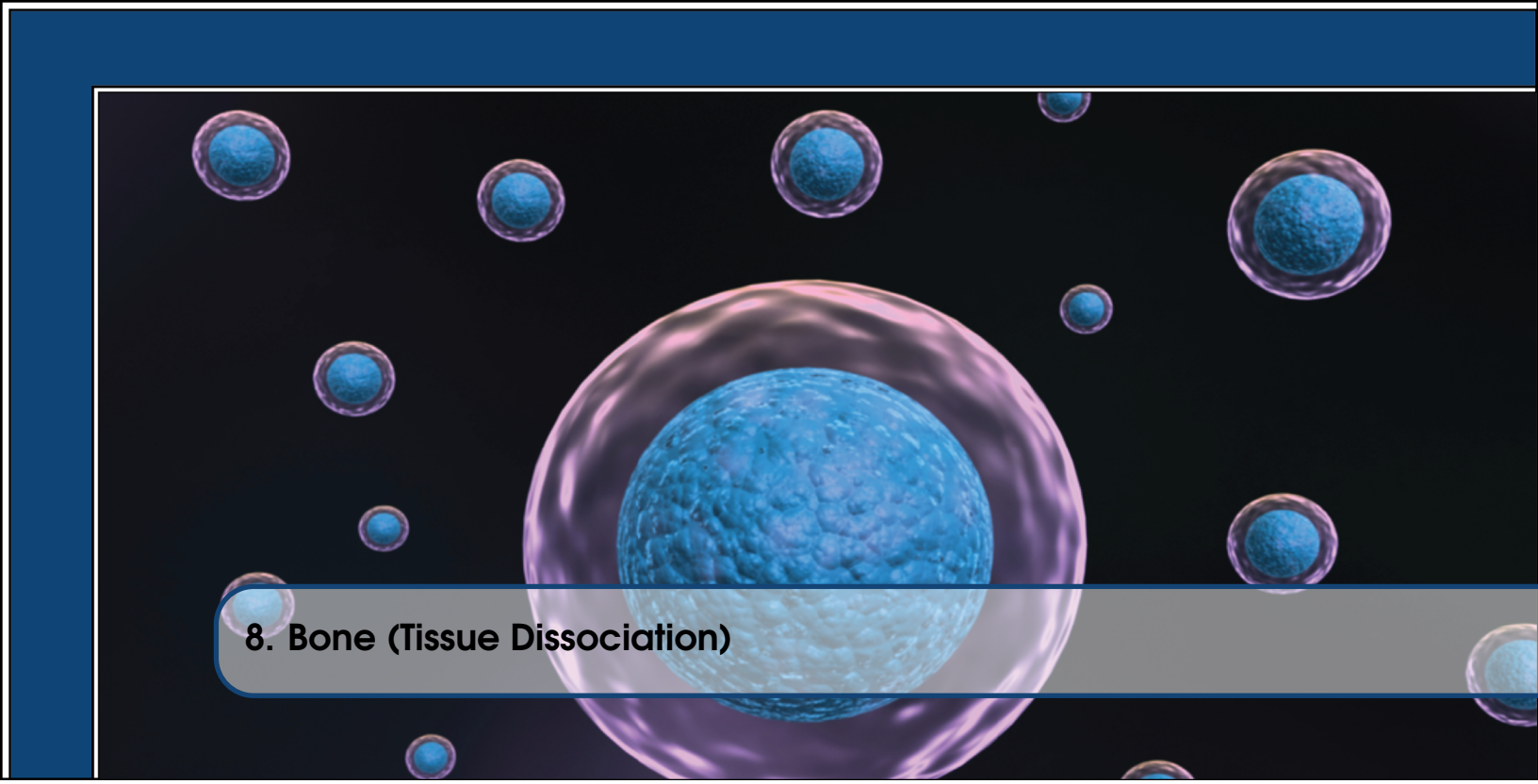

8. Bone (Tissue Dissociation)

Species: Bovine

Table 8.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Buschmann	Chondrocytes	Collagenase Type 2: 0.4%	DMEM

Species: Chicken

Table 8.2: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Gay	Osteoblasts	Trypsin: 0.03%	DMEM
3	Schiltz	Vertebrae chondroblasts	Trypsin: 0.25%	Simm's, CMF
4	Rosselot	Chondrocytes	Trypsin: 0.25%	Ham's F12

Species: Human

Table 8.3: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
5	Meikle	Osteoblasts	Trypsin: 0.1%	DMEM
6	Fedarko	Osteoblasts	Collagenase Type 4: 250 u/ml	DMEM
7	Kneser U	Osteoblasts	Trypsin: 0.5%	Basal Medium
8	Chen X.	Bone Cells, Osteoblasts	Collagenase Type 2: 200-250 u/ml	DMEM

Species: Mouse

Table 8.4: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
9	Igarashi	Bone Stromal cells	Collagenase Type 1: 0.3%	DMEM
10	Lee	Osteoblasts	Collagenase Type 1: 1.0% Neutral Protease: 1.6%	MEM
11	Nakamura	Endosteal cells	Collagenase Type 1: 0.3%	DMEM
12	Morikawa	Bone marrow	Collagenase: 0.2%	DMEM
8	Chen X.	Bone Cells, Osteoblasts	Collagenase Type 2: 200-250 u/ml	DMEM
13	Stern	Osteocytes	Collagenase Type 1: 300 u/ml	MEM
14	Takanashi	Osteoblast-like Cells Stromal Cell Lines Hematopoietic Blast Cells	Trypsin: 0.1%	Eagle's MEM
15	Sakai	Osteoclasts	Collagenase Type 3: 0.1%	DMEM
16	Chen	Neonatal bone	Collagenase Type 2: 0.20%	Tris-buffered saline

Species: Rat

Table 8.5: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
17	Owen	Calvaria	Collagenase: 0.2%	MEM
18	Peck	Calvaria	Collagenase: 0.01%-0.6%	Tris-buffered saline
19	Ernst	Osteoblastlike cells	Collagenase Type 2: 0.3%	MEM

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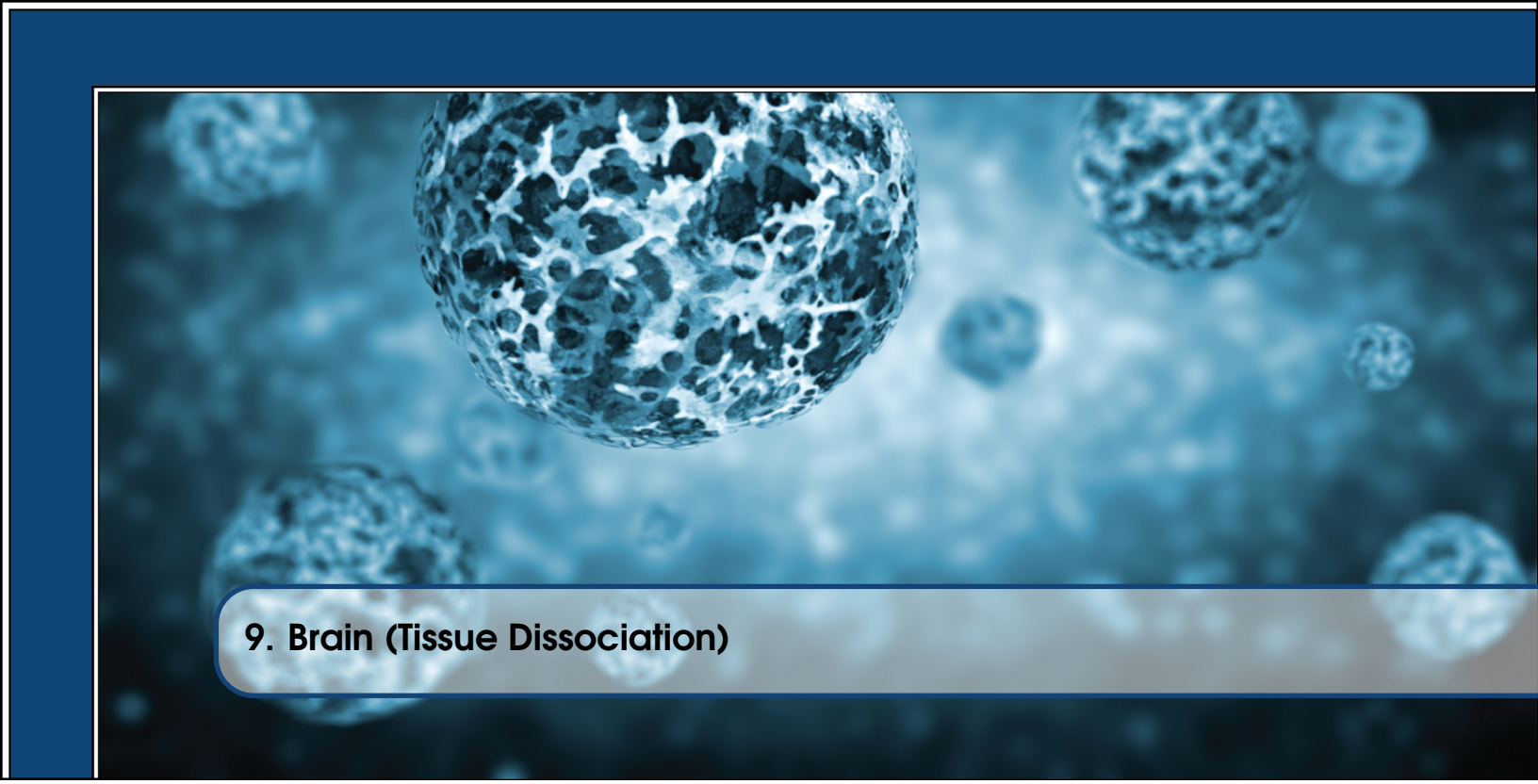

9. Brain (Tissue Dissociation)

Species: Bovine

Table 9.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Kanda	Microvascular endothelial	Neutral Protease: 0.005%	Medium 199
2	Wolburg H	Brain endothelial cells	Collagenase Type 2: 0.35%	DMEM
3	Miller	Endothelial	Neutral Protease: 0.125%	MEM
4	Estrada	Capillary endothelial	Collagenase: 0.1%	DMEM
5	Audus	Endothelial	Neutral Protease: 0.5%	MEM
6	Goetz	Endothelial Brain arteries	Collagenase Type 2: 0.2%	Dulbecco's PBS
7	Machi	Cerebral artery Endothelial	Collagenase: 0.2%	HBSS
8	Poduslo	Oligodendroglia Neural	Trypsin: 0.1%	See Reference

Species: Guinea-Pig

Table 9.2: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
9	Kay	Neurons	Trypsin: 0.06-0.08%	PIPES saline

Species: Human

Table 9.3: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
10	Sauvageot	Tumor	Collagenase Type 4: 0.1% Hyaluronidase: 0.07% Deoxyribonuclease I: 0.04%	See Reference
11	Klegeris Andis	Microglia	Trypsin: 0.25% Deoxyribonuclease I: .005%	DMEM/F12
12	Gerhart	Microvessels	Collagenase Type 4: 0.1%	DMEM
13	Roher	Neuronal	Deoxyribonuclease I: 10 μg/ml	Tris-HCl, 50 mM, CaCl2, 2 mM

14	Vinters	Microvessels	Collagenase: 0.1%	Serum-free modified Lewis medium

Species: Insect

Table 9.4: **Insect**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
15	Sicaeros	Neurons	Papain: 4 u/ml	DMEM
16	Gu	Neurons	Papain: 20 u/ml	Saline

Species: Monkey

Table 9.5: **Monkey**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
17	Marcondes MC	Brain cells	Collagenase Type 2: 500 u/ml Deoxyribonuclease I: 28 u/ml	HBSS

Species: Mouse

Table 9.6: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
18	Baruch	Chloroid plexus	Collagenase Type 4: 400 u/ml	PBS
19	Ryg-Cornejo	Brain-sequestered leukocytes	Collagenase Type 4: 0.05% Deoxyribonuclease I: 2 u/ml	RPMI
20	Shah. G.	Cerebral pericytes	Collagenase Type 2: 0.1% Deoxyribonuclease I: 30 u/ml	DMEM
21	Oliver	Granule cell precursors, pre-neoplastic and tumor cells	Papain: 10 u/ml Deoxyribonuclease I: 250 u/ml	Neurobasal/B27
22	Klein C	Neurosperes	PDS kit: per instructions	DMEM/F12
23	Hernandez	Cortical neurons	PDS kit: per instructions	Neurobasal
24	Miyazawa K	Cerebellar granule cell precursors	Papain: 0.435% Deoxyribonuclease I: 0.05%	EBSS
25	Lim	Astrocytes	Trypsin: 0.25%	DMEM
26	Shrier	Neural	Trypsin NF 1:250: 50 0.25%	BSS
27	Seaberg	Neural progenitor	PDS kit: per instructions	See Reference
28	Martin-Aparicio	Neurons and glia	PDS kit: per instructions	Neurobasal
29	O'Meara	Oligodendrocytes, dorsal root ganglia	Papain: 0.15% Deoxyribonuclease I: 0.006%	DMEM
30	Smeyne Michelle	Postnal substantia nigra	PDS kit: per instructions	See Reference
31	Nishioku T	Microglia	Papain: 90 u/ml Deoxyribonuclease I: 2000 u/ml	Eagle's MEM
32	Lee	Neurons	PDS kit: with modifications	HBSS
33	Chung	Vascular smooth muscle cells	Papain: 0.05% Collagenase Type 4: 0.15% Elastase: 0.05%	PBS
34	Saxena	Neurons	PDS kit: with modifications	DMEM/F12
35	Estivill-Torrus	Cortical progenitors	PDS kit: per instructions	Serum free medium
36	Hilgenberg	Cortical neurons	Papain: 4-10 u/ml	Neurobasal
37	Fasano	Neurons	Papain: 20 u/ml	Neurobasal
38	Jun K	Hippocampal cells	Papain: 10 u/ml	DMKM
39	Haseleu	Glial	Papain: 20 u/ml Deoxyribonuclease I: 0.0005%	EBSS
40	Sher	Astrocytes	Trypsin: 0.25% Deoxyribonuclease I: 1,000 u/ml	HBSS
41	O'Donnell SL	Microglia	Trypsin: 0.125% Collagenase Type 2: 0.01% Deoxyribonuclease I: .005%	RPMI-1640

42	Spielman	Papillae, taste receptor	Pronase E: 0.15%	Carbonate-Phosphate buffer See Reference

Species: Ovine

Table 9.7: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Poduslo	Oligodendroglia Neural	Trypsin: 0.1%	See Reference

Species: Porcine

Table 9.8: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
43	Robinson	Microvascular	Collagenase: 0.1%	HBSS

Species: Rat

Table 9.9: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
44	Acheson	Fibroblasts	Trypsin: 0.2%	HEPES buffered DMEM
45	Giulian	Cerebral cortices	Trypsin: 0.25%	PBS
46	Jekabsons MB	Hippocampal neurons	Papain: 0.2%	Hibernate
47	Naeve GS	Hippocampal and cortical neurons	PDS kit: per instructions	Neurobasal media
48	Khakh Baljit S	Hippocampal neuron	Papain: 10 u/ml	MEM
49	Swaiman	Cortical	Trypsin: 0.25%	Puck's D1 See Reference
50	Rothman	Hippocampi	Trypsin: 0.1%	HBSS
51	Bartlett	Hippocampal neurons	Trypsin: 0.25%	HBSS, CMF
52	Chen	Hippocampal neurons	Papain: 0.2%	Hibernate A
53	Banker	Hippocampal neurons	Trypsin: 0.1%	HBSS
54	Shigetomi	Astrocytes	Papain: 20 u/ml	EBSS
55	Richler Esther	Hippocampal neurons	Papain: 10 u/ml	EBSS
56	Peptan	Dura mater	Collagenase Type 1: 0.075%	DMEM
57	Holzwarth	Astrocytes	Trypsin: 0.25%	DMEM, HBSS
58	Akanda	Hippocampal	Trypsin: 0.1%	Neurobasal
59	Ahmed	Neural	Trypsin: 0.25%	DMEM
60	Cao	Suprachiasmatic nucleus neurons	Papain: 100 u/ml	MEM
61	Rowe	Pineal	Trypsin: 0.25%	DMEM, MEM
62	Mattson	Hippocampal neurons	Trypsin: 0.2% Deoxyribonuclease I: 10 ug/ml	HBSS
63	Tanaka	Cerebellar granule neurons	PDS kit: per instructions	PBS
64	Doron	Endothelial	Collagenase Type 2: 0.5%	Medium 199
65	Varney MA	Cortical neurons	PDS kit: with modifications	Neurobasal Medium
66	Behar	Cortical cells	Papain: 20 u/ml Deoxyribonuclease I: .005%	EBSS
67	Matsuda	Fetal rat brain	Collagenase Type 4: 0.1%	DMEM
68	Peterfreund	Cerebral cortex Hypothalmus	Deoxyribonuclease I: 0.001%	HEPES
69	Brewer	Hippocampal neurons	Papain: 0.2%	HibernateA/B27
70	Pixley	Glial	Trypsin: 0.0625%	MEM, sterile
71	Bowman	Capillary endothelium Pericytes	Neutral Protease: 0.1%	Medium 199
72	Velasco Myrian	Hippocampal neurons	Trypsin: 0.05%	DMEM

73	Mizoguchi Y	Visual cortical	PDS kit: per instructions	EBSS
74	Brzezinska AK	Cerebral artery smooth muscle cells	Papain: 1.5 mg/ml Collagenase Type 4: 1.5 mg/ml	Physiological Salt Solution
75	Phillips	Endothelial	Trypsin: 0.5%	BSS
76	Floris S	Cerebral endothelial	Collagenase Type 3: 0.2%	MEM
77	Williams	Microvessels Endothelial	Collagenase Type 2: 0.75%	Ringers-HEPES buffer
78	Boehm S	Hippocampal cells	Papain: 1 mg/ml	DMEM
79	Goldman	Germinal matrix	Trypsin: 0.25%	HBSS
80	Abney	Glial	Trypsin: 0.05%	Eagle's MEM/DMEM
81	Diglio	Endothelial Cerebral	Collagenase Type 2: 0.05%	HBSS

Species: Shellfish

Table 9.10: **Shellfish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
82	Hadley	Neurons Buccal ganglia	Trypsin: 0.2%	Saline, Sterile

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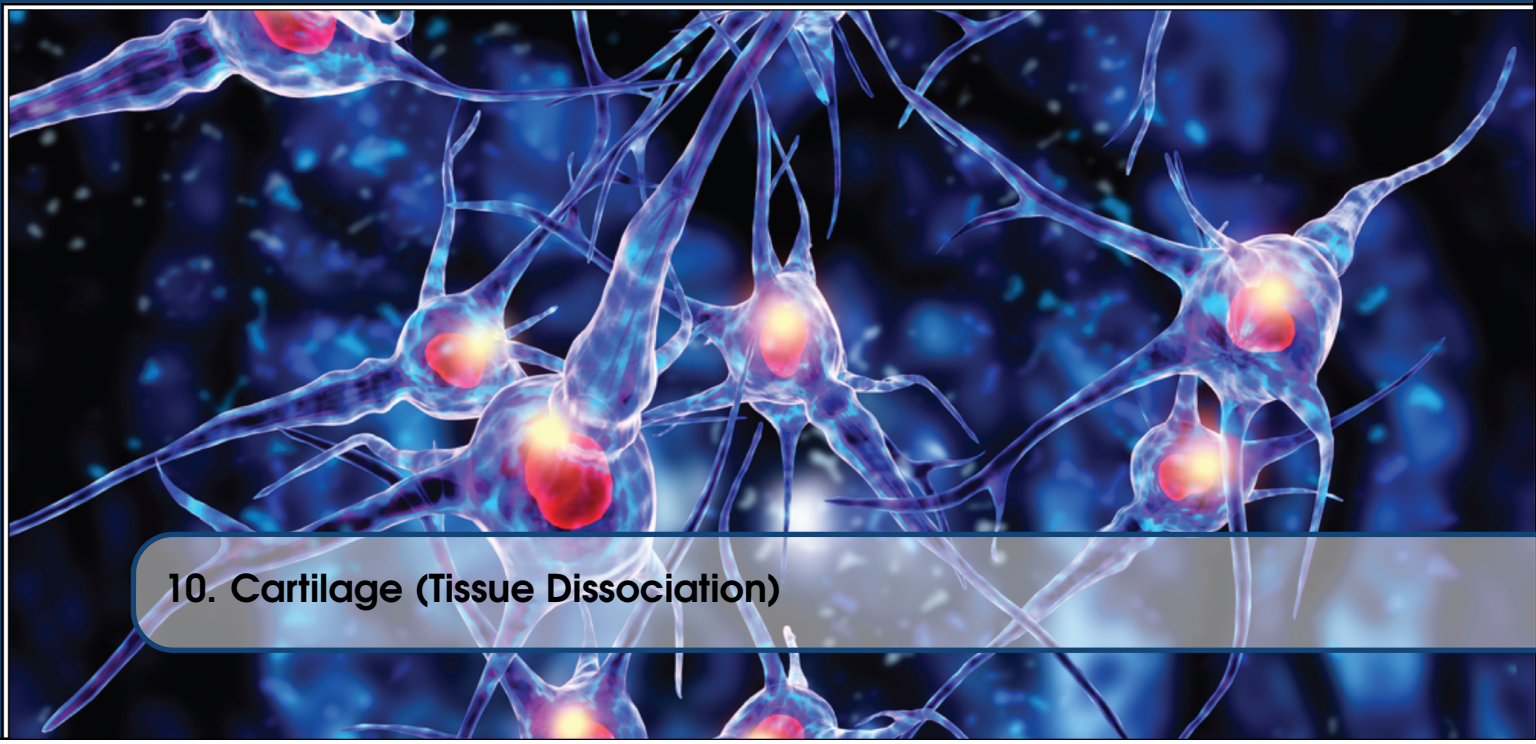

10. Cartilage (Tissue Dissociation)

Species: Bovine

Table 10.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Lima	Chondrocytes	Collagenase Type 4: 390 u/ml	hgDMEM
2	White	Chondrocytes	Collagenase Type 1: 0.1%	DMEM
3	Hwang	Chondrocytes	Collagenase Type 2: 0.2%	DMEM
4	Klagsbrun	Chondrocytes	Collagenase Type 2: 0.20%	PBS
5	Mackintosh	Chondrocytes	Trypsin: 0.20%	HBSS

Species: Canine

Table 10.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
6	Lee	Chondrocytes	Collagenase Type 2: 0.3% Trypsin: 0.25%	Ham's F12

Species: Chicken

Table 10.3: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Capasso	Chondrocytes	Trypsin: 0.75%	Coon's modified F-12
8	Wong	Mesenchymal	Trypsin: 0.1%	DMEM See Reference
9	Ahrens	Wing buds	Trypsin: 0.1%	Saline G
10	Gionti	Fibroblasts Epithelial-like	Trypsin: 0.25%	E 199 medium
11	Genge	Matrix vesicles	Trypsin: 0.1%	See Reference
12	Genge	Matrix vesicles Epiphyseal growth plate	Trypsin: 0.1%	Tris-buffered saline

Species: Equine

Table 10.4: **Equine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
13	Ley	Chondrocytes	Collagenase Type 2: 0.08%	DMEM/F12

Species: Goat

Table 10.5: **Goat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
14	Bekkers	Chondrocytes	Collagenase Type 2: 2%	DMEM

Species: Human

Table 10.6: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
15	Capsoni	Chondrocytes	Collagenase Type 2: 0.15%	DMEM
16	Kim Wan-Uk	Synoviocytes	Collagenase Type 1: 0.4%	DMEM
17	Sarkissian M	Synovial fibroblasts	Collagenase: 0.4%	DMEM
18	Dunham B P	Septal chondrocytes	Collagenase Type 2: 0.2% Hyaluronidase: 0.01% Deoxyribonuclease I: 0.015%	DMEM/F12
19	Dayer	Synovial tissue	Trypsin: 0.05%	DMEM See Reference
20	Srivastava	Articular chondrocytes	Trypsin: 0.2%	BSS
21	Rotter N	Chondrocytes	Collagenase Type 2: 0.2%	DMEM
22	Marsano A	Meniscus and cartilage	Collagenase Type 2: 0.15%	DMEM
23	Manning	Chondrocytes	Collagenase:	GBSS
24	Pallu	Chondrocytes	Collagenase Type 2: 0.2% Pronase: 0.15%	DMEM/F12
25	Tallheden T	Chondrocytes	Collagenase Type 2: 0.08%	DMEM/F12
26	Liagre B	Synovial	Collagenase: 0.15% Hyaluronidase: 0.1% Deoxyribonuclease I: 0.015%	DMEM
27	Jakob M	Chondrocytes	Collagenase Type 2: 0.15%	DMEM
28	McEvoy A.	Synoviocytes	Collagenase Type 1: 0.1%	RPMI

Species: Mouse

Table 10.7: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
29	Terpstra	Chondrocytes	Collagenase: 0.2%	DMEM
30	Otsuru	Chondrocytes	Trypsin: 0.25% Collagenase Type 1: 86.5 u/ml	DMEM

Species: Ovine

Table 10.8: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
31	Kojima Koji	Chondrocytes	Collagenase Type 2: 0.3%	Ham's F-12

Species: Porcine

Table 10.9: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
32	Chowdhury	Chondrocytes	Collagenase Type 1: 0.2%	DEMEM
33	Graff RD	Chondrons	Neutral Protease: 0.3% Collagenase: 0.2%	PBS

Species: Rabbit

Table 10.10: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
34	Schindler	Chondrocytes	Trypsin: 0.1%	Saline G, CMF
35	Green	Articular chondrocytes Hyaline	Trypsin: 0.2%	Gey's BSS
36	Bentley	Epiphyseal Articular cartilage	Trypsin: 0.25%	Eagle's basal medium
37	Ju	Chondrocytes	Collagenase Type 2: 0.025% Pronase: 0.2%	DMEM
38	Mehraban F	Chondrocytes	Hyaluronidase: .05% Collagenase Type 2: 0.2% Trypsin: 0.2%	Gey's solution
39	Plaas	Chondrocytes	Protease XIV: 5 mg/g of tissue	Ham's F-12
40	Benya	Chondrocytes	Trypsin: 0.2%	Gey's BSS

Species: Rat

Table 10.11: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
41	Shimomura	Chondrocytes	Trypsin: 0.2%	Ham's F-12 medium

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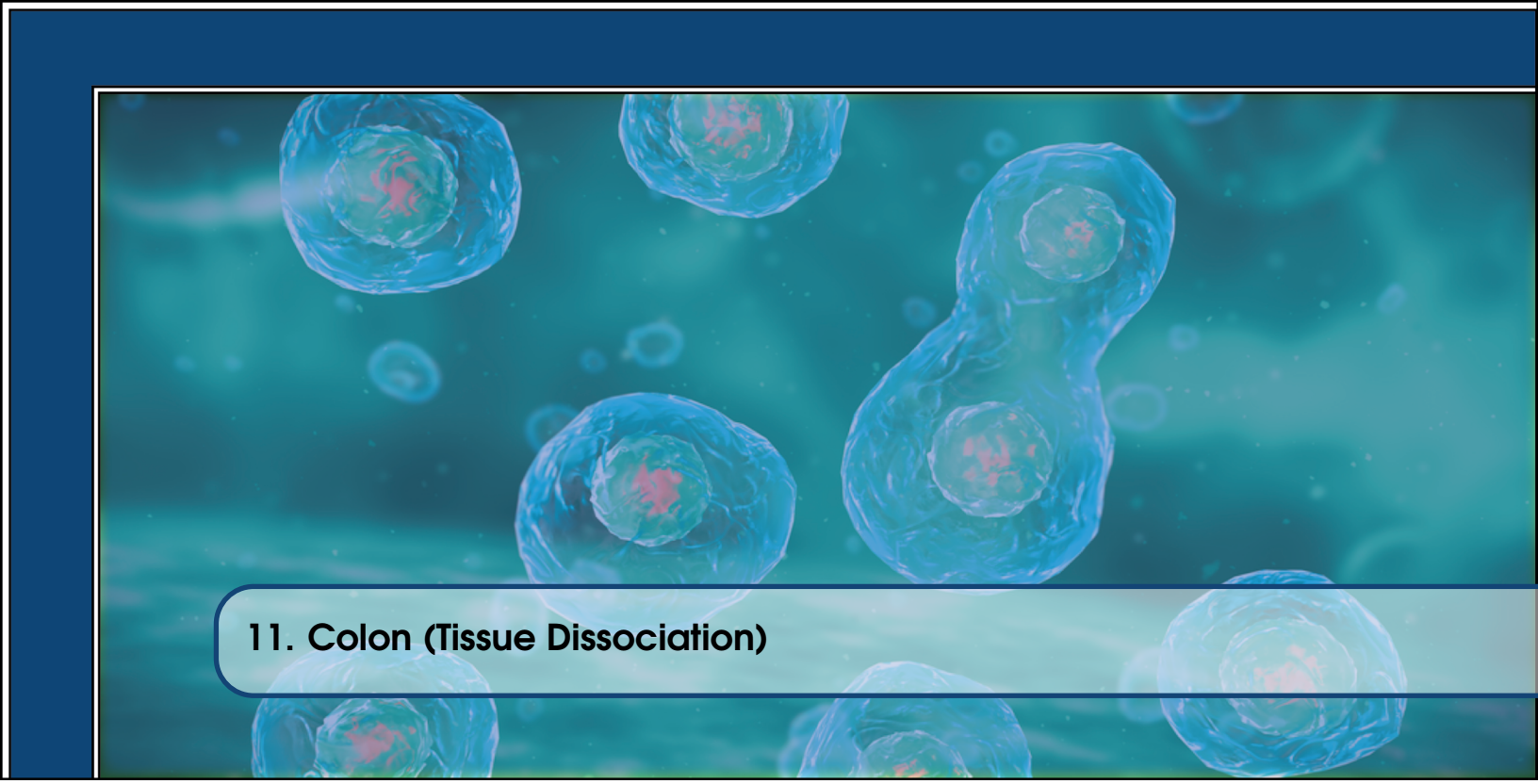

11. Colon (Tissue Dissociation)

Species: Guinea-Pig

Table 11.1: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Raghupathi	Enterochromaffin	Trypsin: 0.05% Collagenase Type 1: 0.1%	DMEM
2	Kang	Myenteric ganglia	Collagenase Type 4: 0.2% Protease: 0.1%	Kreb's solution

Species: Human

Table 11.2: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Raghupathi	Enterochromaffin	Trypsin: 0.05% Collagenase Type 1: 0.1%	DMEM
3	Roig	Colonic epithelial	Collagenase: 150 u/ml Neutral Protease: 0.04 mg/ml	Basal X media
4	Zhou	Colorectal cancer	Collagenase Type 4: 1% Deoxyribonuclease I: 0.2%	HBSS
5	Huang	Colonic epithelial	Collagenase Type 4: 0.1%	not listed
6	Varnat	Colon cancer	Collagenase Type 1: 300 u/ml Hyaluronidase: 100 u/ml	DMEM/F12
7	Dalerba Piero	Cancer stem cell	Collagenase Type 3: 200 u/ml Deoxyribonuclease I: 100 u/ml	RPMI-1640
8	Fukushima	Colonic epithelial	Collagenase: Neutral Protease: 0.3% Deoxyribonuclease I: 0.05%	RPMI 1640
9	Wang D.	Colonic endothelial cells	Collagenase Type 2: 0.25%	HBSS/5%FBS
10	Emenaker N	Colonocytes	Collagenase:	DMEM/F12
11	Hisamatsu	Epithelial and mucosal lymphocytes	Neutral Protease: 0.1% CLSPA: 0.02% Deoxyribonuclease I: 0.01%	RPMI 1640

12	Gibson	Colonic epithelial	Neutral Protease: 1.2 u/ml Collagenase Type 4: 50 u/ml	HBSS
13	Ueyama H	Lamina propria lymphocytes	Collagenase: 25 u/ml	HBSS

Species: Mouse

Table 11.3: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
14	Atarashi	Lamina propria	Collagenase Type 2: 0.1% Neutral Protease: 0.1% Deoxyribonuclease I: 0.004%	RPMI 1640
15	Weigmann Benno	Lamina propria mononuclear cells	Collagenase: 0.05% Deoxyribonuclease I: 0.05% Neutral Protease: 0.3%	HBSS
16	Abe	Dentritic	Collagenase: 300 u/ml Deoxyribonuclease I: 0.002%	RPMI 1640
17	Annacker O	Lympocytes	Collagenase/Dispase: 100 u/ml	RPMI 1640
7	Dalerba Piero	Cancer stem cell	Collagenase Type 3: 200 u/ml Deoxyribonuclease I: 100 u/ml	RPMI-1640
18	Totsuka T	Lamina propria lymphocytes	Collagenase Type 1: 0.2% Deoxyribonuclease I: 0.01%	HBSS
19	Wirtz S.	Lamina propria mononuclear cells	Collagenase Type 2: 0.015% Deoxyribonuclease I: 0.01%	RPMI

Species: Rat

Table 11.4: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
20	Somara	Colon smooth muscle	Soybean Trypsin Inhibitor: 0.01% Collagenase Type 2: 0.1%	DMEM

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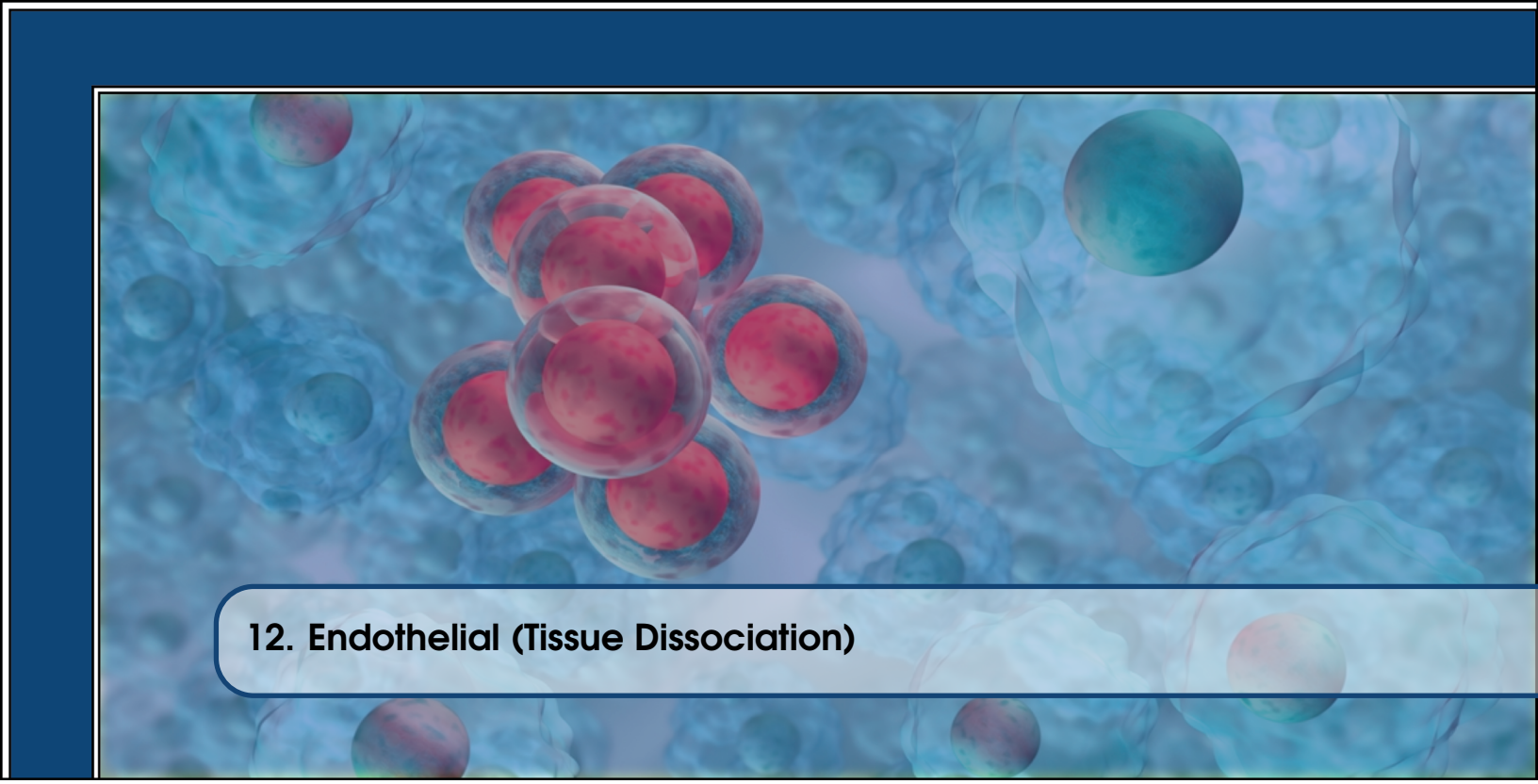

12. Endothelial (Tissue Dissociation)

Species: Bovine

Table 12.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Ricken Albert M	Bovine umbilical cord (BUVEC)	Collagenase: 0.1%	Dulbecco's/Ham F-12
2	Yu	Pulmonary artery endothelial and smooth muscle cells	Collagenase: 0.04-0.05% Soybean Trypsin Inhibitor: 0.04%	RPMI-1640
3	Martin	Endothelial, pulmonary artery	Collagenase: 0.1%	CMF-Dulbecco's PBS
4	DeNucci	Endothelial Aortic	Trypsin: 0.05%	Krebs Ringer solution
5	Carson	Endothelial Aorta	Collagenase Type 2: 0.1%	PBS
6	Gospodarowicz	Endothelial	Collagenase: 0.5%	DMEM/Ham's F-12
7	Kinsella	Endothelial Aortic	Collagenase Type 2: 0.1%	DMEM
8	Goetz	Endothelial Brain arteries	Collagenase Type 2: 0.2%	Dulbecco's PBS
9	Robinson	Endothelial, Corneal	Trypsin: 0.05%	0.01M Phosphate buffer with 0.02% EDTA 0.9% NaCl See Reference
10	Scott	Endothelial Corneal	Trypsin: 0.2%	PBS: DMEM
11	Olander	Endothelial Subclavian vein	Collagenase Type 1: 0.10%	PBS
12	Makarski	Aortic Pulmonary artery	Collagenase Type 2: 0.10%	PBS
13	Folkman	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum
14	Eskin	Saphenous Vein Aorta	Collagenase: 0.01%	PBS
15	Schwartz	Endothelial Thoracic aorta Saphenous veins	Collagenase Type 2: 0.1%	PBS
16	Ryan	Pulmonary artery	Collagenase Type 2: 0.25%	Puck's solution

17	Del Vecchio	Endothelial Pulmonary	Collagenase: 1000 u/ml	PBS, CMF
??	Lee	Endothelial Pulmonary artery	Collagenase Type 1: 0.2%	RPMI 1640 w/1% Fetal Bovine Serum
19	Machi	Cerebral artery Endothelial	Collagenase: 0.2%	HBSS
20	Cotta-Pereira	Aorta	Collagenase Type 1: 125 u/ml	Dulbecco's PBS with calcium and magnesium
21	Ryan	Pulmonary artery	Collagenase: 0.1%	Medium 199
22	Vender	Endothelial	Trypsin: 0.25%	HEPES
23	Voyta	Endothelial Smooth muscle	Collagenase: 0.75%	DMEM
24	Howard	Endothelial	Collagenase Type 2: 0.1%	DMEM
25	Rosen	Endothelial	Collagenase Type 1: 0.25%	PBS

Species: Canine

Table 12.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
26	Ford	Endothelium Jugular vein	Trypsin: 0.1%	Earle's PBS, CMF
27	Gerhart	Microvessels	Collagenase Type 4: 0.1%	DMEM

Species: Guinea-Pig

Table 12.3: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
28	Buxton I L	Coronary endothelial	Collagenase Type 2: 0.1%	See Reference

Species: Human

Table 12.4: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
29	Ganguly	Vascular endothelial	Collagenase Type 1: 0.1%	HBSS
30	Davis	HUVEC	Collagenase Type 1: 0.1%	HBSS
31	Moss	Endothelial and vascular smooth muscle	Collagenase Type 1: 0.2%	HBSS
32	Silva AP	HUVEC	Collagenase Type 4: 0.1%	RPMI 1640
33	Patel	Endothelial	Collagenase Type 2: 0.1%	DMEM
34	Rafiee	Esophageal microvascular endothelial	Collagenase Type 2: 0.2%	MCDB-131
35	Wang D.	Colonic endothelial cells	Collagenase Type 2: 0.25%	HBSS/5%FBS
36	Takano Manabu	HUVEC	Collagenase Type 2: 0.1%	PBS
37	Muller AM	Pulmonary vascular endothelial cells	Neutral Protease: 1.18 u/ml Elastase: 10 u/ml	M199
38	Goolcharran	Endothelial	Trypsin: 2%	PBS
39	Kwak HJ	HUVEC, porcine pulmonary arterial endothelial cells	Collagenase Type 2: 0.2%	DMEM
40	Schonbeck U	Vascular endothelial cells	Collagenase: 0.1%	DMEM
41	Sanyal AJ	Hepatic endothelial cells	Collagenase: 0.2%	DMEM
42	Wojta J	Foreskin microvascular endothelial	Trypsin: 0.3%	HBSS
43	Farber HW	Vascular endothelial	Neutral Protease: 0.15% Trypsin: 0.25%	M-199
44	Lee	Endothelial	Trypsin: 0.3.%	HBSS See Reference
45	Grant	Umbilical vein HUVEC	Collagenase: 0.1%	Cord Buffer See Reference
46	Whitehead	Crypt cells	Collagenase: 125 u/ml	RMPI 1640

47	Fischer	Endothelial	Collagenase Type 1: 0.2%	Medium 199
48	Muller WA	Human umbilical vein endothelial cells	Collagenase Type 2: 75 u/ml	M199
49	Hoshi	Umbilical cord Smooth muscle	Collagenase: 0.1%	HEPES
50	Kubota	Endothelial/HUVEC Foreskin & umbilical cord	Trypsin: 0.3%	HBSS/PBS, Medium 199 See Reference
27	Gerhart	Microvessels	Collagenase Type 4: 0.1%	DMEM
51	Sharefkin	Endothelial Saphenous vein	Collagenase Type 2: 0.1%	PBS, CMF
52	Hoshi	Endothelial	Collagenase: 0.1%	HEPES
53	Marks	Endothelial Dermal	Trypsin: 0.3%	PBS
54	Gordon	Fibroblasts Foreskin	Hyaluronidase: 0.10%	DMEM
55	Glassberg	Iliac arteries	Collagenase: 0.25%	PBS w/Ca^{++}, Mg^{++}, & BSA See Reference
56	Sherer	Microvascular endothelial Neonatal foreskins	Neutral Protease: at 1000 u/ml	Konigsberg's modification of HBSS See Reference
57	Jaffe	Umbilical vein	Trypsin: 100 μg/ml	Tris-HCl,0.2 M
58	Gimbrone Jr.	Umbilical vein	Collagenase Type 1: 125 u/ml	Dulbecco's PBS
59	Gimbrone	Umbilical cord	Collagenase: 0.1%	Dulbecco's PBS
60	Jaffe	Umbilical cord	Collagenase: 0.2%	Cord buffer See Reference
61	Lewis	Umbilical cord	Trypsin NF 1:250: 0.125%	Saline, normal
62	Fryer	Umbilical cord	Trypsin NF 1:250: 0.25%	CMF solution
13	Folkman	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum
63	Li Wei	Corneal endothelial	Collagenase: 0.2% Neutral Protease: 1.0%	DMEM/F12
64	Ashida	Peripheral blood mononuclear Monocytes T cells Endothelial	Collagenase: 0.25%	RPMI 1640

Species: Mouse

Table 12.5: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
65	Wang	Endothelial lung	Collagenase Type 1: 0.2%	DMEM
66	Kazerounian	Tumor endothelial	Collagenase Type 1: 0.2%	HBSS
67	Sapatino	Cerebrovascular Endothelial	Collagenase/Dispase: 0.1%	PBS
68	Kondo	Endothelial kidney	Collagenase Type 1: 0.1%	DMEM
69	Su X	Retinal endothelial cells	Collagenase Type 1: 0.1%	DMEM
70	Follenzi	Liver endothelial	Collagenase: 0.03%	DMEM
71	Imoukhuede	Endothelial	Collagenase Type 4: 0.2%	HBSS
72	Braren Rickmer	Endothelial	Collagenase Type 3: 200 u/ml Deoxyribonuclease I: 0.001%	PBS
73	Izawa D	Endothelial cells from lymph node	Collagenase Type 1: 0.1%	PBS
74	Cha	Microvascular endothelial	Neutral Protease: 0.005% Collagenase Type 1: 4%	DMEM

Species: Porcine

Table 12.6: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
75	Gould	Valvular endothelial	Collagenase Type 2: 300-600 u/ml	DMEM
39	Kwak HJ	HUVEC, porcine pulmonary arterial endothelial cells	Collagenase Type 2: 0.2%	DMEM
76	Shasby	Endothelial	Trypsin: 0.25%	Medium 199
77	Vischer	Endothelial	Trypsin: 0.05%	DMEM
78	Dickinson	Endothelial Aortic	Collagenase Type 2: 0.1%	Dulbecco-Vogt MEM w/o serum
79	Nugent	Endothelial	Collagenase Type 1:	DMEM w/ 10% calf serum
80	Slater	Endothelial Aortas Veins	Collagenase: 0.1%	Medium 199 w/BSS and HEPES or $NaHCO_3$
81	Merrilees	Endothelia Aortic	Collagenase Type 4: 0.025%	Medium 199
82	Balaoing	Valvular endothelial	Neutral Protease: 2 u/ml Collagenase Type 2: 60 u/ml	PBS
83	Hill-Kapturczak N	Porcine pulmonary endothelial	Collagenase Type 1: 0.3%	RPMI 1640
84	Coulson	Aorta	Trypsin: 0.1%	Phosphate buffer See Reference

Species: Rabbit

Table 12.7: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
85	Choi	Corneal endothelial cells (CEC)	Hyaluronidase: 0.05%	DMEM
86	Haley	Endothelial, aortic	Elastase: 0.2%	Hanks solution

Species: Rat

Table 12.8: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
13	Folkman	Foreskin	Collagenase: 0.5%	Dulbecco's MEM w/10% calf serum
87	Merrilees	Endothelial Thoracic aorta	Trypsin: 0.05%	Medium 199 and 0.01M EDTA
88	Schwertschlag	Smooth muscle, aorta	Soybean Trypsin Inhibitor: 0.25%	HBSS with 0.2 mM Ca++
89	Friedman	Lipocytes Kupffer Sinusoidal endothelial	Collagenase: 0.015%	DMEM/Ham's F-12
90	Phillips	Endothelial	Trypsin: 0.5%	BSS
91	Nagelkenke	Endothelial Kupffer Parenchymal	Pronase: 0.25%	HBSS
92	Diglio	Endothelial Cerebral	Collagenase Type 2: 0.05%	HBSS

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13. Epithelial (Tissue Dissociation)

Species: Bovine

Table 13.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Schumann	Epithelial Tracheal	Neutral Protease: 2%	Dissociation medium, CMF

Species: Canine

Table 13.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Virmani	Tracheal	Pronase: 0.1%	DMEM

Species: Chicken

Table 13.3: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Simon-Assmann	Intestinal mesenchymal and epithelial	Collagenase: 0.03%	DMEM

Species: Fish

Table 13.4: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Karnaky	Rectal gland	Collagenase: 0.2%	Ringer's solution
5	Valentich	Rectal gland	Collagenase: 0.2%	Ringer's solution
6	Dickman	Renal tubule	Trypsin: 0.2%	CMF solution

Species: Frog

Table 13.5: **Frog**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Heinke	Colonic epithelial	Collagenase Type 4: 0.1%	Kreb's

Species: Guinea-Pig

Table 13.6: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Chaminadas	Endometrial	Collagenase: 0.25%	HBSS
9	Rutten	Epithelial	Collagenase Type 1: 0.1%	DMEM

Species: Hamster

Table 13.7: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
10	Lee	Tracheal	Trypsin: 0.05%	Medium 199
11	Niles	Tracheal	Pronase: 0.1%	MEM with Hepes, CMF
12	Goldman	Tracheal	Trypsin: 0.25%	PBS with EDTA
13	McDowell	Tracheal	Trypsin: 0.1%	Ham's F-12

Species: Human

Table 13.8: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
14	Widdicombe JH	Human tracheal epithelium	Protease Type XIV: 0.04%	DMEM/F12
15	Fukushima	Colonic epithelial	Collagenase: Neutral Protease: 0.3% Deoxyribonuclease I: 0.05%	RPMI 1640
16	Fahlgren	Intestinal epithelial	Collagenase Type 4: 72.5 u/ml	HBSS
17	Espana EM	Corneal limbal epithelial sheet	Neutral Protease: 5%	See Reference
18	Smoot	Gastric epithelial cells	Collagenase Type 2: 200 u/ml Neutral Protease: 1.2 u/ml Soybean Trypsin Inhibitor: 0.125%	L-15
19	Halbert CL	Nasal polyp epithelial	Neutral Protease: .004% Trypsin: 0.1%	See Reference
20	Sarosiek	Gastric	Collagenase Type 4: 0.01%	F-12 medium
21	Sabatini	Epithelial	Trypsin: 0.05%	DMEM
22	Robinson	Epithelial	Trypsin: 0.2%	MEM, PBS
23	Wood	Epithelial Sweat gland	Collagenase Type 2: 0.2%	See Reference
24	Emerman	Epithelial	Collagenase: 2.0%	DMEM/Ham's F-12
25	Gruenert	Epithelial	Pronase: 0.1%	PBS
26	Widdicombe	Epithelial	Deoxyribonuclease I: 0.01%	HEPES with 5.9mM Glucose,5mM DTT
27	Yankaskas	Epithelial	Protease Type XIV: 0.1%	Eagle's MEM
28	Lechner	Epithelial Prostate	Trypsin: 0.1%	HBSS
29	Auersperg	Epithelial Ovary	Trypsin: 0.125%	HBSS, CMF
30	Munson	Endometrial epithelial	Trypsin:	DMEM/Ham's F-12

Species: Mouse

Table 13.9: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
31	Durban	Submandibular salivary	Collagenase Type 2 or 3: 0.16%	DMEM
32	Katayama	Esophageal	Trypsin: 0.25%	PBS, CMF
33	Yang	Mammary tumors Epithelial	Collagenase: 1.0%	HBSS
34	Durban	Epithelial Submandibular salivary gland	Collagenase Type 3: 0.16% , 1:1 v/v	DMEM with 15 mM HEPES
35	Yang	Epithelial Submandibular gland	Collagenase Type 3: 0.1%	HBSS
36	Mueller	Epithelial	Collagenase Type 3: 25 u/ml Hyaluronidase: 0.1% Protease XIV: 0.05% Deoxyribonuclease I: 0.04%	DMEM/F12
37	Wirtz S.	Lamina propria mononuclear cells	Collagenase Type 2: 0.015% Deoxyribonuclease I: 0.01%	RPMI
38	Gualdoni	Cilary epithelial	PDS kit: per instructions	EBSS
39	Lillehaug	Epithelial	Collagenase: 0.10%	DMEM
40	Ishimaru N	Salivary gland epithelial	Collagenase Type 1: 750 u/ml Hyaluronidase: 500 u/ml	DMEM/F12
41	Reiser	Epithelial	Pepsin: 0.1%	HBSS
42	Fukamachi	Uterine	Trypsin: 0.25%	HBSS
3	Simon-Assmann	Intestinal mesenchymal and epithelial	Collagenase: 0.03%	DMEM
43	Breggia	Renal tubular epithelial	Collagenase: 200 u/ml Soybean Trypsin Inhibitor: See Reference	HBSS
44	Ghosh	Uterine	Trypsin: 0.2%	HBSS

Species: Porcine

Table 13.10: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
45	De Buysscher	Trachea	Neutral Protease: 0.2%	HBSS
46	Wiencke	Retinal pigment epithelial cells	Collagenase: 2%	DMEM

Species: Rabbit

Table 13.11: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
47	Logsdon	Epithelial Gastric	Collagenase Type 3: 0.10%	HBSS
48	Nicosia	Mesothelial and surface epithelial Ovaries	Trypsin: 0.125%-0.5%	Medium 199
49	Vidrich	Colon	Neutral Protease: 0.3%	PBS
50	Chew	Gastric Parietal and chief	Collagenase Type 2: 0.08%	Sodium phosphate buffer

Species: Rat

Table 13.12: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
51	Mei	Mammary epithelial	Collagenase Type 3: 0.35%	HBSS
52	Masuda	Epthelial	Collagenase Type 3: 0.1%	Eagle's MEM Serum-free
53	Wang N	Retinal pigment epithelial cells	Collagenase Type 1: 65 u/ml Hyaluronidase: 220 u/ml	CF Hanks with EDTA
54	Evans GS	Rat intestinal epithelial	Neutral Protease: 0.01% Collagenase: 300 u/ml	DMEM
55	Chang CW	Retinal pigment epithelial	Neutral Protease: 2%	DMEM
56	Heimann	Interlobular duct fragments	Papain:	DMEM/Ham's F-12
57	Shannon JM	Tracheal epithelial	Collagenase Type 4: 0.05% Deoxyribonuclease I: Neutral Protease:	DMEM/F12
3	Simon-Assmann	Intestinal mesenchymal and epithelial	Collagenase: 0.03%	DMEM
58	Williams	Epithelial-like	Trypsin: 0.25%	PBS
59	Williams	Epithelial	Hyaluronidase: 0.0075%	KCl-NaCl HEPES Buffer
60	Herring	Epithelial	Trypsin: 0.05%	HBSS CMF
61	Babcock	Epithelial Esophagus	Hyaluronidase: 0.1%	HEPES BSS
62	Chang	Tracheal epithelial	Pronase: 0.5%	DMEM
63	Planus	Alveolar epithelial	Elastase: 40 u/ml	DMEM
64	Yassin	Colon	Deoxyribonuclease I: 10 μg/ml	See Reference
65	Klinefelter	Epididymal epithelial	Collagenase Type 2: 0.1%	HBSS
66	Abou-Haila	Seminiferous tubules	Trypsin: 0.05%	Krebs-Ringer bicarbonate buffer See Reference
67	Cohen	Epithelial, cancer and tumor	Collagenase: 0.1%	Eagles's MEM
68	Dial	Epithelial Stomach	Pronase: 0.15%	Medium 199
69	Malan-Shibley	Epithelial	Trypsin: 0.05%	HBSS, CMF
70	Jassal	Epithelial	Trypsin: 0.1%	HBSS
22	Robinson	Epithelial	Trypsin: 0.2%	MEM, PBS

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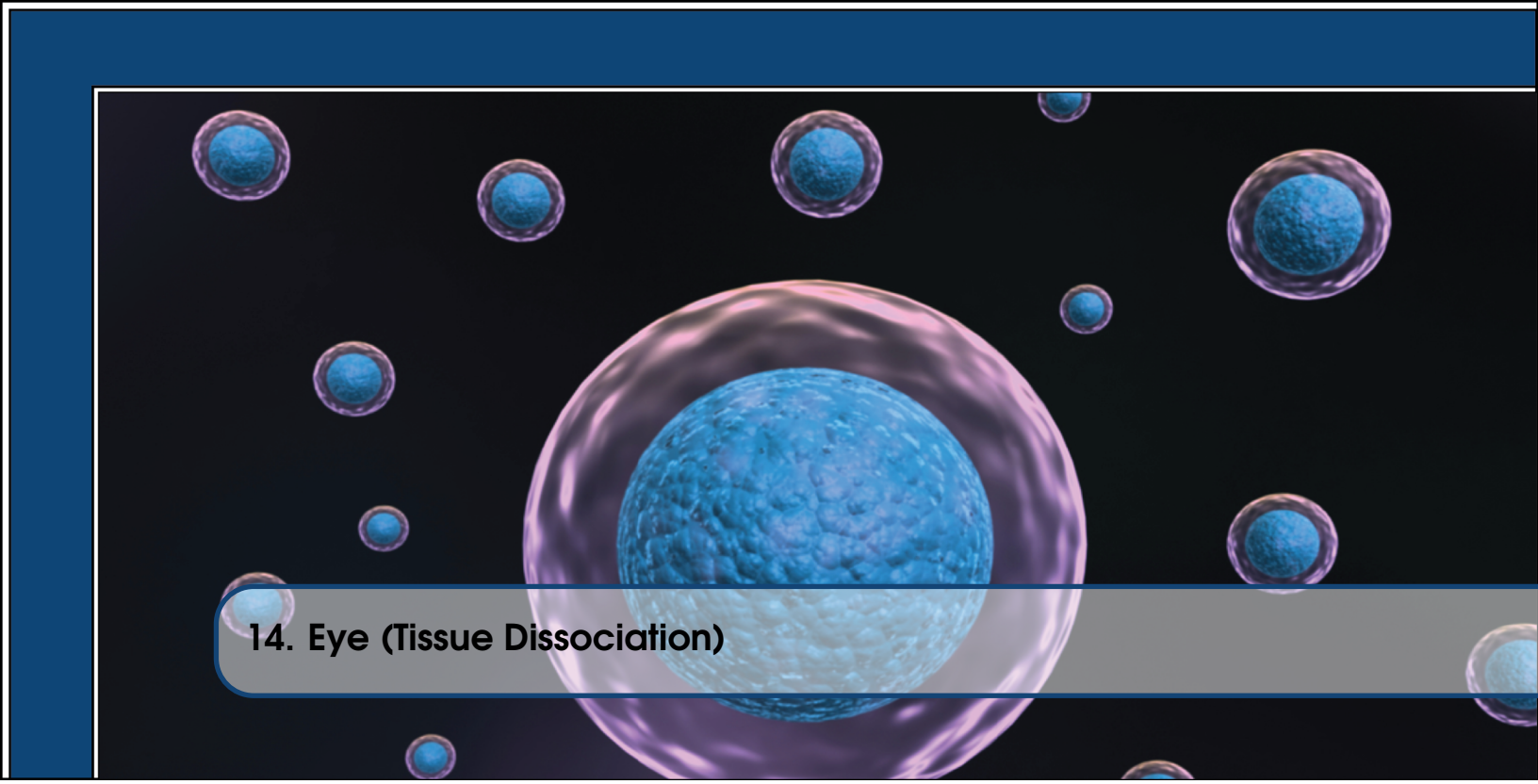

14. Eye (Tissue Dissociation)

Species: Bovine

Table 14.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Bryan	Pericyte	Collagenase: 0.2%	DMEM
2	Bowman	Microvascular endothelial	Collagenase/Dispase: 0.1%	MEM

Species: Chicken

Table 14.2: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Reenstra Wende R	Corneal epithelia	Collagenase: 0.08% Trypsin: 0.08%	HBSS
4	Seigel	Retinal	Trypsin: 0.6%	DMEM
5	Moyer	Flat, retina	Trypsin: 0.1%	Tyrode's solution, CMF
6	Jacob Vanessa	Retinal cells	Trypsin: 0.005% Deoxyribonuclease I: 0.005%	DMEM/F12

Species: Fish

Table 14.3: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Diekmann	Retinal	Papain: 10 u/ml	L-15

Species: Human

Table 14.4: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Du	Corneal stromal stem	Neutral Protease: 1.2 u/ml Collagenase: 0.1%	DMEM
9	Espana EM	Corneal limbal epithelial sheet	Neutral Protease: 5%	See Reference
10	Li Wei	Corneal endothelial	Collagenase: 0.2% Neutral Protease: 1.0%	DMEM/F12
11	Von Recum	Retinal pigment epithelial (RPE)	Trypsin: 0.25%	HBSS

Species: Monkey

Table 14.5: **Monkey**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
12	Han	Retinal	Papain: 20-40 u/ml Deoxyribonuclease I: 400 u/ml	Ames' solution
13	Whittum-Hudson JA	Conjunctival lymphocytes	Collagenase Type 1: 0.02%	RPMI 1640

Species: Mouse

Table 14.6: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
14	Schmidt	Retinal ganglion	CLSPA: 240 u/ml Hyaluronidase: 0.2%	Ames
15	Amadi-Obi	Retinal	Collagenase: 0.1% Deoxyribonuclease I: 0.001%	RPMI
16	Maxeiner Stephan	Mouse retinal and bipolar	Papain: 20 u/ml Deoxyribonuclease I: 200 u/ml	HBSS
17	Su X	Retinal endothelial cells	Collagenase Type 1: 0.1%	DMEM
18	Balmer	Photoreceptors	PDS kit: per instructions	Neurobasal
19	Singh	Retinal	PDS kit: per instructions	DMEM
20	Feodorova	Retinal photoreceptor	PDS kit: with modifications	EBSS
21	Gualdoni	Cilary epithelial	PDS kit: per instructions	EBSS
22	Jadhav AP	Retinal	Papain: 50 u/ml	DMEM/F-12
23	Jiang	Retinal progenitor	Collagenase: 0.1%	HBSS

Species: Porcine

Table 14.7: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
24	Wiencke	Retinal pigment epithelial cells	Collagenase: 2%	DMEM

Species: Rabbit

Table 14.8: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
25	Hao	Corneal keratocytes	Collagenase: 0.2% Hyaluronidase: 0.05%	DMEM
26	Brockway LM	Retinal neurons	Papain: 26 u/ml	DMEM
27	Stramer Brian M	Corneal keratocytes	Trypsin: 0.25% Collagenase: 0.5%	PBS
28	Johnson-Muller	Epithelial	Trypsin: 0.25%	HBSS/DMEM

Species: Rat

Table 14.9: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
29	Wang N	Retinal pigment epithelial cells	Collagenase Type 1: 65 u/ml Hyaluronidase: 220 u/ml	CF Hanks with EDTA
30	Chang CW	Retinal pigment epithelial	Neutral Protease: 2%	DMEM
31	Akagi T	Ciliary-derived eye	PDS kit: with modifications	DMEM/F12
4	Seigel	Retinal	Trypsin: 0.6%	DMEM
32	Suzuki	Retinal	PDS kit: per instructions	MEM
33	Huettner	Neurons, visual cortex	Papain: 20 u/ml	BSS See Reference
34	Tan	Retinal ganglion	PDS kit: with modifications	Neurobasal
35	Jing	Retina	Papain: 120 U	HBSS, PBS
36	Ma	Retinal ganglion	Papain: 0.2%	Neurobasal
37	Sarthy PV	Retina	Trypsin: 0.25%	Ham's F-12

Species: Salamander

Table 14.10: **Salamander**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
38	Clarke	Neurons	Papain: 14 u/ml	Ringers
39	Bader	Photoreceptors, retina	Papain: 0.05%	See Reference
40	Townes-Anderson	Retina	Papain: 14 u/ml	Saline

Species: Turtle

Table 14.11: **Turtle**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
41	Lam	Retinal	Papain: 0.1% (13.5 u/mg)	Kreb's Ringer

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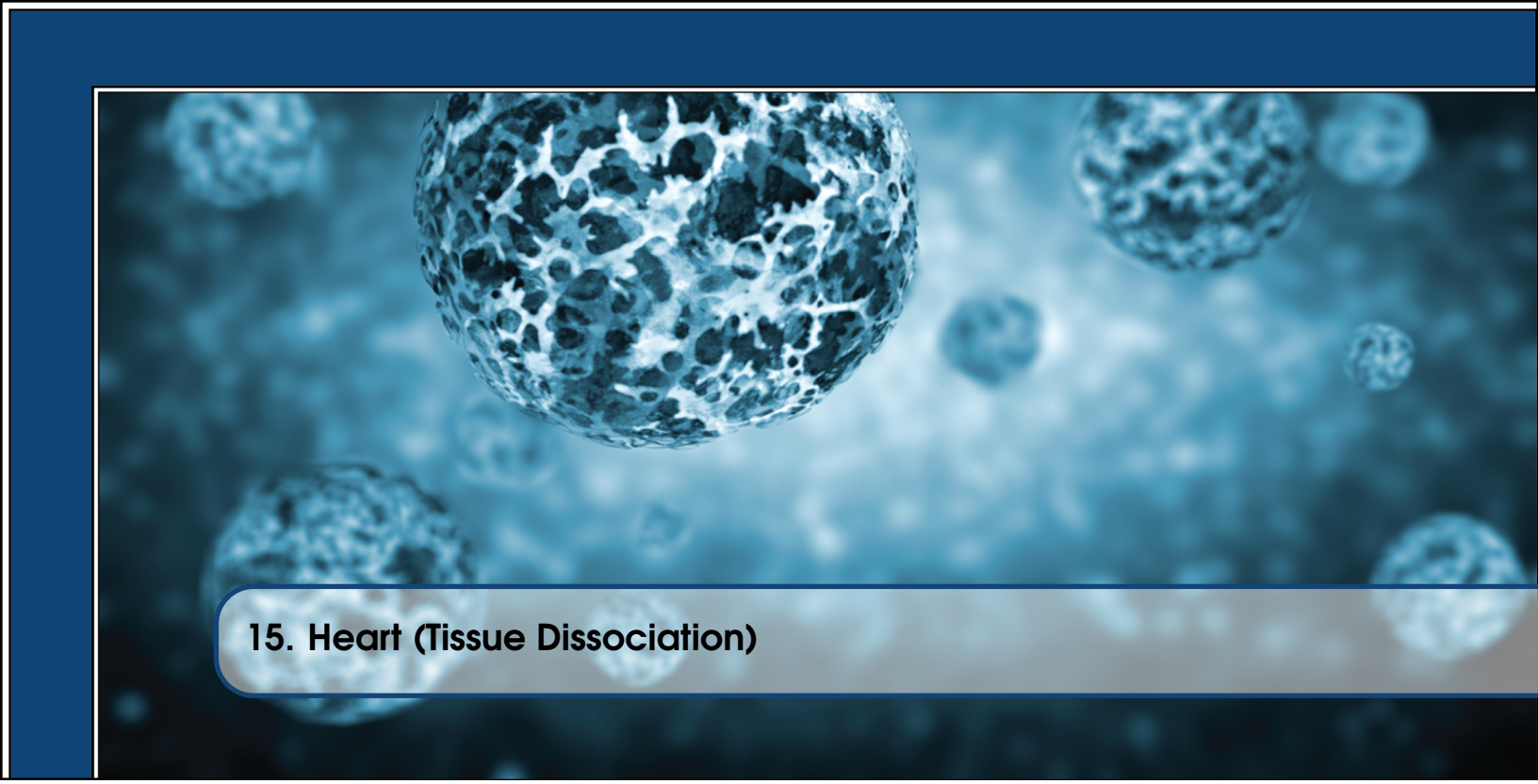

15. Heart (Tissue Dissociation)

Species: Bovine

Table 15.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Trifaro	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF

Species: Canine

Table 15.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Zhang	Cardiomyocytes	Collagenase Type 2: 150 u/ml	Tyrode's solution
3	Gan	Atrial Myocytes	Collagenase Type 2: 0.03%	Tyrode's solution
4	Harleton	Cardiomyocytes	Collagenase Type 2: 60 u/ml Protease XIV: 0.5 u/ml	See reference
5	Zhang	Atrial and Ventricular Myocytes	Collagenase Type 2: 0.05-0.08%	See reference
6	Xi	Cardiomyocytes	Collagenase Type 2: 300 u/ml Protease: 0.03%	Tyrode's solution
7	Schotten	Atrial and Ventricular myocytes	Collagenase Type 2: 0.065%	Tyrode's solution
8	Baba	Atrial myocytes	Collagenase Type 2: 0.013%	HEPES
9	Gintant	Ventricular and Atrial Myocytes	Collagenase Type 4: 0.04% Collagenase Type 2: 125 u/ml	HEPES/Tyrode's
10	Burashnikov	Cardiomyocytes	Collagenase Type 2: 0.05%	Tyrode's solution
11	Sun	Cardiomyocytes	Collagenase Type 2: 110 u/ml	Tyrode's solution
12	Bonilla	Atrial and Ventricular Myocytes	Collagenase Type 2: 0.065%	See reference
13	Calloe	Cardiomyocytes	Collagenase Type 2: 0.05% Protease Type XIV: 0.01%	HEPES
14	Gavi	Cardiomyocytes	Collagenase Type 2: 0.05% Protease: 0.008%	M199
15	Spanier	Myocytes	Hyaluronidase: 0.1%	Joklik's MEM

Species: Chicken

Table 15.3: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
16	Steinberg	Heart Liver	Trypsin: 3.0%	Tyrode's solution, CMF
17	Kim	Ventricular	Trypsin: 0.025%	HBSS, CMF
18	Gross	Myocytes	Trypsin NF 1:250: 0.25%	Rinaldini's buffer solution, CF
19	Jacob	Cardiomyocytes	Trypsin: 0.05%	HBSS, CMF
20	Murphy	Myocytes	Trypsin: 0.025%	CMF solution
21	Wang	Cardiomyocytes	Trypsin: 0.17%	HBSS, CMF
22	Eschenhagen	Cardiomyocytes	Trypsin: 0.25%	PBS
23	Blech-Hermoni	Cardiomyocytes	Trypsin: 0.13% Collagenase Type 2: 0.13% Deoxyribonuclease I: 0.033%	HBSS
24	Dehann	Heart	Trypsin: 0.025%	Medium 199

Species: Feline

Table 15.4: **Feline**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
25	Silver	Myocytes Ventricular	Collagenase Type 2: 0.12%	Kreb's Henseleit, CF

Species: Fish

Table 15.5: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
26	Sander	Cardiomyocytes	Collagenase Type 2: 0.5% Collagenase Type 4: 0.5%	MEM

Species: Frog

Table 15.6: **Frog**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
27	Arrio-Dupont	Myocytes	Trypsin: 0.04%	CF Ringer

Species: Guinea-Pig

Table 15.7: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
28	Preisig-Muller	Endothelial	Collagenase Type 2: 0.15%	Perfusing solution See Reference
29	Zorn-Pauly K	Cardiomyocytes	Collagenase Type 2: 100 u/ml	M-199
30	Buxton I L	Coronary endothelial	Collagenase Type 2: 0.1%	See Reference
31	Dhamoon	Cardiomyocytes	Collagenase Type 2: 100-200 u/ml	See reference
32	Ishihara	Cardiomyocytes	Collagenase: 0.04%	Tyrode solution, CF
33	Bridge	Myocytes	Hyaluronidase: 0.10%	Bicarbonate buffer, CF
34	Stemmer	Myocytes	Hyaluronidase: 0.02%	Krebs Henseleit bicarbonate buffer

Species: Human

Table 15.8: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
35	Holt-Casper	Cardiac	Collagenase: 0.045% Pancreatin: 0.1%	DPBS
36	Voigt	Atrial myocytes	Collagenase Type 1: 286 u/ml Protease: 5 u/ml	See References
37	Anderson	Myofibers	Collagenase Type 1: 0.3%	See Reference
38	Voigt	Atrial cardiomyocytes	Collagenase Type 1: 286 u/ml Protease: 5 u/ml	See Reference
39	Jensen	Coronary artery smooth muscle	Collagenase Type 2: 0.1% Elastase: 0.05%	DMEM
40	Mathew	Smooth muscle aortic	Collagenase Type 1: 0.2% Collagenase Type 2: 0.1% Elastase: 0.025%	DMEM
41	Todor Anastassia	Cardiomyocytes	Collagenase Type 2: 0.05% Collagenase Type 1: 0.025% Protease XIV: 0.013%	HEPES solution
42	Hoppe	Heart Myocytes	Collagenase Type 2: 200 u/ml	Tyrode's solution
43	Van Wagoner	Atrial myocytes	Collagenase Type 2: 0.1% Protease Type XIV: 0.04%	See Reference
44	Hassler	Thoracic aorta	Collagenase: 0.2%	Phosphate buffer w/NaCl
45	Mukerjee	Myocardial	Hyaluronidase: 0.05%	Medium 199
46	Bugaisky	Myocytes	Trypsin: 0.25%	Joklik's MEM
47	Smith	Myocardial Atrial	Collagenase: 0.14%	HBSS, CMF
48	Goldman	Myocytes	Hyaluronidase: 0.1%	HBSS with Calcium

Species: Invertebrate

Table 15.9: **Invertebrate**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
49	Altimiras J.	Systemic heart cardiomyocytes	Collagenase: 0.025% Trypsin: 0.02%	See Reference

Species: Mouse

Table 15.10: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
50	Touchberry	Cardiomyocytes	Collagenase Type 2: 360 u/ml Papain: 0.4 u/ml Deoxyribonuclease I: 40 u/ml	L-15
51	Hennessey	Cardiomyocytes	Collagenase Type 2: 150 u/ml	MEM
52	Butcher	Aortic adventitial leukocytes	Collagenase Type 2: 300 u/ml Elastase: 5.6 u/ml	PBS
53	Verheye	Smooth muscle cells	Collagenase Type 2: 300 u/ml Elastase: 5 u/ml	F10 Ham
54	Chen	Cardiomyocytes	Collagenase Type 1: 0.17%	PBS
55	Lu Tong	Ventricular myocytes and mesenteric arterial SMC	Collagenase Type 2: 0.06% Papain: 0.175%	Krebs-Ringer
56	Kobayashi	Aortic endothelial	Collagenase Type 2: 0.2%	DMEM
57	Fukumoto Yoshihiro	Smooth muscle cells	Collagenase Type 1: 0.1% Elastase: 0.0125%	DMEM
58	Rybkin	Cardiomyocytes	Collagenase Type 2: 0.2% Pancreatin: 0.06%	DMEM/F-12
59	Christensen G	Cardiomyocytes	Collagenase Type 2: 150 u/ml	Joklik's MEM
60	Soonpaa	Cardiomyocyte	Collagenase: 0.17 %	PBS
61	Felzen	Myocytes	Collagenase Type 2: 0.04%	Tyrode's solution

18	Gross	Myocytes	Trypsin NF 1:250: 0.25%	Rinaldini's buffer solution, CF
62	Wobus	Myocytes	Collagenase Type 1: 0.1%	DMEM
63	Potts	Cardiomyoctes	NCIS kit: per instructions	EBSS
64	Lader	Cardiomyocytes	NCIS kit: with modifications See Reference	L-15
65	Lader	Cardiomyocytes	NCIS kit: with modifications See Reference	L-15
66	Wang	Cardiomyocytes	Trypsin: 0.25%	FBS-MEM
67	Watzka SB	Cardiomyocytes	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.002%	PBS
68	Henn	Ventricular myocytes	Collagenase Type 2: 0.12%	See Reference
69	Zhou YY	Ventricular myocytes	Collagenase Type 2: 0.05% Collagenase Type 4: 0.05% Protease XIV: 0.002%	MEM
70	Christel	Sinoatrial node	Collagenase Type 2: 229 u/ml Elastase: 1.9 u/ml Protease Type XIV: 0.9 u/ml	Tyrode's solution
71	Wang	Cardiac progenitor	Collagenase Type 1: 0.1%	DMEM
72	Bradshaw AD	Fibroblasts, mesangial, smooth muscle	Trypsin: 0.25% Collagenase: See Reference Soybean Trypsin Inhibitor: .05%	DMEM
73	Qian	Vascular smooth muscle	Papain: 10 u/ml Elastase: .005% Collagenase: 0.05% Deoxyribonuclease I: 1000 u/ml PDS kit: with modifications	EBSS
74	Valenzuela	Ventricular and atrial myocytes	Collagenase Type 2: 95 u/ml Hyaluronidase: 172.5 u/ml Trypsin: 0.002% Deoxyribonuclease I: 60u/ml	DMEM/Tyrodes
75	Santos Nascimento	Cardiac Progenitor	Collagenase Type 2: 600 u/ml Deoxyribonuclease I: 60 u/ml	MEM
76	Kohncke	Cardiomyocytes	Collagenase Type 2: 0.08%	See Reference
77	Flynn	Cardiomyocytes	Collagenase Type 1: 0.1-0.4% Protease Type XIV: 0.004-.02%	See Reference
78	Zhang	Ventricular myoctes	Collagenase: 0.1%	M199
79	O'Connell Timothy D	Cardiomyocytes	Collagenase: See Reference	HBSS
80	Shioya	Cardiomyocytes	Collagenase Type 2: 0.1% Trypsin: 0.006% Protease XIV: 0.006%	See Reference
81	Kabaeva	Cardiomyocytes	Collagenase Type 2: 620 u/ml Protease XIV: 0.104 u/ml Deoxyribonuclease I: 0.0015%	Myocyte buffer See Reference
82	Zhang Sui	Cardiomyocytes	Collagenase Type 2: 0.02% Elastase: 0.03% Pancreatin: 0.06%	See Reference
83	Makino	Coronary endothelial cells	Collagenase Type 2: 0.1% Neutral Protease: 0.6 u/ml	M199
84	Carley	Cardiomyocytes	Collagenase Type 2: 59 u/ml	MEM
85	Takahashi N	Cardiomyoctes	Collagenase Type 1: 150 u/ml Trypsin: 0.01%	M199
86	Bettahi	Atrial myocytes	NCIS kit: with modifications See Reference	L-15
87	Schreiber	Cardiomyocytes	Collagenase Type 2: 0.03-0.2%	Joklik's MEM
88	Nelson	Myocytes	Collagenase Type 2: 150 u/ml	Joklik's MEM
89	Arber	Cardiomyocytes	Collagenase: 0.045%	DMEM, Medium 199

Species: Ovine

Table 15.11: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
90	Dibb	Cardiomyocytes	Collagenase Type 2: 0.055% Protease Type XIV: 0.006%	See reference
31	Dhamoon	Cardiomyocytes	Collagenase Type 2: 100-200 u/ml	See reference

Species: Porcine

Table 15.12: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
91	Bowles	Coronary smooth muscle	Trypsin: 0.1%	Krebs bicarbonate solution
92	Butcher	Aortic smooth muscle and interstitial cells	Collagenase: 600 u/ml	PBS
93	Christ	Coronary vascular smooth muscle (PCVSMCs)	Trypsin: 0.037%	HEPES

Species: Rabbit

Table 15.13: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
53	Verheye	Smooth muscle cells	Collagenase Type 2: 300 u/ml Elastase: 5 u/ml	F10 Ham
94	Anumonwo JM	Tricuspid valve cells	Collagenase: 0.8 mg/ml	HEPES Tyrode solution
95	Dani	Myocytes	Hyaluronidase: 0.007%	Kreb's Ringer
96	Spitzer	Myocytes	Collagenase Type 2: 0.1%	HEPES
97	Driesen Ronald B	Cardiomyocytes and fibroblasts	Collagenase: 0.06%	Medium 199
98	Farkas	Cardiomyocytes	Collagenase Type 1: 0.05% Hyaluronidase: See Reference Protease: See Reference	Krebs-Henseleit
99	Sedarat	Myocytes	Collagenase Type 2: 0.05%	EGTA-KB
100	Buxton	Cardiomyocytes	Hyaluronidase: 0.5%	Eagle's MEM

Species: Rat

Table 15.14: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
51	Hennessey	Cardiomyocytes	Collagenase Type 2: 150 u/ml	MEM
101	Zilberman	Thoracic aorta, smooth muscle	Elastase: 0.02%	See Reference
1	Trifaro	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF
102	Chen	Cardiomyocytes	NCIS kit: per instructions	F-10
103	Yang Y	Cardiomyocytes	NCIS kit: per instructions	L-15
104	Xie	Cardiomyocytes	NCIS kit: per instructions	MEM
105	Pedram	Cardiomyocytes	NCIS kit: per instructions	DMEM/F-12
106	Reinecke H	Cardiomyocytes	Collagenase Type 2: 95 u/ml Pancreatin: 0.06%	DMEM
107	Matsui	Cardiomyocytes	NCIS kit: per instructions	RPMI
108	Adderley SR	Neonatal rat cardiomyocytes	Collagenase Type 2: 80u/ml Pancreatin: 0.06%	DMEM/F12
109	Bishop	Myocytes	Collagenase Type 1: 0.1%	Saline
110	Nemec	Atrial	Trypsin: 0.03%	HBSS or EBSS
111	LaFramboise	Cardiomyocytes	Collagenase Type 2: 0.05% Pancreatin: 0.1%	DMEM
112	Speicher	Myocytes	Trypsin: 0.01%	Saline A

113	Ye	Cardiomyocytes	Collagenase Type 2: 300 u/ml	PBS
114	Aberle II NS	Ventricular myocytes	Collagenase: 223 u/ml Hyaluronidase: 0.01% Trypsin: 0.002%	Medium 199
115	Karakikes	Ventricular myocytes	Collagenase Type 2: 0.2%	Medium 199
116	Louch	Cardiomyocytes	Collagenase Type 2: 0.025%	See Reference
117	Xu	Myocytes	Collagenase Type 2: 0.05% Protease XIV: 0.02%	Media 199
118	Ren J	Ventricular myocytes	Collagenase: 223 u/ml Hyaluronidase: 0.01% Trypsin: 0.002%	Tyrode solution
119	Moustafa	Myocytes	Collagenase: 0.1%	Perfusing solution
120	Richards SM	Cardioomyocytes	Collagenase Type 4: 0.12%-adult Collagenase Type 2: 0.05%-neonatal	DMEM/medium 199
121	McMahon	Myocytes, atria	Collagenase Type 1: 100 u/ml	Kreb's Ringer bicarbonate - HEPES buffer
122	Gordon Jennifer M	Cardiomyocytes	Collagenase Type 2: 178 u/ml Hyaluronidase: 0.01%	Krebs-Henseleit, CF
123	Nag	Myocytes	Collagenase Type 2: 0.05%	Joklik's MEM
124	Grosso	Myocytes	Collagenase: 0.1% Hyaluronidase: 0.1%	MEM CF
125	Vahouny	Myocytes	Trypsin: 0.1%	Saline A
126	Berry	Myocytes	Hyaluronidase: 0.20%	Hank's solution, CF
127	Liu Qinghang	Ventricular myocytes	Collagenase Type 2: 0.1%	Ringer solution
128	Claycomb	Myocytes	Collagenase Type 2: 0.1%	Joklik's MEM
129	Nag	Myocytes	Hyaluronidase: 0.1%	Kreb's Ringers phosphate
130	Tamamori-Adachi Mimi	Cardiomyocytes	Collagenase Type 2: 0.1%	HEPES
131	Schwartzbauer	Cardiomyocytes	Trypsin:	DMEM/Ham's F-12
132	Bierman	Myocytes	Trypsin: 0.05%	Versene buffer
133	Kim	Aorta, smooth muscle	Trypsin: 0.025%	Medium 199 with 20% FBS medium
134	Farmer	Myocytes	Hyaluronidase: 82 u/ml	Kreb's Henseleit buffer
135	Hunton DaciaL	Cardiomyocytes	Collagenase Type 1: 0.03% Protease: 0.01%	HBSS
136	Kitta	Cardiac myocytes	Protease: 0.55 u/ml	Tyrode's solution
137	Nag	Muscle	Hyaluronidase: 0.1%	Krebs Ringer phosphate buffer, CMF
138	Dittami	Cardiomyocytes	NCIS kit: per instructions	L-15
139	Guan	Cardiomyocytes	Trypsin: 0.07% Collagenase Type 2: 0.1%	DMEM/M199
140	Smith	Cardiomyocytes	NCIS kit: per instructions	L-15
141	Robinet	Cardiomyocytes	NCIS kit: per instructions	L-15
142	Tamamori-Adachi	Cardiomyocytes	Collagenase Type 2: 0.1%	DMEM
143	Chen Hsiao-Huei	Cardiac myocytes	Collagenase Type 2: 0.5%	HBSS
144	Li TS	Cardiomyocytes	NCIS kit: per instructions	L-15
145	Guo K	Cardiomyocytes	NCIS kit: per instructions	DMEM/M199
146	Webster DR	Cardiomyocytes	NCIS kit: per instructions	L-15
147	Marino	Cardiomyocytes	Collagenase Type 2: 0.12%	Krebs-Henseleit Buffer, CF
148	Vanwinkle	Cardiomyocytes	Trypsin: 0.05%	DMEM
149	Lam ML	Ventricular cardiomyocytes	Collagenase Type 2: 0.7-1%	DMEM
150	Kim	Atrial	Trypsin: 0.06%	DMEM
151	Horne	Valve Interstitial cells	Collagenase Type 2: 600 u/ml	DMEM
152	Buxton	Myocytes	Collagenase Type 2: 0.1%	Kreb's Ringer w/ calcium
153	Clark	Cardiomyocytes	Trypsin: 0.025% Collagenase Type 2: 1.0%	HBSS
154	Wagner DR	Cardiomyocytes	NCIS kit: per instructions	DMEM/F-12
155	Zhang	Cardiomyocytes	Trypsin: 0.06% Collagenase Type 2: 220 u/ml	DMEM

156	Kim	Cardiomyocytes	NCIS kit: per instructions	L-15
157	Muller-Bore	Cardiomyocytes	NCIS kit: per instructions	L-15
158	Arutunyan A	Cardiomyocytes	Trypsin: 0.01% Collagenase Type 2: 0.08%	HBSS
159	Tardif Annie	Cardiomyocytes	Collagenase Type 2: 0.05% Deoxyribonuclease I: 0.02%	Krebs-Ringer
160	Jang	Cardiomyocytes	Collagenase Type 2: 0.1%	DMEM/F-12
161	Fang	Cardiomyocytes	Collagenase Type 2: 0.05%	DMEM
162	Entcheva	Cardiomyocytes, fibroblasts	Trypsin: 0.1% Collagenase: 0.1%	Medium 199
163	Toraason	Cardiomyocytes Fibroblasts	Trypsin: 0.1%	HBSS
98	Farkas	Cardiomyocytes	Collagenase Type 1: 0.05% Hyaluronidase: See Reference Protease: See Reference	Krebs-Henseleit
55	Lu Tong	Ventricular myocytes and mesenteric arterial SMC	Collagenase Type 2: 0.06% Papain: 0.175%	Krebs-Ringer
164	Swift Luther	Cardiomyocytes	Collagenase Type 2: 0.05-0.1%	Joklik's MEM
165	Sharma VK	Cardiomyocytes	Collagenase Type 2: 0.05%	Joklik's
166	Niederbichler Andreas D	Cardiomyocytes	Collagenase Type 2: 0.05% Hyaluronidase: 0.02%	DMEM
167	Yu L	Cardiomyocytes	Collagenase Type 2: 0.04%	See Reference
168	Lee	Ventricular myocytes	Collagenase: 0.08% Pronase: 0.004%	Tyrode's Solution
169	Puri S	Heart microvascular cells	CLSPA: 250u/ml Papain: 5 u/ml Elastase: 0.8 u/ml	L-15
170	Bugaisky	Myocytes	Collagenase: 0.1%	Joklik's medium
171	Fischer	Cardiomyocytes	Collagenase: 0.11%	See Reference
172	Frangakis	Myocytes	Hyaluronidase: 0.10%	Joklik MEM, CF
173	Glick	Heart ventricles, beating	Collagenase: 0.05-0.2%	Phosphate buffer
174	Piper	Myocytes	Collagenase Type 1: 0.06%	Krebs Ringer bicarbonate buffer
175	Powell	Myocytes Ventricular myocardium	Collagenase Type 1: 0.20%	Kreb's Ringer bicarbonate buffer
176	Westfall M V	Ventricular cardiac myocytes	Collagenase Type 2: 0.5% Hyaluronidase: 0.2%	Krebs-Henseleit buffer
177	Welder	Myocardial	Collagenase Type 2: 0.1%	Joklik's MEM, Kreb's-Henseleit buffer, CF
178	Li RK	Cardiomyocytes	Collagenase: 0.1% Trypsin: 0.2%	PBS
179	Mellor	Cardiomyocytes	Collagenase Type 2: 0.056%	Krebs
180	Stagg Mark A	Ventricular myocytes	Collagenase: 0.13% Hyaluronidase: 0.06%	DMEM
181	Dai L	Cardiomyocytes	Collagenase Type 2: 140 u/ml	Krebs-Henseleit
182	Cornwell	Aortic smooth muscle	Trypsin: 0.05%	DMEM
183	Pretlow II	Myocytes	Hyaluronidase: 0.05%	See Reference
184	Laughlin	Myocytes Ventricles	Collagenase: 0.07%	Joklik's MEM
185	Kubli	Cardiomyocytes	Collagenase Type 2: 0.1%	J-MEM
186	De Young	Myocytes, ventricular	Collagenase Type 1: 90 and 100 u/ml	Joklik's solution with and without calcium
187	Head	Cardiomyocytes	Collagenase Type 2: 250 u/ml	Cardioplegic solution
188	Sun L	Cardiomyocytes	Collagenase Type 2: 0.06%	M199
189	Kim	Myocytes, heart	Hyaluronidase: 0.03%	Bicarbonate-buffered medium
190	Rahman A	Cardiac myocytes	NCIS kit: per instructions	L-15
191	DeAlmeida	Peritoneal mast	Hyaluronidase: 100 u/ml	DMEM See Reference
192	Freerksen	Myocytes	Trypsin: 2.4 u/ml	DMEM
193	Maki T	Ventricular myocytes	Collagenase Type 2: 0.08%	DMEM
194	Shimizu Tatsuya	Cardiomyocytes	Collagenase Type 2: 80u/ml	M199

195	Kinugawa	Cardiomyoctes	Collagenase Type 2: 80 u/ml	HBSS
196	Castillo	Cardiomyocytes	NCIS kit: per instructions	L-15
197	Mark	Myocytes	Trypsin: 0.125%	Gey's BSS
198	Eckerle	Cardiomyocytes	Collagenase Type 2: 150 u/ml	See Reference
199	Cowan DB	Cardiomyocytes	NCIS kit: per instructions	DMEM-F12
200	Grynberg	Myoblast, cardiac	Trypsin: 0.1%	Standardized Medium See Reference
201	Calderon-Sachez	Ventricular myocytes	Collagenase Type 2: 251 u/ml	Tyrode solution
202	Pyle	Ventricular myocytes	Collagenase Type 2: 0.1%	Ringer solution
203	Brinckmann	Cardiomyocytes	NCIS kit: per instructions	L-15
204	Sambandam N	Cardiomyocytes	Collagenase Type 2: 228 u/ml	Joklik MEM
205	Butler	Cardiomyocytes	NCIS kit: per instructions	L-15
206	Natarajan AR	Cardiomyocytes	NCIS kit: per instructions	DMEM
207	Berg	Myocytes	Collagenase Type 1: 100 u/ml and 150 u/ml	Krebs Ringer bicarbonate buffer
208	Engelmann	Ventricular Cardiomyocytes	Collagenase Type 2: 0.05 - 0.08%	See reference
209	MacGregor	Cardiomyocytes	NCIS kit: per instructions	HBSS
210	Miller	Ventricular myocytes	Collagenase Type 2: 95 u/ml Protease: 0.1 u/ml	HEPES

Species: Shellfish

Table 15.15: **Shellfish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
211	Wen	Myocytes Fibroblasts	Collagenase Type 1: 2%	L15 medium
212	Le Duff	Haemocytes	Trypsin:	L15 medium

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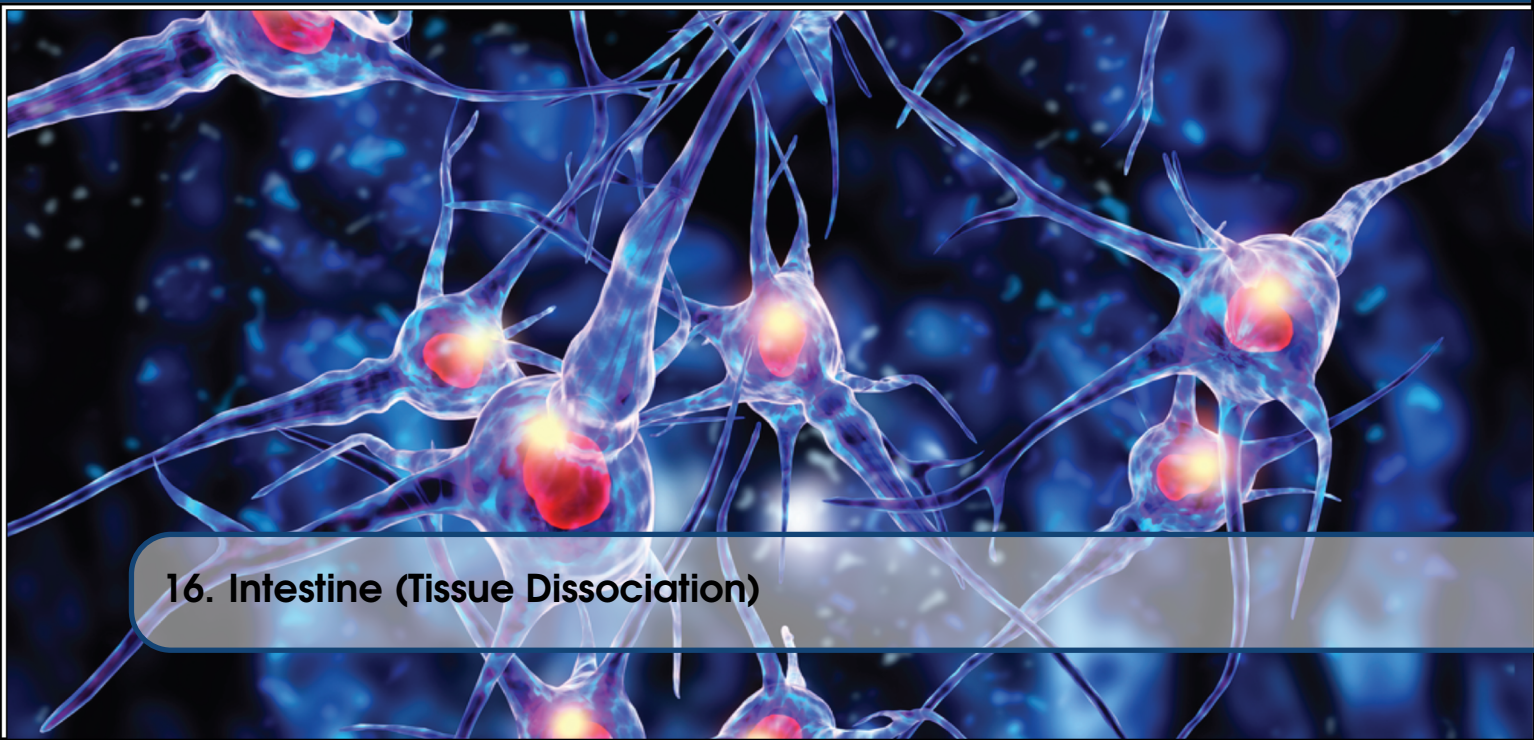

16. Intestine (Tissue Dissociation)

Species: Canine

Table 16.1: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Damholt AB	Intestinal L-cells	Collagenase Type 1: 75 u/ml	HBSS

Species: Human

Table 16.2: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Ebert	Lamina propria lymphocytes	Collagenase: 20 u/ml	Medium
3	Kanai Takanori	Lamina propria mononuclear cells	Collagenase: 0.02%	HBSS
4	Fahlgren	Intestinal epithelial	Collagenase Type 4: 72.5 u/ml	HBSS
5	Stallmach A	Mucosal mononuclear cells	Collagenase Type 3: 0.01% Deoxyribonuclease I: 0.01% Soybean Trypsin Inhibitor: 0.01%	RPMI

Species: Mouse

Table 16.3: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
6	Xue	colon tumor organoids	Collagenase Type 4: 200 u/ml Neutral Protease: 0.01%	DMEM
7	Barthel	Intestinal organoids	Collagenase Type 1: 800 u/ml Neutral Protease: 0.013%	DMEM
8	Geem	Dendritic, macrophages	Collagenase: 0.15% Deoxyribonuclease I: 0.004%	HBSS
9	Ito	Lamina propria lymphocytes	Collagenase Type 1: 0.3% Deoxyribonuclease I: 0.01%	RPMI 1640
10	Forbes	Intestinal mononuclear	Collagenase Type 4: 0.1%	RPMI 1640
11	Wu Y	Lamina propria lymphocytes	Collagenase Type 4: 300 u/ml	PBS

12	Lee Young Mee	Interstitial cells of Cajal	Collagenase Type 2: 0.13%	Hanks
13	Goto Kazunori	Interstitial cells	Collagenase: 0.04% Trypsin: 0.02%	See Reference
14	Ordag Tamas	Interstitial cells of Cajal	Collagenase Type 2: 0.13%	HBSS
15	Sakagami Y	Intestinal mesenchymal	Collagenase Type 2: 0.03%	HBSS
16	Kidd	Enterochromaffin cells	Collagenase: 0.025% Pronase E: 0.07%	HBSS
17	Joseph	Myenteric plexus	Collagenase Type 4: 0.025-0.1% Papain: 10 u/ml Deoxyribonuclease I: 100 u/ml	HBSS
18	Hotta	Enteric neural crest progenitors	Neutral Protease: 0.5% Collagenase animal free: 0.05%	DMEM/F12

Species: Rat

Table 16.4: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
19	Quaroni A	Intestinal epithelial	Collagenase Type 1: 0.1%	DMEM
20	Kruger	Gut Neural Crest Stem Cells	Collagenase Type 4: 0.1% Trypsin: 0.025%	HBSS
21	Bixby	Sciatic Nerve and Gut Neural Crest Stem Cells	Collagenase Type 4: 0.025% Trypsin: 0.005% Deoxyribonuclease I: 0.05%	HBSS

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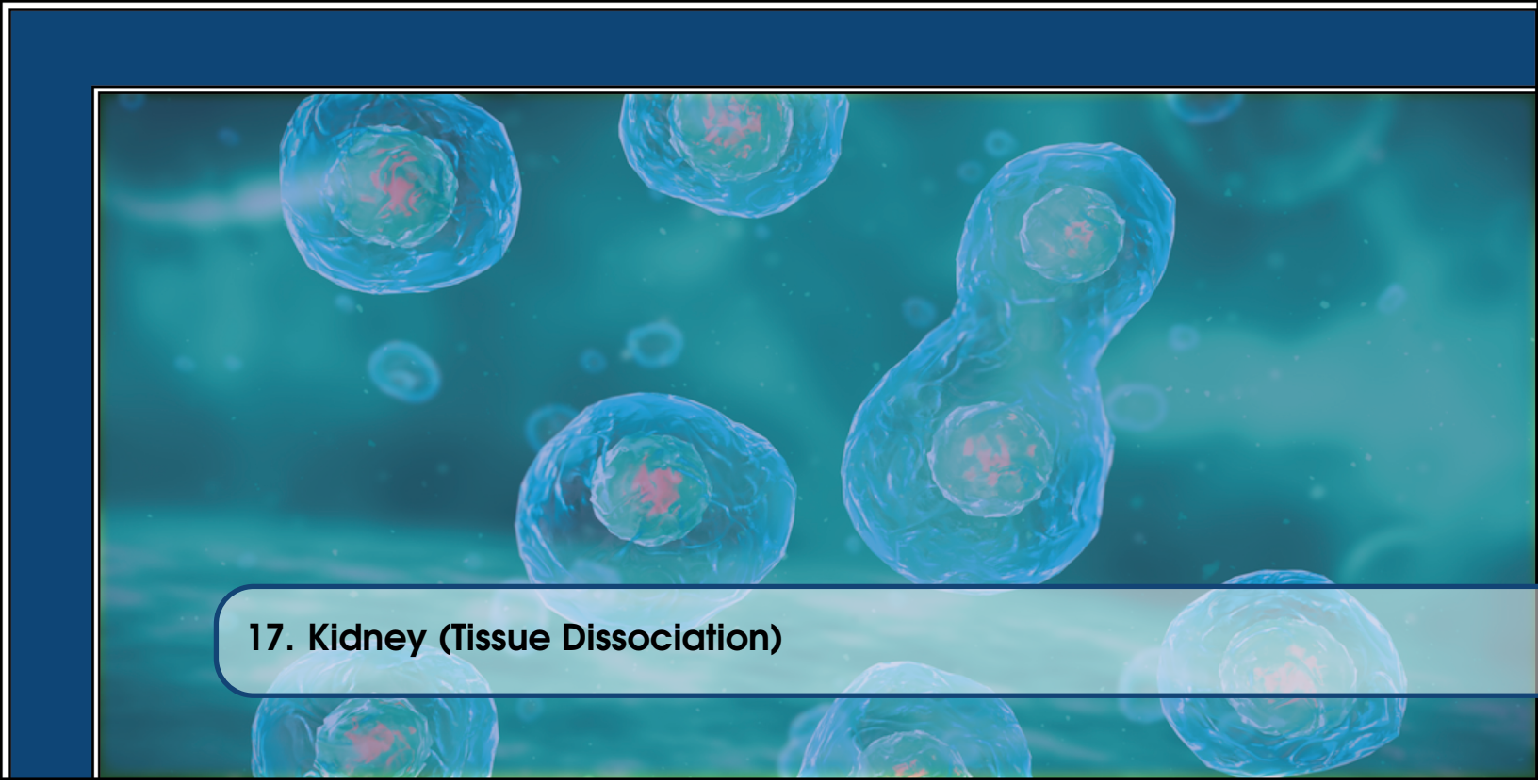

17. Kidney (Tissue Dissociation)

Species: Avian

Table 17.1: **Avian**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Reich	Tubule and glomeruli	Collagenase Type 1: 0.1%	RPMI 1640
2	Goldstein D.	Tubule segments	Collagenase Type 2: 0.1%	HBSS

Species: Bovine

Table 17.2: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Husted	Papillary duct	Hyaluronidase: 0.2%	Keri's buffer HEPES buffered saline

Species: Canine

Table 17.3: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Basu	Renal adipose derived cells	Collagenase Type 1: 0.3%	DMEM
5	States	Proximal tubules	Collagenase Type 1: 0.15%	Krebs Ringer bicarbonate buffer
6	Yau	Proximal tubular	Deoxyribonuclease I: 0.0125%	See Reference

Species: Fish

Table 17.4: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Dickman	Renal tubule	Trypsin: 0.2%	CMF solution See Reference

Species: Guinea-Pig

Table 17.5: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Phillips	Single cells	Trypsin: 0.25%	CF salt solution

Species: Hamster

Table 17.6: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Husted	Papillary duct	Hyaluronidase: 0.2%	Keri's buffer HEPES buffered saline

Species: Human

Table 17.7: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Basu	Renal adipose derived cells	Collagenase Type 1: 0.3%	DMEM
9	Presnell	Renal cells	Collagenase Type 4: 300 u/ml Neutral Protease: See Reference	HBSS
10	McAteer	Renal cortex	Trypsin: 0.1%	Tissue Culture Grade Water
11	Trifillis	Papillary duct	Collagenase: 400 u/ml	Eagle's MEM-HEPES buffer w/L-glutamine
12	Heieren	Mesangial	Trypsin: 0.25%	DMEM/Ham's F-12
13	Hemstreet	Malignant Stromal	Papain: 0.009%	Sacks solution
14	De Oca	Renal	Trypsin: 0.25%	See reference
15	Valente	Renal tumor cells and proximal tubular epithelial	Collagenase Type 2: 0.1%	DMEM/F12
16	Johnson	Renal proximal tubule and cortical fibroblasts	Collagenase Type 2: 383 u/ml	DMEM/F-12
17	Yang	Tubular	Collagenase: 250 u/ml	PBS
18	Trifillis	Tubular	Collagenase: 100 u/ml	Joklik's MEM

Species: Monkey

Table 17.8: **Monkey**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
19	Melnick	Kidney	Trypsin: 0.01% - 0.00001%	Eagle's MEM

Species: Mouse

Table 17.9: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
20	DiRocco	Collecting duct epithelial	Collagenase Type 2: 0.1%	DMEM
21	Kabgani	Glomeruli	Collagenase Type 4: 0.1%	RPMI 1640
22	Wright	Proximal tubule	Collagenase Type 2: 0.013%	See Reference
23	Taub	Epithelial	Collagenase Type 4: 1%	DMEM /F-12
24	Park	Proximal tubule	Collagenase Type 1: 0.1%	See reference
25	Bradshaw AD	Fibroblasts, mesangial, smooth muscle	Trypsin: 0.25% Collagenase: See Reference Soybean Trypsin Inhibitor: .05%	DMEM
26	Kondo	Endothelial kidney	Collagenase Type 1: 0.1%	DMEM
27	Radeke HH	Glomerular mesangial cells	Collagenase Type 4: 0.1%	RPMI 1640
28	Syal Ashu	Cortex, proximal tubule	Collagenase: 0.15%	DMEM
29	Brown	Kidney	Collagenase Type 1: 0.25% Pancreatin: 1.0% Deoxyribonuclease I: 1 u/ml	HBSS
30	Cunningham	Renal proximal tubule cells	Collagenase Type 2: 0.1% Soybean Trypsin Inhibitor: 0.25%	DMEM/F-12

31	Sindic	Cortical collecting duct	Collagenase: 54-178 u/ml Protease: See Reference	MEM
32	Breggia	Renal tubular epithelial	Collagenase: 200 u/ml Soybean Trypsin Inhibitor: See Reference	HBSS
33	Haverty	Proximal tubular epithelial	Deoxyribonuclease I: 15 μg/ml	RPMI 1640

Species: Porcine

Table 17.10: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
34	Heussner	Kidney	Collagenase Type 1: 0.1%	HBSS

Species: Rabbit

Table 17.11: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
35	Nowak	Renal proximal tubular	Collagenase Type 1: 60 u/ml	DMEM/F12
3	Husted	Papillary duct	Hyaluronidase: 0.2%	Keri's buffer HEPES buffered saline
36	Schafer	Nephron segments	Collagenase Type 2: 0.05% Deoxyribonuclease I: 50 u/ml	Eagle's MEM
37	Taub	Proximal tubule cells	Collagenase Type 4: 0.005% Soybean Trypsin Inhibitor: 0.00025%	DMEM/F-12
38	Rodeheaver	Renal proximal tubules	Deoxyribonuclease I: 70 u/ml	Modified DME-F12
39	Naray-Fejes-Toth	Duct	Soybean Trypsin Inhibitor: 0.025%	Hank's Solution with calcium and HEPES
40	Grenier	Collecting tubule	Trypsin: 0.05%	Kreb's Ringer buffer
41	Allen	MTALH cells RCCT cells	Collagenase: 0.1%	DMEM
42	Dworzack	Papillary collecting duct	Collagenase: 0.2%	See Reference

Species: Rat

Table 17.12: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
43	Panico	Proximal tubule	Collagenase Type 2: 0.1%	See Reference
44	Arystarkhova	Cortical tubule cells	Collagenase: 0.6%	DMEM
45	Wallach	Fibroblasts Kidney	Trypsin: up to 0.25%	Dulbecco-Vogt MEM
46	Ishikawa	Renal	Collagenase: 0.1%	DMEM
47	Brion	Inner medullary collecting duct Papillae	Collagenase Type 2: 0.1%	PBS
4	Basu	Renal adipose derived cells	Collagenase Type 1: 0.3%	DMEM
48	Elliget	Epithelial Proximal tubule	Protease: 0.1%	HBSS/CMF
49	Valencia L.	Cortical collection duct	Collagenase Type 2: 0.15%	DMEM/Ham's F12
50	Amiri F	Glomerular mesangial cells	Collagenase Type 1: 250 u/ml	DMEM
51	Li	Microvessels	Collagenase Type 2: 0.1%	PSS
52	Mattson D.	Renal	Collagenase Type 2: 0.2%	See Reference
53	Gupta	Fetal kidney	Collagenase Type 4: 0.1%	MEM
54	Eitle E	Proximal tubule suspensions	Collagenase Type 4: 0.1% Pronase E: 2 u/ml	HEPES buffer
36	Schafer	Nephron segments	Collagenase Type 2: 0.05% Deoxyribonuclease I: 50 u/ml	Eagle's MEM
55	Amiri Farhad	Glomerular mesangial cells	Collagenase Type 1: 250 u/ml	DMEM
56	Silva	Medullary thick ascending limb	Collagenase: 0.1%	HEPES-saline
57	Miyata	Renal tubules	Collagenase: 0.1%	See Reference

58	Deng Aihua	Renal proximal tubules	Collagenase Type 2: 0.2%	DMEM/F12
59	Wang	Glomerular mesangial	Collagenase: 0.025%	RPMI 1640
60	Barlet-Bas	Renal target	Collagenase: 1.0% (also 0.7%)	Eagle's MEM
61	Deng	Renal proximal tubules	Collagenase Type 2: 0.2%	DMEM/F-12
62	Gesek	Proximal tubules	Collagenase: 0.2%	Krebs-Henseleit buffer
63	Vinay	Proximal tubules	Collagenase: 0.15 %	Krebs Henseleit solution

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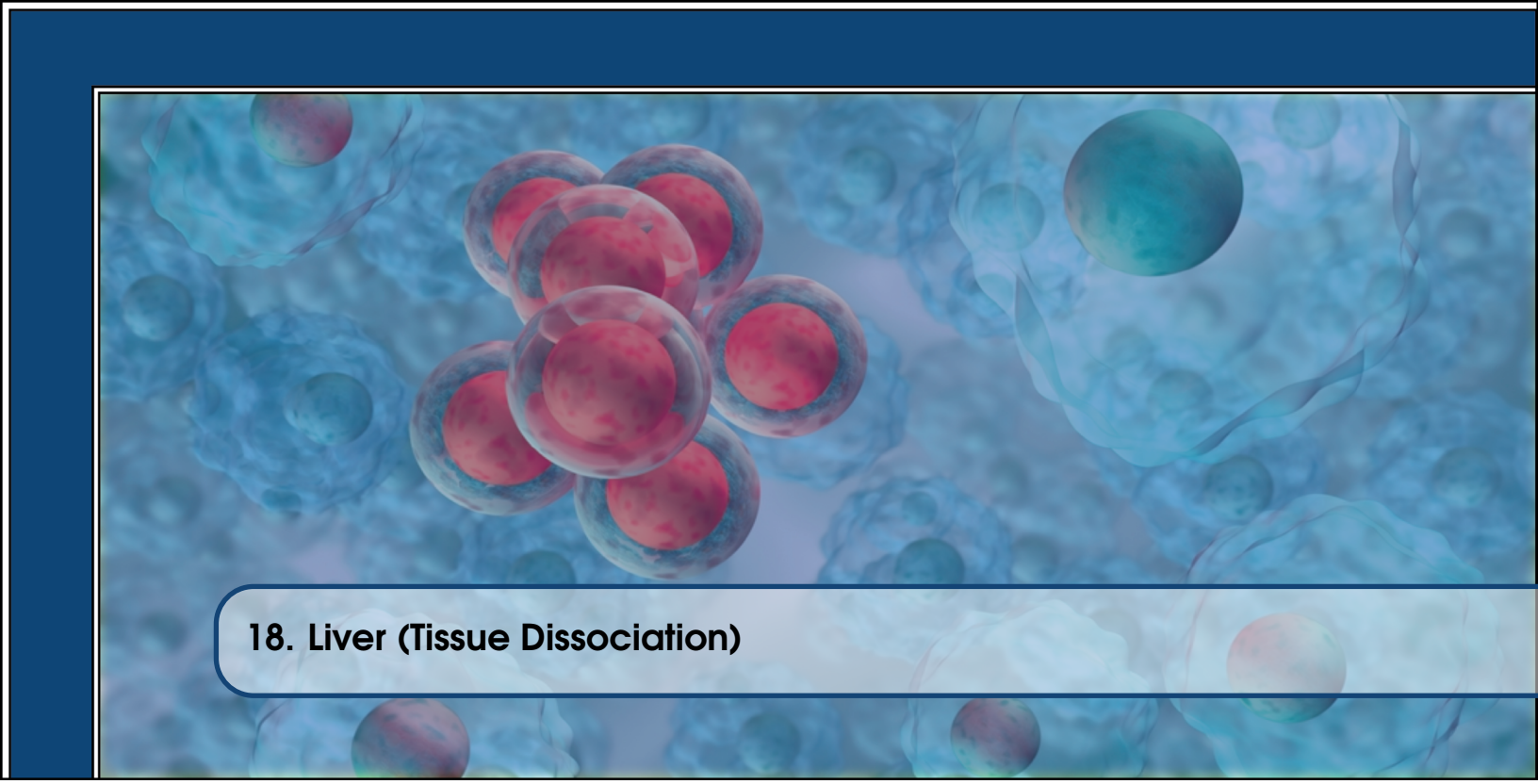

18. Liver (Tissue Dissociation)

Species: Avian

Table 18.1: **Avian**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Else	Hepatocytes	Collagenase Type 1: 0.1%	See Reference
2	Lee J.	Hepatocytes	Collagenase: 0.05% Hyaluronidase: 0.05%	DMEM/ F12

Species: Canine

Table 18.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Reese	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate
4	Vickrey	Hepatocytes	Trypsin: 0.1%	HBSS, CMF

Species: Chicken

Table 18.3: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
5	Rinaldini	Various tissues (heart, liver, skeletal, cardiac)	Trypsin: various grades	CMF Tyrode's solution
6	Steinberg	Heart Liver	Trypsin: 3.0%	Tyrode's solution, CMF
7	Roseman	Hepatocytes	Collagenase Type 4: 6000 units	Medium A
8	Fraslin	Hepatocytes	Collagenase: 0.02%	HEPES, CF
9	Tarlow	Hepatocytes	Deoxyribonuclease I: 0.00125%	PBS

Species: Equine

Table 18.4: **Equine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
10	Bakala A.	Hepatocytes	Collagenase Type 4: 0.1%	HBSS

Species: Fish

Table 18.5: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
11	Blair	Hepatocytes	Collagenase Type 2: 0.045%	Kreb's-Ringer bicarbonate buffer, CF
12	Klaunig	Hepatocytes	Collagenase: 100 u/ml	HBSS/CMF
13	Klaunig	Hepatocytes	Collagenase: 100u/ml	HBSS
14	Bailey	Hepatocytes	Hyaluronidase: 0.08%	See reference
15	Lipsky	Hepatocytes	Collagenase Type 2: 0.045%	HBSS with 0.05M HEPES

Species: Guinea-Pig

Table 18.6: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
16	Arinze	Hepatocytes	Collagenase Type 2:	Kreb's Ringer bicarbonate buffer
3	Reese	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate

Species: Human

Table 18.7: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
17	Torok	Hepatocytes	Collagenase Type 1: 0.05%	Williams E
18	Wauthier	Hepatic stem cells and hepto-blasts	Collagenase Type 4: 0.014-0.06%	various
19	Pichard L	Hepatocytes	Collagenase: 0.05%	HEPES
20	Hughes	Hepatocytes	Collagenase: 0.05%	EBSS
21	Hussain	Hepatic side population	Collagenase: 0.02-0.05%	HBSS
22	Duanmu Z.	Hepatocytes	Collagenase Type 4: 0.05%	Williams E
23	Donato M	Hepatocytes	Collagenase: 0.05%	Williams E
24	Parzefall	Hepatocytes	Collagenase Type 1: 0.025%	Williams E
25	Gomez-Lechon	Hepatocytes	Collagenase: 0.05%	HEPES buffer See Reference
26	Kaighn	Hepatocytes	Trypsin: 0.1%	HBSS, CMF
27	Gugen-Guillouzo	Hepatocytes	Collagenase: 0.05%	HEPES
28	LeBot	Hepatocytes	Collagenase: 0.05% & 0.025%	HEPES See Reference
3	Reese	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate
29	Cho JJ	Hepatocytes	Collagenase: 0.6%	RPMI 1640
30	Vatakis	Liver Hematopoietic	Collagenase Type 4: 0.1% Hyaluronidase: 0.1% Deoxyribonuclease I: 2 u/ml	RPMI
31	Malhi	Epithelial progenitor	Collagenase: 0.03%	DMEM
32	Begue	Hepatocytes	Collagenase: 0.05%	HEPES buffer
33	Dandri	Hepatocytes	Collagenase Type 1: 0.05%	Leffert's buffer

Species: Monkey

Table 18.8: **Monkey**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
34	Ulrich	Hepatocytes	Trypsin: 160 u/ml	HEPES buffer
35	Weber	Hepatocytes	Collagenase Type 1: 129 u/ml	DMEM/F12

Species: Mouse

Table 18.9: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
36	Guenthner	Hepatocytes	Collagenase Type 3: 100 u/ml Collagenase Type 4: 100 u/ml	DMEM
37	Sin	hepatocytes	Collagenase Type 4: 100 u/ml	DMEM
38	Brundert	Hepatocytes & non-parenchymal liver cells	Collagenase: 0.03-0.05%	DMEM
39	Kang	Hepatocytes	HIS kit: with modifications	DMEM
40	Rountree	CD133+ stem	Collagenase Type 4: 0.05% Pronase: 0.05% Deoxyribonuclease I: 0.01%	DMEM/F12
41	Holl	Hepatacytes	Collagenase Type 1: 0.05%	Williams E
42	Bosschaerts	Liver non-parenchymal	Collagenase Type 3: 100 u/ml	HBSS
43	Li Wen-Lin	Liver epithelial progenitor cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.05%	DMEM
44	Jiang Guo-qiang	Hepatocytes	Collagenase Type 1: 0.033%	Leffert's buffer
45	Benten Daniel	Liver sinusodial endothelial	Collagenase: 0.03%	DMEM
46	Sazani P.	Hepatocytes	Collagenase Type 1: 0.053%	DMEM/ F-12
23	Donato M	Hepatocytes	Collagenase: 0.05%	Williams E
47	Ling W	Nonparenchymal liver	Collagenase Type 1: 0.05%	Hanks
48	Beldi	Liver sinusoidal endothelial cells	Collagenase Type 1: 0.05% Neutral Protease: 0.025%	HEPES
49	Crisp	Parenchymal and non-parenchymal	Hyaluronidase: 0.1%	Hank's w/ Insulin, CMF
50	Angele MK	Kupffer cells	Collagenase Type 4: 0.05%	HBSS
51	Follenzi	Liver endothelial	Collagenase: 0.03%	DMEM
52	Kotton	Liver derived stem cells	Collagenase Type 1: 0.1% Neutral Protease: 2.4 u/ml	HBSS
53	Lillehaug	Epithelial	Collagenase: 0.10%	DMEM
54	Oliva	Hepatocytes	Collagenase Type 1: 100 u/ml Elastase: 0.1 u/ml	Williams E
55	Chung	Hepatocytes	Collagenase Type 1: 0.03%	Williams E
56	Lingohr Melissa K	Hepatocytes	Collagenase Type 2: 100 u/ml	HBSS
57	Hatano E	Hepatocytes	Collagenase Type 1: 0.04%	Waymouth's medium
58	Mathijs	Hepatocytes	Collagenase Type 4: 0.05%	HBSS

Species: Porcine

Table 18.10: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
59	Zhou X	Hepatocytes	Collagenase: 0.05% Neutral Protease:	MEM
60	Li	Hepatocytes	Collagenase Type 4: 125 u/ml	Williams E
61	Raman Priya	Hepatocytes	Collagenase: 0.05%	DMEM
62	Gerlach	Hepatocytes	Collagenase: 0.8%	PBS
63	Meng	Hepatocytes	Collagenase Type 4: 0.05% Neutral Protease: 0.84% Deoxyribonuclease I: See Reference	Williams E

64	Wang Y.	Hepatocytes	Collagenase Type 4: 0.05%	RPMI 1640
65	Terner	Hepatocytes	Collagenase Type 1: 0.07%	Williams E

Species: Rabbit

Table 18.11: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Reese	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate

Species: Rat

Table 18.12: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
66	Budick-Harmelin	Hepatocytes	Collagenase Type 2: 0.05%	DMEM
67	Gopalakrishnan	Hepatocytes and SEC's	Collagenase Type 1: 0.1-0.2%	RPMI
45	Benten Daniel	Liver sinusodial endothelial	Collagenase: 0.03%	DMEM
68	Alston-Smith	Hepatocytes	Collagenase: 0.04% - 0.06%	PBS
69	Matsumoto	Hepatocytes	Collagenase: 0.05%	DMEM
70	VanBezodijen	Parenchymal	Collagenase Type 1: 0.05% - 0.06%	HEPES buffer
71	Gravela	Hepatocytes	Collagenase: 0.05%	Hank's solution, CF
72	Haung	Hepatocytes	Hyaluronidase: 0.10%	HBSS, CF
73	Mula	Hepatocytes	Collagenase Type 1: 0.067%	HBSS
74	Chang	Hepatocytes	Collagenase Type 2: 166 u/ml	DMEM
75	Shen	Hepatocytes	Collagenase Type 2: 1000 U	William's
76	Wen	Portal fibroblasts	Collagenase Type 2: 0.3%	DMEM/F-12
77	Jensen C.	Hepatocytes	Collagenase Type 1: 0.1% Pronase: 1% Deoxyribonuclease I: 0.007%	Williams E
78	Braun	Hepatocytes and Nonparenchymal	Pronase: 0.1%	HEPES buffer with calcium
79	Gerschenson	Hepatocytes	Hyaluronidase: 0.10%	HBSS
80	Nagy	Hepatocytes	Collagenase: 0.05%	Ringer's biocarbonate buffer
81	Williams	Epithelial	Hyaluronidase: 0.0075%	KCl-NaCl HEPES Buffer
82	Herring	Epithelial	Trypsin: 0.05%	HBSS CMF
83	Francavilla	Hepatocytes	Collagenase Type 1: 125 - 250 u/ml	MEM See Reference
84	Laishes	Hepatocytes	Collagenase: 0.05%-0.10%	Williams E
85	Blouin	Epithelial	Collagenase: 0.05% Neutral Protease: 0.48% Hyaluronidase: 0.075%	Eagles's MEM
86	Mathis	Bile ductular epithelial	Collagenase Type 1: 220 u/ml	L-15
87	Leffert	Hepatocytes	Collagenase: 0.3%	Modified Eagle's w/ Serum
88	Williams	Epithelial-like	Trypsin: 0.25%	PBS
89	Williams	Hepatocytes	Collagenase Type 1: 100 u/ml	HBSS See Reference
90	Isabel Zvibel	Hepatocytes	Collagenase Type 1: 0.22%	HBSS
91	Chan	Hepatocytes	Collagenase Type 1: 0.5%	RPMI 1640
92	Smith	Hepatocytes	Collagenase Type 2: 0.05%	Williams E
93	Matsuura T	Hepatocytes	Collagenase: 60 u/ml	HEPES
94	Sosef MN	Hepatocytes	Collagenase: See References	DMEM
95	Holstege A	Hepatocytes, Kupffer, endothelial	Collagenase: 0.05%	Gey's BSS
96	Bettinger	Hepatocytes	Collagenase Type 2: 0.1%	DPBS
97	Low-Baselli A.	Hepatocytes	Collagenase Type 4: 0.02%	Williams E
98	Bausher	Hepatocytes	Trypsin: 0.25%	PBS, CMF

99	Handy	Hepatic stellate cells	Pronase: 0.02% Collagenase: See Reference	Medium 199
100	Chung	Hepatocytes	Collagenase: 0.05%	HBSS
3	Reese	Hepatocytes	Collagenase: 90 u/ml	CF EGTA perfusate
101	Charbonneau	Hepatocytes	Collagenase Type 1: 0.1%	See Reference
102	Pillai	Hepatocytes	HIS kit: per instructions	Waymouth's MB
103	Parasrampuria	Hepatocytes	HIS kit: per instructions	Krebs-Henseleit
104	Perepelyuk	Hepatocytes and sinusoidal endothelial	Collagenase Type 4: 0.05% Deoxyribonuclease I: 0.003%	HBSS
105	Li	Portal Fibroblasts	Collagenase: 0.03% Pronase: 0.033% Hyaluronidase: 0.036%	DMEM/F-12
106	Acosta	Liver	Collagenase: 0.05%	HBSS modified See Reference
107	Davila	Parenchymal hepatocytes	Collagenase Type 4: 80 u/ml	HBSS
108	Berry	Parenchymal	Hyaluronidase: 0.10% Collagenase Type 1: 0.05%	HBSS, CF
109	Bissell	Parenchymal	Collagenase Type 1: 0.05%	Hank's solution, CF
110	Howard	Parenchymal	Hyaluronidase: 0.10%	HBSS, CF
111	Bonney	Parenchymal	Hyaluronidase: 0.1%	Hank's solution, CMF
112	Davis	Hepatocytes	Collagenase Type 1: 0.065%	DMEM See Reference
113	Schwarz	Hepatocytes	Collagenase: 0.5%	Krebs Ringer bicarbonate buffer
114	Johnson	Hepatocytes	Hyaluronidase: 0.08%	HBSS, CF
115	Burczynski	Hepatocytes	Collagenase Type 2: 0.025%	Williams E
116	Oka	Hepatocytes	Collagenase: 100 - 200 μg/g body weight	Eagle's Eagle's w/HEPES HBSS
117	Reddy	Hepatocytes	Collagenase:	Kreb's Ringer bicarbonate buffer
118	Gupta	Hepatocytes	Collagenase: 0.05%	MEM
119	Rana	Hepatocytes	Collagenase: 0.05%	Medium 199
120	Rubin	Hepatocytes	Collagenase Type 1: 100 u/ml	Buffers 1 & 2 See Reference
61	Raman Priya	Hepatocytes	Collagenase: 0.05%	DMEM
121	Kreamer	Hepatocytes	Collagenase: 0.05%	HBSS
122	Witters	Hepatocytes	Collagenase Type 1: 0.05%	MEM
123	Voss	Hepatocytes	Collagenase: 0.05%	HEPES
124	Kuddus	Hepatocytes	Collagenase Type 1: 0.1%	HBSS
125	Doleh	Hepatocytes	Collagenase Type 2: 0.1%	HEPES
126	Yamada	Parenchymal	Collagenase Type 2: 0.05%	Kreb's Henseleit bicarbonate buffer
127	Dixit	Hepatocytes	Collagenase Type 4: 200 u/ml	RPMI 1640
128	Liu	Hepatocytes	Collagenase Type 2: 0.05%	HBSS, CMF
129	Brass	Hepatocytes	Collagenase: 0.04%	Bicarbonate buffer with calcium added
130	Friedman	Lipocytes Kupffer Sinusoidal endothelial	Collagenase: 0.015%	DMEM/Ham's F-12
131	Rodriguez de Turco	Hepatocytes	Collagenase Type 2:	HBSS
132	Gabriel	Stellate	Protease: 0.02%	HBSS
133	Studer	Hepatocytes	Collagenase: 100 u/ml	Krebs Henseleit bicarbonate buffer
134	Davila	Hepatocytes	Collagenase Type 4: 0.05%	Hanks' BSS, CF
64	Wang Y.	Hepatocytes	Collagenase Type 4: 0.05%	RPMI 1640
135	Malan-Shibley	Epithelial	Trypsin: 0.05%	HBSS, CMF
136	Berg	Hepatocytes	Hyaluronidase: 0.10%	HBSS, CF
137	Putz G	Hepatocytes	Collagenase Type 2: 0.05%	Williams E
138	Annaert	Hepatocytes	Collagenase Type 1: 200 u/ml	DMEM
139	Li	Hepatocytes	Collagenase Type 2: 0.033%	Williams E
140	Liu	Hepatocytes	Collagenase Type 2: 0.05%	Serum-free medium
141	Okumura	Hepatocytes	Trypsin: 0.005%	Williams E

142	Zvibel	Hepatic stellate cells	Collagenase Type 1: 0.025-0.1% Pronase: 0.025-0.13%	DMEM
143	Cotariu	Hepatocytes	Collagenase: 0.05%	HBSS
144	Gugen-Guillouzo	Hepatocytes	Collagenase: 0.025%	HEPES buffer
145	Wolz E	Hepatocytes	Collagenase Type 2: 120 u/ml	HBSS
146	De Gerlache	Hepatocytes	Hyaluronidase: 0.02%	Kreb's buffer
147	Cai	Parenchymal Kupffer	Collagenase Type 2: 0.05%	HBSS with $CaCl_2$
148	Kindberg	Parenchymal	Collagenase Type 1:	HEPES, modified
149	Poli	Hepatocytes	Hyaluronidase: 460 u/ml	Saline
150	McAbee DD and Weigel PH	Hepatocytes	Collagenase Type 1: 0.05%	HEPES
151	Seglen	Hepatocytes	Collagenase: 0.01 - 0.08%	HEPES
152	Nagelkenke	Endothelial Kupffer Parenchymal	Collagenase: 0.05%	HBSS
153	Iype	Hepatocytes	Hyaluronidase: 1.0%	HBSS, CMF
154	Kuiper	Parenchymal Endothelial Kupffer	Collagenase Type 1: 0.05%	Krebs Henseleit
155	Goldstein	Hepatocytes	Collagenase Type 2: 0.30%	Dulbecco-Vogt arginine free Eagle's
156	Weigel	Hepatocytes	Collagenase Type 3 & 4:	HEPES

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19. Lung (Tissue Dissociation)

Species: Bovine

Table 19.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Del Vecchio	Pulmonary microvessel endothelial	Collagenase Type 2: 1000 u/ml	PBS

Species: Guinea-Pig

Table 19.2: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Phillips	Single cells	Trypsin: 0.25%	CF salt solution
3	Sikpi	Alveolar type II	Elastase: 40 u/ml	PBS See Reference

Species: Human

Table 19.3: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Barkauskas	Alveolar epithelial	Neutral Protease: 2 u/ml Trypsin: 0.25% Elastase: 10 u/ml	Bronchial Epithelial Growth Medium
5	Comhair	Lung endothelial	Collagenase Type 2: 0.2%	PBS
6	Fujino	Lung	Neutral Protease: 2 u/ml Collagenase/Dispase: 0.1% Deoxyribonuclease I: 0.01%	DMEM
7	Zhu	Lung Fibroblasts	Trypsin: 0.05%	DMEM
8	Lechner	Epithelial	Trypsin: 0.02%	Medium 199
9	Hinz	Lung	Collagenase: 0.01%	HBSS
10	Kan	Fibroblasts	Trypsin: 0.01%	Eagle's MEM
11	Liley	Alveolar type II Fetal	Trypsin: 50 ug/ml	Ham's F-12, Eagle's MEM

Species: Mouse

Table 19.4: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
12	Mohapatra	Alveolar	Collagenase Type 1: 300 u/ml Elastase: 4 u/ml Neutral Protease: 5 u/ml Deoxyribonuclease I: 200 u/ml	HBSS
13	Kim	Pulmonary	Collagenase Type 4: 0.16% Deoxyribonuclease I: 0.1%	See Reference
14	Guimond	Lung	Collagenase Type 4: 0.3%	PBS
15	Vaughan	Lung tumor	Neutral Protease: 50 u/ml Collagenase: 400 u/ml Deoxyribonuclease I: 50 u/ml	DMEM
16	Hardy	Lung	Collagenase Type 3: 0.1% Deoxyribonuclease I: 0.0025%	RPMI 1640
17	Rayamajhi	Lung draining	Collagenase Type 4: 0.1-0.125%	HBSS
18	Rock	Lung	Collagenase Type 1: 450 u/ml Elastase: 4 u/ml Neutral Protease: 5 u/ml Deoxyribonuclease I: 0.33 u/ml	DMEM/F12
19	Lancelin	Dendritic	Collagenase Type 1: 0.05% Deoxyribonuclease I: 0.002%	HBSS
20	Ferreira	Lung	Collagenase Type 2: 300 u/ml Deoxyribonuclease I: 0.015%	RPMI 1640
21	Flano	Dendritic	Collagenase Type 1: 0.5%	HBSS
22	Zhao	Lung	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.01%	HBSS
23	Jones	Lung	Collagenase Type 2: 0.2%	PBS
24	Finotto	Lung	Collagenase Type 2: 300 u/ml Deoxyribonuclease I: 0.001%	Dulbecco's PBS
25	Huaux F	Pulmonary T lymphocytes	Collagenase Type 3: 10 mg/lung Deoxyribonuclease I: 250 ug/lung	RPMI medium
26	Paine R 3rd	Alveolar epithelial cells	Neutral Protease: Deoxyribonuclease I: 0.01%	DMEM
27	Freedman SD	Lung	Collagenase: 100 u/ml Deoxyribonuclease I: 200 u/ml	Krebs-Henseleit Buffer
28	Ebeling	Lung	Collagenase Type 3: 0.17%	HBSS
29	Andonegui G.	Murine pulmonary endothelial	Collagenase Type 2: 0.25%	HBSS
30	Abonia	Mononuclear Cells	Collagenase Type 4: 0.1%	RPMI 1640
31	Jungblut	Leukocytes and Endothelial	Collagenase: 0.2% Deoxyribonuclease I: 40-80 u/ml	PBS
32	Vermaelen KY	Lung and lymph node cells	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.002%	RPMI 1640
33	Stampfli MR	Lung cells	Collagenase Type 3: 150 u/ml	HBSS
34	Chow	Lung mesenchymal stem	Collagenase Type 2: 0.2%	HBSS
35	Driscoll	Lung progenitor	Collagenase/Dispase: 0.2% Neutral Protease: 5 u/ml Deoxyribonuclease I: 0.0025%	DMEM
36	Woolard MD	Mononuclear cells	Collagenase Type 1: 300 u/ml Deoxyribonuclease I: 50 u/ml	RPMI 1640 medium
37	Trotter	Alveolar and fibroblast	Trypsin: 2.5% Deoxyribonuclease I: 0.2% Collagenase Type 1: 1250 u/ml	MEM
38	Hamilton-Easton A	Antigen presenting cells	Collagenase: 150 u/ml Deoxyribonuclease I: 30 u/ml	RPMI 1640
39	Dong QG	Murine endothelial cells	Collagenase Type 1: 1 mg/ml	DMEM
40	Breslow	Lung mononuclear	Collagenase Type 4: 500 u/ml Deoxyribonuclease I: 0.002%	HBSS

Species: Porcine

Table 19.5: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
9	Hinz	Lung	Collagenase: 0.01%	HBSS

Species: Rabbit

Table 19.6: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
41	Finkelstein	Alveolar type II	Trypsin: 0.0025%	Joklik's MEM
42	Devereux	Clara cells	Protease: 0.1%	HEPES
43	Scott	Alveolar type II	Trypsin: 0.05%	HBSS
44	Gould	Lung	Pronase: 0.2%	Kreb's serum substitute solution, CMF

Species: Rat

Table 19.7: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
23	Jones	Lung	Collagenase Type 2: 0.2%	PBS
45	Douglas	Lung	Collagenase: 0.1%	Moscona saline, CMF
46	Fraslon-Vanhulle	Fetal alveolar epithelial type II	Trypsin: 0.1%	DMEM
47	Post	Fibroblasts & type II	Trypsin: 0.05%	HBSS: serum free MEM, CMF
48	Douglas	Alveolar pneumonocytes, type II	Trypsin: 0.1 %	HBSS, CMF
49	Mangum	Alveolar type II pneumocytes	Elastase: 40 u/ml	HEPES buffer See Reference
50	Steinmuller C	Interstitial lung macrophages	Collagenase Type 1: 100 u/ml Deoxyribonuclease I: 50 u/ml	RPMI-1640
51	Planus	Alveolar epithelial	Elastase: 40 u/ml	DMEM
52	Bakhramov	Pulmonary arterial myocytes	Collagenase: 0.15% Papain: 0.15% Elastase: 0.05%	PBS
53	Liebler	Alveolar type I & II	Elastase: 2.5-8 u/ml Collagenase Type 1: 1.0%	DMEM/F12
54	Sunil VR	Type II alveolar epithelial cells	Elastase: 4.2 u/ml Deoxyribonuclease I: 0.0001%	DMEM
55	Chen J.	Alveolar epithelial	Elastase: 3-4.5 u/ml	RPMI 1640
56	King J	Pulmonary endothelial cells	Collagenase Type 2: 1000 u/ml	DMEM/F-12
57	Berk	Interstitial	Trypsin: 1.125%	HEPES buffer
58	Weller	Alveolar type I	Trypsin: 0.05%	DMEM
59	Kikkawa	Alveolar type II	Trypsin: 1.0%	Joklik's medium
60	Kim	Alveolar epithelial	Elastase: 2 u/ml	EBSS
61	Brown	Pneumocytes type II	Trypsin: 0.30%	BSS
62	Goodman	Alveolar type II	Elastase: 4 u/ml	Auto-Pow Eagle's modified MEM
63	Dobbs	Alveolar type II	Elastase: 4.3 u/ml	HEPES See Reference
64	Ma	Alveolar type II	Elastase: 40 u/ml	Phosphate-buffered medium See Reference
65	Kemp	Alveolar	Elastase: 2.0 - 2.5 u/ml	DMEM/Ham's F-12 See Reference
66	Mason	Alveolar type II	Trypsin: 0.30%	BSS
67	Jassal	Epithelial	Trypsin: 0.1%	HBSS
68	Batenburg	Alveolar type II	Trypsin: 0.1%	RPMI 1640
69	Fraslon	Alveolar epithelial type II	Trypsin: 1%	Eagle's MEM
70	King	Alveolar type II	Trypsin: 0.50%	Earle's MEM

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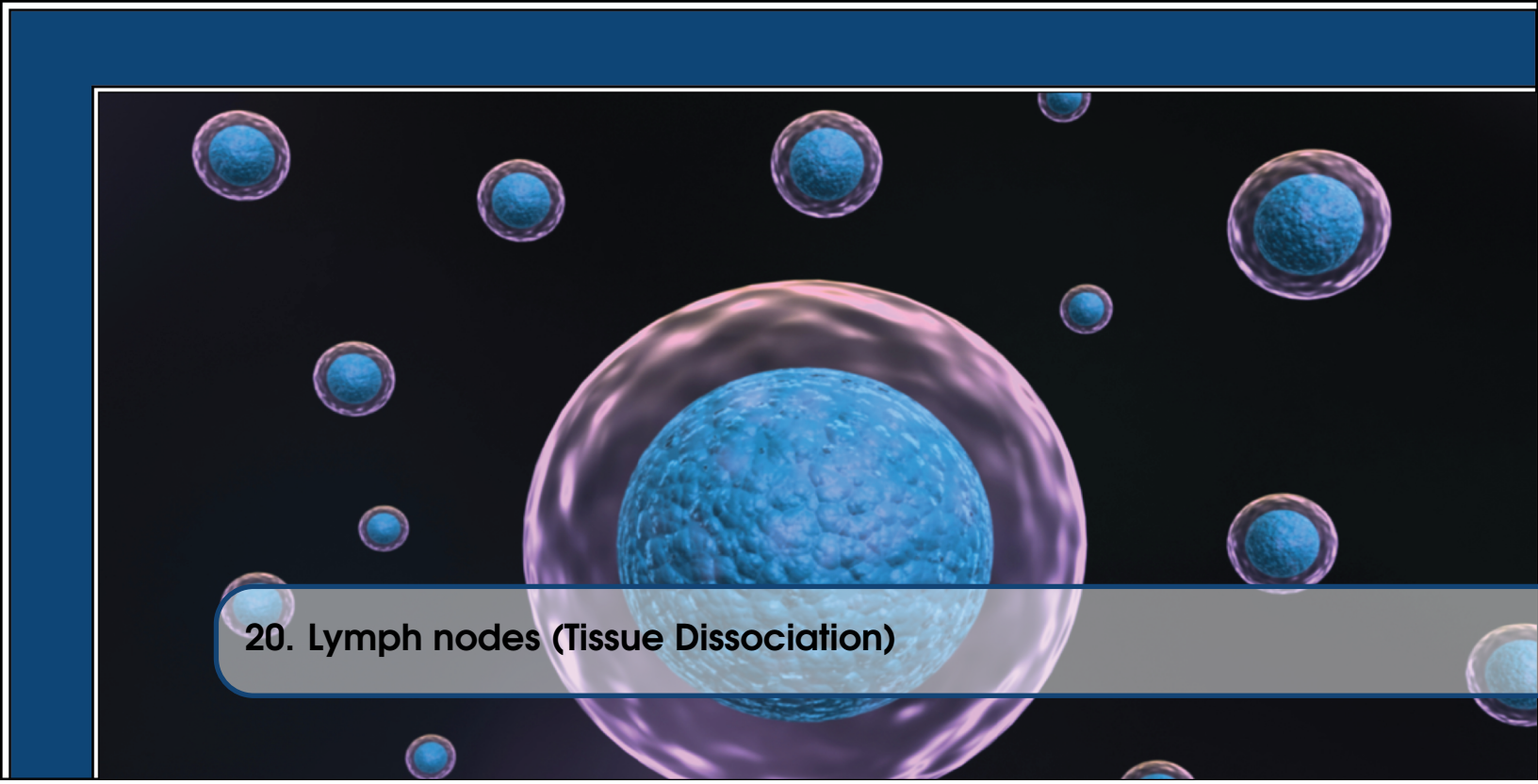

20. Lymph nodes (Tissue Dissociation)

Species: Mouse

Table 20.1: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Rayamajhi	Lung draining	Collagenase Type 4: 0.1-0.125%	HBSS
2	Kapasi ZF	Follicular dentritic	Collagenase Type 4: 0.25% Deoxyribonuclease I: 0.5%	HBSS

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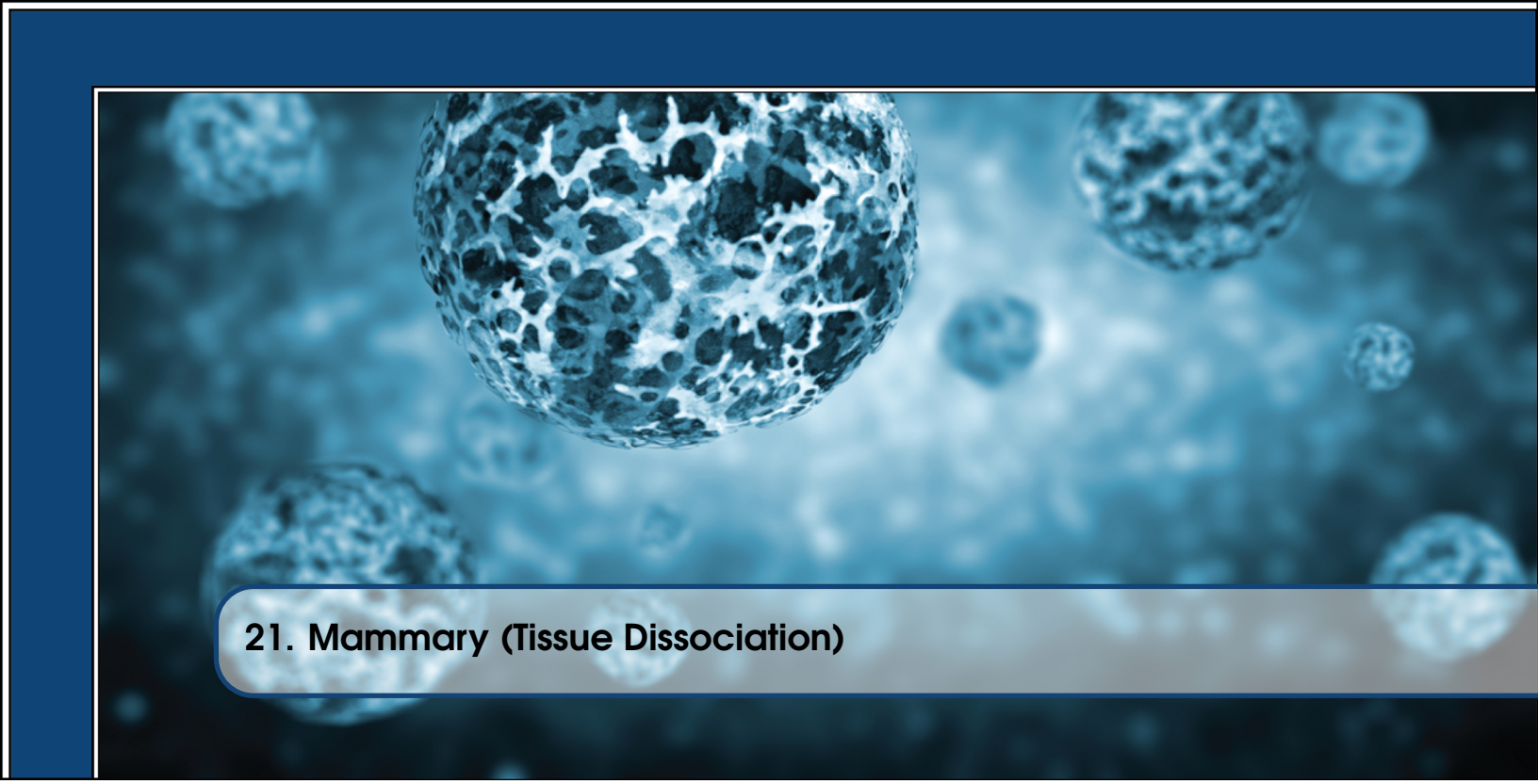

21. Mammary (Tissue Dissociation)

Species: Bovine

Table 21.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Gibson	Epithelial	Hyaluronidase: 0.005%	HBSS
2	Baumrucker	Epithelial	Deoxyribonuclease I: 0.04%	HBSS/Medium 199
3	Schingoethe	Secretory Mammary gland	Collagenase: 0.02 - 0.03%	HBSS or EBSS
4	Weber M.	Mammary epithelial	Collagenase Type 2: 1% Hyaluronidase: 1% Deoxyribonuclease I: 0.03%	M-199
5	Miranda	Mammary epithelial	Collagenase Type 3: 400 u/ml Hyaluronidase: 100 u/ml Deoxyribonuclease I: 2 u/ml	HBSS
6	Anderson	Mammary	Collagenase: 0.30 %	HBSS or EBSS

Species: Goat

Table 21.2: **Goat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Blanco	Mammary gland	Collagenase: 0.02 - 0.03%	HBSS or EBSS

Species: Guinea-Pig

Table 21.3: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Turba	Mammary gland	Trypsin NF 1:250: 0.25%	Dulbecco phosphate

Species: Human

Table 21.4: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
9	Labarge	Mammary Epithelial	Collagenase: 200 u/ml Hyaluronidase: 100 u/ml	DMEM/F-12
10	Huss	Mammary epithelial cells	Collagenase Type 1: 0.1%	DMEM
11	Ogmundsdottir	Epithelial Fibroblasts	Collagenase Type 1: 450 IU/ml	DMEM/Ham's F-12
12	Ronnov-Jessen	Myofibroblasts	Collagenase: 900 IU/ml	DME - F12
13	Emerman	Epithelial	Collagenase: 2.0%	DMEM/Ham's F-12
14	Leung	Tumor, breast	Neuraminidase: 0.8 u/ml	HBSS
15	Stampfer	Epithelial	Hyaluronidase: 100 u/ml	DMEM/Ham's F-12
16	Berthon	Epithelial	Hyaluronidase: 150 IU/ml	DMEM
17	Ronnov-Jessen L.	Fibroblasts	Collagenase Type 3: 900 u/ml	DMEM/F-12

Species: Mouse

Table 21.5: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
18	Liu	Mammary tumor	Collagenase: 0.15% Hyaluronidase: 0.020%	DMEM/F12
19	Boulanger CA	Mammary epithelial stem	Collagenase Type 3: 0.1% Hyaluronidase: 0.1% Pronase: 1.25% Deoxyribonuclease I: 0.2%	DMEM
20	Ceriani	Mammary	Hyaluronidase: 0.1%	CMF medium
21	Bandyopadhyay	Epithelial	Pronase: 0.01%	Hepes buffered medium 199
22	White	Epithelial Mammary gland	Collagenase Type 3: 0.1%	HBSS
23	Yang	Mammary tumors Epithelial	Collagenase: 1.0%	HBSS
24	Taketani	Epithelial	Deoxyribonuclease I: 0.0001%	Medium 199
25	Ehmann	Epithelial	Collagenase Type 3: 0.1%	DMEM
26	Riser	Epithelial	Pepsin: 0.1% and 0.05%	HBSS with 0.2% EDTA, CMF
27	Emerman	Epithelial	Collagenase Type 3: 0.12%	HBSS
28	Cheng	Fibroblasts, carcinoma	Collagenase Type 4: 0.5% Trypsin: 0.2% Deoxyribonuclease I: 0.004 Hyaluronidase: 1,000 u/ml	DMEM
29	Mueller	Epithelial	Collagenase Type 3: 25 u/ml Hyaluronidase: 0.1% Protease XIV: 0.05% Deoxyribonuclease I: 0.04%	DMEM/F12
30	Taddei Ilaria	Mammary epithelial	Collagenase: 0.3% Hyaluronidase: 100 u/ml Trypsin: 0.25% Neutral Protease: 0.5% Deoxyribonuclease I: 0.01%	See Reference
31	Lasfargues	Epithelial Mammary	Collagenase: 0.02%	Simm's
32	Kanazawa	Epithelium	Collagenase: 250 u/ml	HBSS
33	Ehmann	Epithelial	Collagenase Type 3: 0.1%	DMEM
34	Beck	Adipocytes	Trypsin: 50 μg/ml	DMEM
35	Asch	Epithelial	Collagenase Type 2: 0.2%	HBSS/DMEM
36	Asch	Swiss 3T3	Collagenase Type 3: 0.2%	PBS CMF
37	DeOme	Nodule-transformed	Hyaluronidase: 0.1%	Medium 199
38	Prop	Mammary	Hyaluronidase: 0.1%	BSS, CMF
39	Jones	Epithelial	Deoxyribonuclease I: 0.1%	DMEM

40	Moore	Epithelial	Collagenase: 0.1%	Eagle's MEM
41	Kerkof	Parenchymal	Collagenase Type 1: 0.3%	Kreb's Ringer bicarbonate buffer
42	Kopelovich	Mammary	Trypsin NF 1:250: 0.25%	HBSS
43	Pitelka	Parenchymal	Collagenase: 0.33%	Kreb's buffer
44	Yang J	Mammary	See Reference:	HBSS
45	Daniel	Mammary	Collagenase: 0.05% - 0.1%	HBSS

Species: Rat

Table 21.6: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
46	Mei	Mammary epithelial	Collagenase Type 3: 0.35%	HBSS
47	Soriano	Epithelial	Collagenase Type 1: 0.4%	DMEM
48	Hahm	Epithelial	Neutral Protease: 0.2%	EBSS
49	Djuric Z.	Mammary gland epithelial	Collagenase Type 3: 0.35%	See Reference
50	Maffini M.	Mammary epithelial	Collagenase Type 3: 0.15%	DMEM/F12
51	McGrath	Epithelial	Hyaluronidase: 0.1%	Medium 199
52	Richards	Epithelial	Collagenase Type 3: 0.1%	Medium 199
53	Ethler	Epithelial	Collagenase: 0.1%	Medium 199
54	Lin	Epithelial Mammary	Collagenase: 0.05%	Medium 199
55	Brake P.	Mammary fibroblasts	Collagenase Type 3: 0.2% Neutral Protease: 0.2% Deoxyribonuclease I: 0.01%	DMEM/F-12
56	Martin	Mammary	Collagenase: 0.2%	Kreb's Ringer bicarbonate buffer
57	Varela L.	Mammary epithelial	Collagenase Type 3: 0.2% Neutral Protease: 0.2%	DMEM/F12
58	Cohen	Epithelial, cancer and tumor	Collagenase: 0.1%	Eagles's MEM
59	Ehmann	Epithelial	Neutral Protease: 3 u/ml	Medium 199
60	Laduca	Epithelial	Collagenase Type 3: 0.5%	EBSS
61	Moon	Mammary	Collagenase: 0.35%	Medium 199
62	Katz	Acini	Collagenase: 0.05%	HBSS

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22. Miscellaneous (Tissue Dissociation)

Species: Equine

Table 22.1: **Equine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Nemoto	Tendon Cells	Collagenase Type 1: 0.1%	DMEM

Species: Human

Table 22.2: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Smoot	Gastric epithelial cells	Collagenase Type 2: 200 u/ml Neutral Protease: 1.2 u/ml Soybean Trypsin Inhibitor: 0.125%	L-15
3	Bonnamain	Dental pulp stem	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM/F12
4	Ozdener	Fungiform taste papillae	Collagenase Type 1: 550 u/ml Elastase: 10 u/ml Soybean Trypsin Inhibitor: 0.09%	See Reference
5	Panetta	Adipose derived stromal	Collagenase Type 2: 0.075%	DMEM
6	Patino Will-marD	Carotid artery plaque macrophage	Collagenase Type 4: 450 u/ml Deoxyribonuclease I: 500 u/ml Soybean Trypsin Inhibitor: 0.1%	HBSS
7	Rafiee	Esophageal microvascular endothelial	Collagenase Type 2: 0.2%	MCDB-131
8	Chen V	Synoviocytes	Collagenase: 0.2%	DMEM/F12
9	Lanas. A.	Peptic cells	Collagenase Type 4: 0.1% Soybean Trypsin Inhibitor: 0.2%	Ringer solution
10	Stern MH	Periapical granuloma	CLSPA: 0.25%	RPMI-1640
11	Salmon	Dental pulp stem cells	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM

Species: Insect

Table 22.3: **Insect**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
12	Goodwin	Lepitopteran	Collagenase Type 3: 0.35% Hyaluronidase: 0.01%	Dulbecco PBS

Species: Mouse

Table 22.4: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
13	Stock	Dentritic	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.001%	DME
14	Jan	Cochleaer	Trypsin: 0.125%	DMEM/F-12
15	Shi	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml	PBS
16	Bertoncello	Bone marrow	Collagenase Type 1: 0.15% Neutral Protease: 0.15%	PBS
17	Futami	Synovial mesenchymal	Collagenase: 0.1% Deoxyribonuclease I: 0.005%	DMEM
18	Minamoto Kanji	Tracheal inflammatory cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 50 u/ml Soybean Trypsin Inhibitor: 0.1%	RPMI 1640
19	Smith	Myenteric plexus	Collagenase Type 2: 0.13%	Neurobasal A
20	Staszkiewicz	Ear mesenchymal stem	Collagenase Type 1: 0.2%	DMEM/F12
21	Ji	Salivary Gland and Stomach	Collagenase Type 4: 0.8% Deoxyribonuclease I: 1.0%	RPMI-1640
22	Xu	Bone marrow mesenchymal stem	Collagenase Type 1: 0.25%	RPMI 1640

Species: Porcine

Table 22.5: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
23	Kean	Synoviocytes	Hyaluronidase: 660 u/ml Trypsin: 0.25% Collagenase Type 2: 583 u/ml	DMEM

Species: Rabbit

Table 22.6: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
24	Zhang	Tenocytes and tendon stem cells	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM

Species: Rat

Table 22.7: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
25	Moghaddami	Synovial cells	Collagenase Type 1: 250 u/ml	RPMI 1640
26	Jain	Synovial macrophages	Collagenase Type 1: 250 u/ml	DMEM
27	Silver	Submandibular acinar	Collagenase: 0.05%	PBS
28	Kaneko	Gingival mitochondria	Collagenase Type 1: 0.115-0.130 %	HBSS

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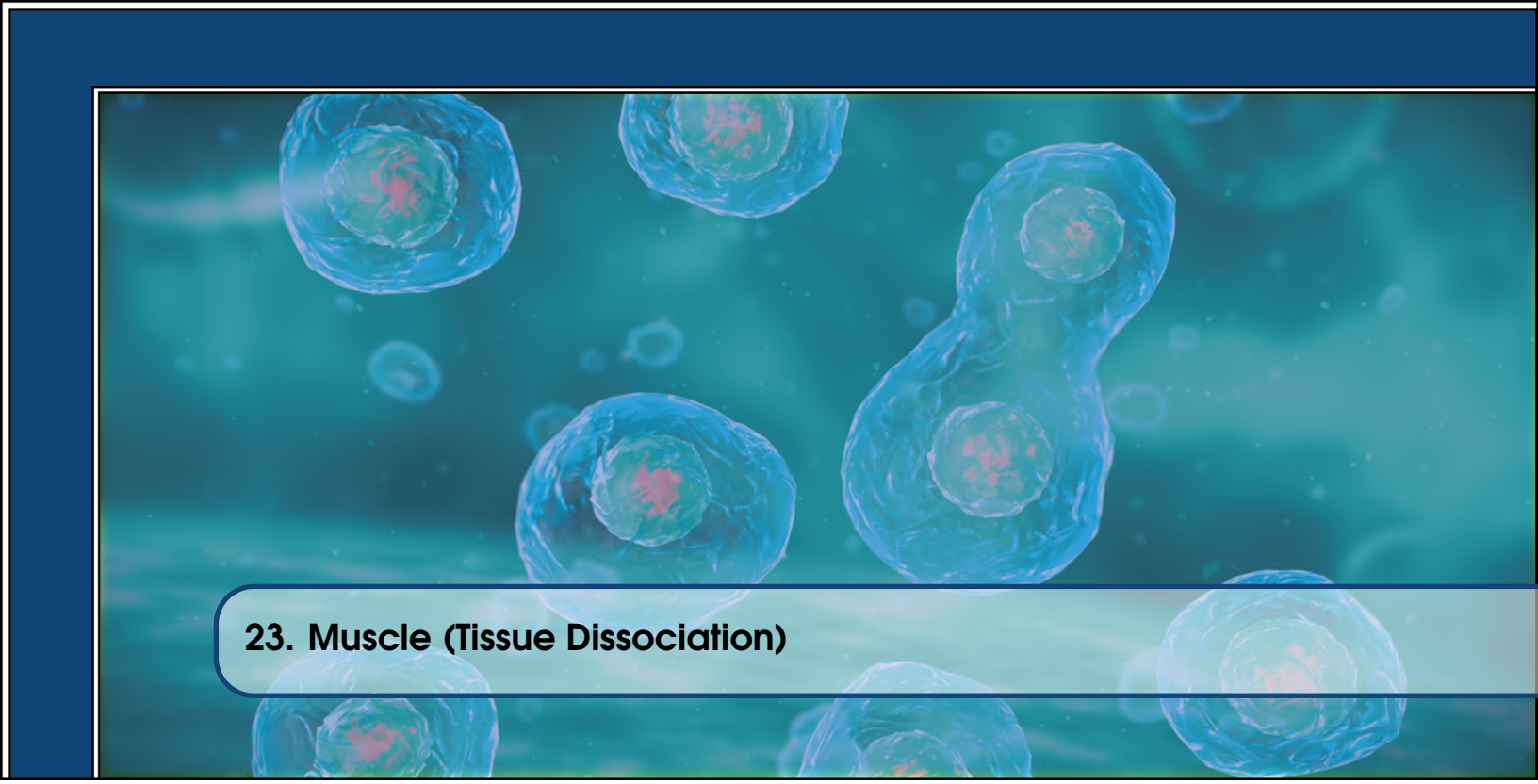

23. Muscle (Tissue Dissociation)

Species: Bovine

Table 23.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Lee	Muscle satellite cells	Collagenase Type 2: 300 u/ml	Krebs-Ringer Bicarbonate
2	Yu	Pulmonary artery endothelial and smooth muscle cells	Collagenase: 0.04-0.05% Soybean Trypsin Inhibitor: 0.04%	RPMI-1640
3	Absher	Smooth muscle	Trypsin: 0.25%	DMEM
4	Warshaw	Vascular smooth muscle	Elastase Type 3: 50 u/ml	PSS
5	Davies	Smooth muscle, fibroblasts	Trypsin: 0.055%	DMEM

Species: Canine

Table 23.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
6	Parker	skeletal muscle cells	Collagenase Type 4: 200 u/ml Neutral Protease: 1 u/ml	DMEM
7	Subramanian	Smooth muscle	Elastase: 50 u/ml	PSS
8	Dobrin	Artery Carotid	Elastase: 80 u/ml	PSS
9	Wilde	Smooth muscle Vascular	Elastase: 34 u/ml	Tyrode's solution w/ calcium

Species: Chicken

Table 23.3: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
10	Chamley-Campbell	Smooth muscle	Trypsin: 0.05% - 0.1%	HBSS
11	Hilfer	Thyroid Muscle Heart	Collagenase: 0.25%	Tyrode's saline, potassium free

12	Rinaldini	Various tissues (heart, liver, skeletal, cardiac)	Trypsin: various grades	CMF Tyrode's solution
13	Hilfer	Muscle	Trypsin: 0.1%	CMF HBSS
14	Bullaro	Muscle	Trypsin: 0.25%	Puck's saline A
15	Tepperman	Muscle	Trypsin: 0.05%	Saline G
16	Dirksen W.	Gizzard and aorta smooth muscle	Collagenase Type 1: 0.15%	HBSS

Species: Feline

Table 23.4: **Feline**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
17	Madden	Cerebral arteries	Elastase: 50 u/ml	Puck's solution
18	Follmer	Myocytes	Collagenase: 0.12%	Kreb's Henseleit, CF

Species: Fish

Table 23.5: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
19	Mitra	Myocytes, heart and stomach	Protease XIV: 0.028%	Solution C See Reference

Species: Frog

Table 23.6: **Frog**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
20	Shepherd	Myocytes	Trypsin: 0.1%	CF Ringer
19	Mitra	Myocytes, heart and stomach	Protease XIV: 0.028%	Solution C See Reference
21	Anderson	Muscle	Trypsin: 0.5%	L15 medium See Reference
22	Stollberg	Muscle	Collagenase: 0.10%	Steinberg's solution

Species: Guinea-Pig

Table 23.7: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
23	Firth	Smooth muscle Gallbladder	Papain: 0.1%	Krebs solution
24	Shieh CC	Bladder smooth muscle	Collagenase Type 2: 0.1-0.2%	Krebs-Ringer bicarbonate
25	Ohya	Smooth muscle Mesenteric artery	Collagenase: 0.3%	CF solution
26	Schnitzler	Capillaries Myocytes	Collagenase Type 2: 0.15%	CF solution
19	Mitra	Myocytes, heart and stomach	Protease XIV: 0.028%	Solution C See Reference
27	Jennings	Smooth muscle Gallbladder	Papain: 0.1%	NaCl, sodium glutamate, MgCl, KCl, glucose, Kreb's, and HEPES
28	Ryder	Myocytes	Protease:	DMEM
29	Ross	Smooth muscle Aortic	Trypsin: 0.05%	Dulbecco-Vogt modification of Eagle's
30	Hu	Smooth muscle	Trypsin: 0.1%	Potassium buffer solution

Species: Hamster

Table 23.8: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
31	Nakamura	Satellite	Trypsin: 0.25%	DMEM

Species: Human

Table 23.9: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
32	Lu	Smooth muscle	Collagenase Type 1: 0.1%	DMEM
33	Nesti	Muscle derived multiprogenitor cells	Collagenase Type 2: 0.05%	DMEM
34	Moss	Endothelial and vascular smooth muscle	Collagenase Type 1: 0.2%	HBSS
35	Kimuli	Urinary tract smooth muscle	Collagenase Type 4: 100 u/ml	DMEM
36	Eskin	Smooth muscle	Trypsin: 0.25%	DMEM
10	Chamley-Campbell	Smooth muscle	Trypsin: 0.05% - 0.1%	HBSS
37	Richardson	Smooth muscle Myometrial	Deoxyribonuclease I: 0.015% and 0.007%	HBSS
38	Casey	Smooth muscle Myometrial	Deoxyribonuclease I: 0.12%	HBSS
5	Davies	Smooth muscle, fibroblasts	Trypsin: 0.055%	DMEM
39	Stadler	Myogenic	Collagenase Type 4: 0.1% Neutral Protease: 2.4 u/ml	HBSS

Species: Lizard

Table 23.10: **Lizard**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
40	Cox	Myoblasts, tail	Collagenase: 0.2%	GM III See Reference

Species: Monkey

Table 23.11: **Monkey**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
41	Chait	Smooth muscle	Trypsin: 0.05%	Dulbecco-Vogt
42	Chamley	Smooth muscle, saphenous vein	Elastase: 0.05%	BSS

Species: Mouse

Table 23.12: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
43	Metz	Vascular Smooth Muscle	Collagenase Type 2: 0.14%	Basal Culture
44	Weisleder	Skeletal muscle fiber	Collagenase Type 1: 0.2%	Tyrode
45	Shi	Myoblast	Collagenase Type 2: 0.2%	DMEM
46	Ieronimakis Nicholas	Myocytes, endothelial	Neutral Protease: 1.2 u/ml Collagenase Type 4: 0.2%	PBS
47	Rezk	Disphragm cells	Collagenase Type 2: 100 u/ml Pronase: 0.125%	PBS
48	Li CX	Intersitial cells of Cajal	Collagenase Type 2: 0.13%	M199
49	Pasut	Myofibers	Collagenase Type 1: 0.2%	DMEM
50	Majka S.	Skeletal muscle progenitor	Collagenase Type 2: 0.2%	DMEM
51	McKinney-Freeman SL	Myocytes	Collagenase Type 2: 0.2% Trypsin: 0.25%	HBSS
52	Winitsky	Precursor cells	Collagenase Type 2: 0.5%	DMEM/F12
53	Johnson	Skeletal muscle myotubes	NCIS kit: per instructions	L-15

54	Fukada S.	Myocytes	Collagenase Type 1: 0.5%	DMEM

Species: Ovine

Table 23.13: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
55	Driska S.	Tracheal smooth muscle cells	Papain: 0.2% Deoxyribonuclease I: 0.1%	MOPS-PSS

Species: Porcine

Table 23.14: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
24	Shieh CC	Bladder smooth muscle	Collagenase Type 2: 0.1-0.2%	Krebs-Ringer bicarbonate
56	Xiong	Smooth muscle, aorta	Collagenase: 0.3%	DMEM
57	Breton	Smooth muscle Aorta	Trypsin: 0.05%	EDTA 0.02%
58	Fehr	Smooth muscle Aortic medial tissue	Collagenase: 0.30%	DMEM
59	Lewis	Skeletal muscle	Collagenase: 10% Neutral Protease: 0.3%	HBSS
60	Wamhoff BR	Arterial smooth muscle	Collagenase Type 2: 294 u/ml Elastase: 6.5 u/ml Deoxyribonuclease I: 0.4 mg/ml Soybean Trypsin Inhibitor: 1 mg/ml	MEM
61	Huckle WR	Coronary smooth muscle cells	Collagenase: 0.3% Elastase: 0.05%	HBSS
62	Korzick	Coronary myocytes	Collagenase Type 2: 294 u/ml Elastase: 6.5 u/ml Deoxyribonuclease I: 0.04% Soybean Trypsin Inhibitor: 0.1%	low calcium physiological saline
63	Sirous ZN	Coronary smooth muscle	Collagenase Type 2: 150 u/ml Elastase: 0.05%	HBSS

Species: Quail

Table 23.15: **Quail**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
64	Konigsberg	Myoblasts	Collagenase Type 2: 0.1%	Puck's solution

Species: Rabbit

Table 23.16: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
19	Mitra	Myocytes, heart and stomach	Protease XIV: 0.028%	Solution C See Reference
65	Benham	Smooth muscle	Elastase: 0.17 - 0.25%	Saline
66	Benham	Smooth muscle, ear artery	Trypsin: 0.1%	CF solution See Reference
67	Peters	Smooth muscle, aorta	Hyaluronidase: 800 u/ml	HBSS
68	Day	Thoracic aorta	Elastase: 0.008%	Kreb's Ringer
69	Croons Valerie	Aortic smooth muscle	Collagenase Type 2: 300 u/ml Elastase: 5 u/ml	F10 Ham's
70	Knodle	Smooth muscle, aortic	Trypsin: 0.038%	MEM
71	Santos	Enterocytes	Trypsin: 0.1%	RPMI 1640 w/ 1% fetal bovine serum PBS
72	Ives	Smooth muscle, aorta	Trypsin: 0.1%	Krebs Ringer HEPES solution
30	Hu	Smooth muscle	Trypsin: 0.1%	Potassium buffer solution

42	Chamley	Smooth muscle, saphenous vein	Elastase: 0.05%	BSS

Species: Rat

Table 23.17: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
44	Weisleder	Skeletal muscle fiber	Collagenase Type 1: 0.2%	Tyrode
73	Pacak	Myogenic	Collagenase Type 2: 1.0% Neutral Protease: 2.4 u/ml	Ham's F-10
74	Bean	Muscle, mesenteric arteries	Trypsin: 0.05%	HEPES KG solution See Reference
30	Hu	Smooth muscle	Trypsin: 0.1%	Potassium buffer solution
75	Barone	Smooth muscle, aortic	Elastase: 0.0125%	DMEM
76	Kono	Muscle	Trypsin: 0.05%	Kreb's Henseleit bicarbonate buffer
77	Kasten	Myocardial	Trypsin NF 1:250: 0.125%	HBSS CMF
78	Das	Myotubes	Trypsin: 0.05%	See Reference
79	Kim	Myoblasts	Collagenase Type 2: 1.0% Neutral Protease: 2.4 u/ml	Ham's F-10
80	Cole	Endothelial, aortic	Trypsin:	RPMI 1640
81	Zeng	Pulmonary arterial smooth muscle	Collagenase Type 2: 0.1% Elastase: 0.05%	Buffer
82	Loch-Caruso	Smooth muscle, myometrial	Trypsin: 150 μg/ml	HBSS or PSS, CMF
83	Gunther S	Mesenteric artery smooth muscle cells	Elastase: .0125% Soybean Trypsin Inhibitor: 0.025% Collagenase Type 1: 0.1%	HBSS
84	Jaggar JH	Arterial smooth muscle	Papain: 0.03% Collagenase: 0.1%	See Reference
85	Su E.	Smooth muscle cells	Collagenase Type 2: 0.2% Elastase: 0.04% Soybean Trypsin Inhibitor: 0.1%	M-199
86	Dennis	Myooids	Neutral Protease: 4 u/ml	Ham's F-12
87	Redmond	Smooth muscle, endothelial	Trypsin: 0.04%	MEM
88	Brock	Smooth muscle, thoracic aorta	Trypsin: 0.0375%	Eagle's MEM with calcium
89	Hrometz	Vascular smooth muscle	Collagenase Type 2: 0.1% Elastase: 0.0125%	DMEM
90	Gordon	Endothelial, aortic	Elastase: 0.05%	Waymouth's culture medium
91	Bolzon	Smooth muscle, tail arteries	Papain: 0.1%	HEPES buffer See Reference
92	McGuire	Smooth muscle, mesenteric artery	Trypsin: 0.05%	MEM
93	Harary	Heart	Trypsin NF 1:250: 250: 0.1%	Saline A (See reference)
94	Wellman	Smooth & skeletal muscle Cardiac myocytes	Protease: 0.01%	PSS
95	Boulanger-Saunier	Myocytes	Collagenase: 0.1%	HBSS

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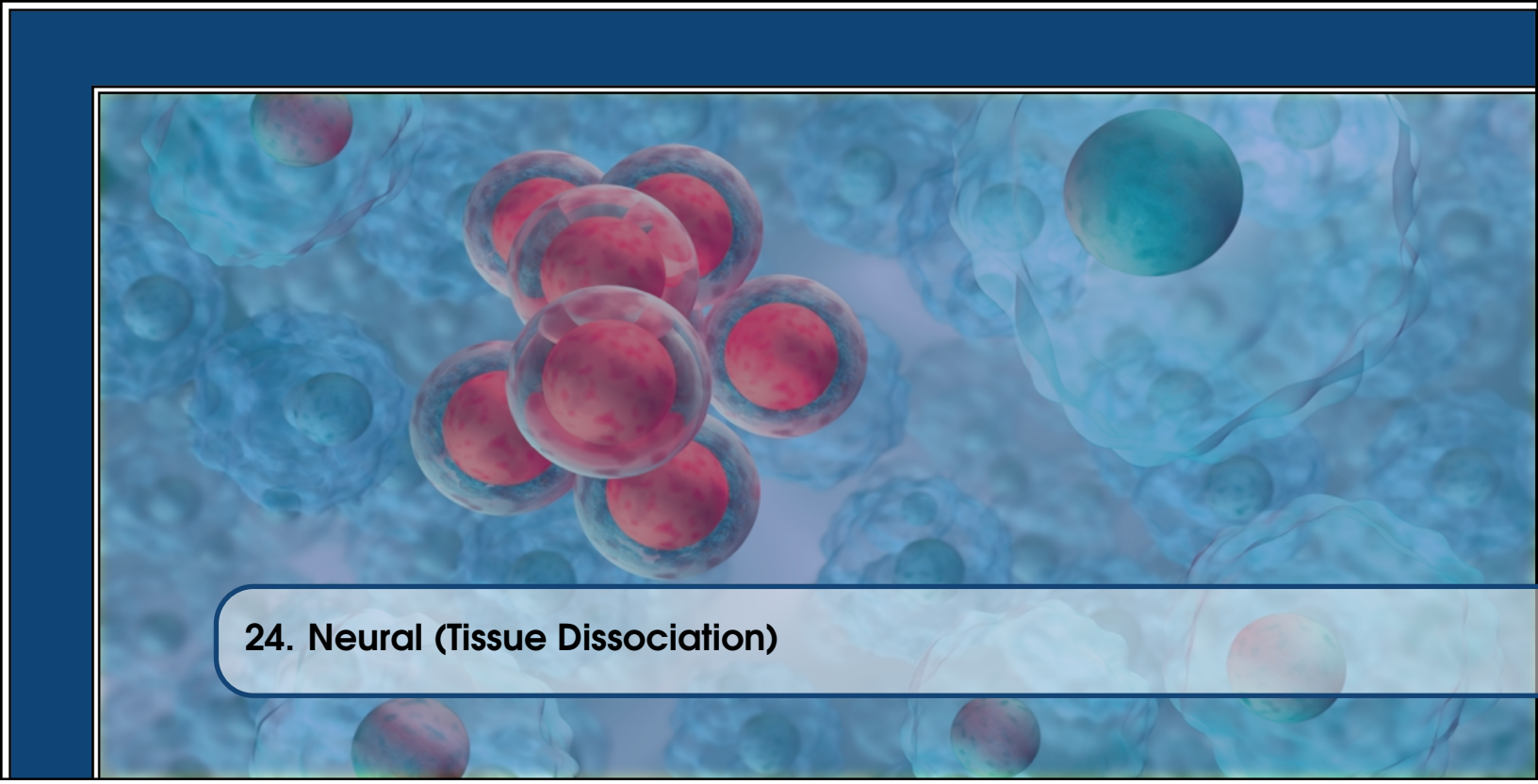

24. Neural (Tissue Dissociation)

Species: Avian

Table 24.1: **Avian**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Temburni	Cilary ganglion neurons	Collagenase Type 1: 0.1%	PBS

Species: Bovine

Table 24.2: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Bowman	Microvascular endothelial	Collagenase/Dispase: 0.1%	MEM
3	Trifaro	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF
4	Poduslo	Oligodendroglia Neural	Trypsin: 0.1%	See Reference

Species: Chicken

Table 24.3: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
5	Choi	Dorsal root ganglion neurons Spinal cord	Trypsin: 0.1%	Puck's saline, CMF
6	McCarthy	Ganglion chains, sympathetic ganglia	Trypsin: 0.25%	Krebs Phosphosaline
7	Moyer	Flat, retina	Trypsin: 0.1%	Tyrode's solution, CMF
8	Bottenstein	Neurons, ganglia	Trypsin: 0.25%	HBSS, CMF
9	Schnaar	Spinal cord	Trypsin: 0.05%	Phosphate buffer See Reference
10	Coates	Cerebral neurons	Trypsin: Trypsin: 0.125-0.25%	DMEM
11	Wiseman	Neural retina	Collagenase: 0.25% Elastase: 0.2% Hyaluronidase: 1.0% Papain: 1.0% Protease: 0.1% Trypsin: 0.05%	HBSS
12	Mudge	Dorsal root ganglia neurons	Collagenase: 0.01%	Eagle's MEM
13	Raman	Neurons	Papain: 40 u/ml	HEPES
14	Tuttle	Ciliary ganglion neurons	Trypsin: 0.25%	Eagle's MEM

Species: Fish

Table 24.4: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
15	Anderson	Neurons, spinal cord	Trypsin: 0.4%	PBS, CMF
16	Sakowski	Motor neurons	Collagenase Type 2: 0.1%	See reference
17	Won	Rohon-Beard neurons	Trypsin: 0.2%	L-15/Hepes
18	Cerda	Neurons	PDS kit: with modifications	EBSS

Species: Frog

Table 24.5: **Frog**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
19	Takahashi	Neuron	Collagenase Type 1: 0.1%	Steinberg's solution

Species: Guinea-Pig

Table 24.6: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
20	Jessen	Neuron, enteric	Trypsin: 0.125%	Medium 199

Species: Hamster

Table 24.7: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
21	Liman ER	Vomeronasal organ neurons	Collagenase Type 1: 0.02% Trypsin: 0.02%	PBS

Species: Human

Table 24.8: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
22	Panchision David M	Neural, various	Papain: 12 u/ml Collagenase/Dispase: See Reference Trypsin: See Reference	See Reference
23	Dib-Hajj SD	Dorsal root ganglion neurons	Collagenase Type 1: 0.2% Neutral Protease: 0.5%	DMEM/F-12
24	Fuja TJ	Neural progenitor cells	Papain: 2.5 u/ml Deoxyribonuclease I: 250 u/ml Neutral Protease: 1 u/ml	DMEM/F-12
25	Von Recum	Retinal pigment epithelial (RPE)	Trypsin: 0.25%	HBSS
26	Dietrich J	Neurons	Collagenase Type 4: 1.33% Papain: 0.07 u/ml Neutral Protease: 1 mg/ml	DMEM/F12
27	Roy NS	Ventricular epithelial	Papain: 11.4 u/ml Deoxyribonuclease I: 10 u/ml	DMEM/F12

Species: Insect

Table 24.9: **Insect**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
28	Sanchez-Soriano	Dendrites	Collagenase: 0.05% Neutral Protease: 0.2%	HBSS
29	Berger	Neuroblasts	Collagenase Type 1: 0.1% Papain: 0.1%	Rinaldini solution
30	Kloppenburg	Giant interneurons	Collagenase: 0.05% Neutral Protease: 0.2%	Leibovitz's L15

Species: Mouse

Table 24.10: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
31	Harris	Oligodendrocytes	Papain: 0.1% Deoxyribonuclease I: 0.0002%	HBSS
32	Williams	hippocampal neurons	PDS kit: per instructions	DMEM
33	Stuart	Neurosperes	Papain: 0.1% Deoxyribonuclease I: 0.1%	NeuroCult NSC
34	Lee	DRG neurons	Collagenase Type 2: 0.1% Trypsin: 0.25%	DMEM/F-12
35	Medina-Rodriguez	Oligodendrocyte precursor cells	Papain: 0.09%	DMEM
36	Li	Neurons	Papain: 100 ul	DMEM
37	Sayed	CNS leukocytes	Collagenase Type 4: 300 u/ml	HBSS
38	Brown	Hippocampal and retinal neurons	Papain: 1% Deoxyribonuclease I: 5 u/ml	HBSS
22	Panchision David M	Neural, various	Papain: 12 u/ml Collagenase/Dispase: See Reference Trypsin: See Reference	See Reference
39	Eagleson	Neurons, spinal cord	Collagenase Type 3: 0.25%	Hank's BSS, CMF
40	Spielman	Papillae, taste receptor	Pronase E: 0.15%	Carbonate-Phosphate buffer See Reference
41	Lee	Neurons, DRG and SCG	Trypsin: 0.25%	L-15 medium
42	Shrier	Neural	Trypsin NF 1:250: 50 0.25%	BSS
43	O'Meara	Oligodendrocytes, dorsal root ganglia	Papain: 0.15% Deoxyribonuclease I: 0.006%	DMEM

44	Savchenko V	Neurons, ganglia	Papain: 20 u/ml Deoxyribonuclease I: 100 u/ml Collagenase: 0.3% Trypsin: 0.05%	HBSS
45	Masaki	Schwann	Collagenase Type 1: 0.01% Trypsin: 0.125%	DMEM
46	Radtke	Olfactory ensheathing	Collagenase: 0.15% Papain: 12 u/ml	DMEM
47	Brewer Gregory J	Neurons, neurospheres	Papain: 0.2%	Hibernate
48	Bracko	Neural stem cells	Papain: 0.01% Neutral Protease: 0.1% Deoxyribonuclease I: 0.01%	DMEM/F12
49	Babona-Pilipos	Neural precursors	Trypsin: 0.13% Hyaluronidase: 0.08%	See Reference
50	Malin	Sensory neurons, DRG	Papain: 20 u/ml Collagenase Type 2: 0.4% Neutral Protease: 0.46%	HBSS
51	Richards L.	Neurons, neuronal precursors	Trypsin: 0.1% Deoxyribonuclease I: 0.001%	DMEM
52	Goetz	Retinal neurons	Papain: 10-20 ul	HBSS
53	Yip	Spinal microganglia	Papain: 0.2%	Hibernate A
54	Gonzalez John M	Brain and spinal cord cells	Trypsin: 0.25%	PBS
55	Okano-Uchida T	Cerebellar granule cell precursors	Papain: 16.5 u/ml Deoxyribonuclease I: 0.008%	Dulbecco's PBS
56	Eide	Neurons	Papain: 0.2%	DMEM
57	Quinn	Neurons, dorsal root ganglion	Trypsin: 0.25%	HBSS
58	Pollari	Spinal cord neurons	Papain: 0.05% Deoxyribonuclease I: 0.004%	PBS/DMEM
59	Conrad	Motorneurons	Trypsin: 0.025%	HBSS
60	Radad Khaled	Dopaminergic neurons	Trypsin: 0.1% Deoxyribonuclease I: 0.02%	DMEM
61	Kitani	Precursor	Trypsin: 0.5%	PBS
62	Roberts	Trigeminal senesory neurons	Papain: 20 u/ml	HEPES buffered saline
63	Stettner	Schwann cells	Collagenase: 0.05-0.1% Trypsin: 0.125-0.25%	DMEM
64	Pedrola	DRG neurons	Collagenase: 0.2% Trypsin: 0.05%	Ham's F12
65	Gill JC	Neurons	PDS kit: per instructions	EBSS
66	Varon	PNS test neurons	Trypsin: 0.08%	Eagle's Basal Medium See Reference
67	Ziegler	Neuroshere	Trypsin: 0.25% Papain: 100 u Deoxyribonuclease I: 0.025%	Pro-N
68	Deshmukh	Neurons	Trypsin: 0.25%	NGF-containing medium

Species: Ovine

Table 24.11: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Poduslo	Oligodendroglia Neural	Trypsin: 0.1%	See Reference

Species: Porcine

Table 24.12: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
69	Si ML	Superior cervical ganglia	Papain: 2 u/ml Collagenase: 0.12% Neutral Protease: 0.48%	HBSS

Species: Quail

Table 24.13: **Quail**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
70	Sieber-Blum	Neural crest	Trypsin: 0.05%	MEM, HBSS

Species: Rat

Table 24.14: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
71	Liu	Neurons, hippocampal	Papain: 15 - 20 u/ml	Eagle's MEM See Reference
72	Stemple	Stem, neural crest	Collagenase: 0.075%	Ringer's solution
3	Trifaro	Heart Adrenal chromaffin Paraneurons	Trypsin: 0.06%	25mM HEPES buffered Locke's solution, CMF
73	Wilding	Neurons, hippocampal	Papain: 20 u/ml	EBSS
74	Thurner	Hippocampal Neurons	Papain: 25 u/ml	L-15
75	Twitchell	Neurons, hippocampal	Papain: 20 u/ml	MEM
76	Robertson	DRG neurons	Papain: 20 u/ml Collagenase Type 1: 150 u/ml Neutral Protease: 0.8%	HBSS
77	Evans J	Neurons	Papain: 0.2%	Hibernate A
78	Mothe	Spinal cord progenitor cells Hybrid toxins	PDS kit: See Reference	Neurobasal A
79	Bosmans	Dorsal Root Ganglion	Papain: 20 u/ml Collagenase Type 1: 0.3% Neutral Protease: 0.4%	L-15
80	Liu QY	Hippocampal neurons	Papain: 20 u/ml Deoxyribonuclease I: 0.01%	MEM
81	Ghogha	Sympathetic neurons	Collagenase Type 2: 0.1% Neutral Protease: 0.5%	DMEM/F-12
82	Dichter	Neurons, cortical	Trypsin: 0.027%	MEM
83	Jahr	Dorsal horn neurons Spinal	Trypsin: 0.025%	Ham's F-12
84	Oyanagi	Cerebral neurons	Papain: 2 u/ml Deoxyribonuclease I: 0.01%	DMEM-PBS
85	Yokosuka Makoto	Hypothalamic neurons and glias	Papain: 0.5 u/ml	DMEM
86	Mabuchi T	Hippocampal neurons	PDS kit: See Reference	DMEM
87	Rheaume	Cortical neurons, DRG	PDS kit: per instructions Collagenase Type 1: 0.17%	Neurobasal
88	O'Connor	Cortical	Papain:	Neurobasal medium and DMEM
89	Lovshin JA	Brainstem and cortical neurons	PDS kit: per instructions	DMEM
90	Wakshull	Neurons, sympathetic	Trypsin: 0.25%	L-15 or HBSS, CMF
91	Bartlett	Hippocampal neurons	Trypsin: 0.25%	HBSS, CMF
92	Mattson	Hippocampal neurons	Trypsin: 0.2%	Eagle's MEM
93	Chen	Hippocampal neurons	Papain: 0.2%	Hibernate A
94	Lie DC	Adult progenitor	Papain: 2.5 u/ml Deoxyribonuclease I: 250 u/ml Neutral Protease: 1 u/ml	DMEM/F-12
95	Huettner	Neurons, visual cortex	Papain: 20 u/ml	BSS See Reference

63	Stettner	Schwann cells	Collagenase: 0.05-0.1% Trypsin: 0.125-0.25%	DMEM
96	Frank	Dopamine neurons	Papain: 20 u/ml	PBS
97	Mains	Neurons, superior cervical ganglia	Trypsin: 0.1%	Basal L-15 medium
98	Reichardt	Neurons, sympathetic	Collagenase Type 1: 0.01%	Hank's solution, CF
99	Acosta	Neurons	Trypsin: 0.25%	MEM10
100	Hatanaka	Septal neurons	Papain: 0.05%	PBS, CMF
101	Leifer	Ganglion, retina	Papain: 12.5 u/ml	HBSS w/5 mM HEPES
102	McFarlane	Neurons, sympathetic	Neutral Protease: 0.24%	HBSS
103	Hall	Neurons, hippocampal	Papain: 20 u/ml	Harvest buffer
104	Pedraza Carlos E	Superior cervical ganglion	Collagenase Type 4: 20 u/ml Trypsin: 0.25%	DMEM
105	Floyd Candace L	Cortical astrocytes	Papain: See Reference	DMEM
106	Yan	Trigeminal ganglia	Papain: 20 u/ml Collagenase Type 2: 0.3%	HBSS
107	Cheng	Hippocampal	Trypsin: 0.2%	HBSS
108	East	Dorsal root ganglia, fibroblast	Collagenase: 0.125%	DMEM
109	Tanaka	Cerebellar granule neurons	PDS kit: per instructions	PBS
110	Novelli	Cerebellar neurons	Trypsin: 0.025%	Eagle's MEM
111	Davies	Neurons, DRG	Neutral Protease: 0.5%	L-15 w/ CO_2
112	Peltier	Neuronal	Papain: 0.2%	Neurobasal E
113	Gavva NR	Dorsal root ganglia	PDS kit: per instructions	MEM/Ham's F12
114	Loktev	Hypothalamic neuronal	PDS kit: per instructions	Neurobasal A
115	Bixby	Sciatic Nerve and Gut Neural Crest Stem Cells	Collagenase Type 4: 0.025% Trypsin: 0.005% Deoxyribonuclease I: 0.05%	HBSS
116	Morrison	Sciatic nerves	Trypsin: 0.025% Collagenase Type 3: 0.1%	L-15 medium See Reference
117	Liu	Hippocampal neurons	Papain: 20 u/ml	Neurobasal/B27
118	Mithen	Schwann, dorsal root ganglia	Trypsin: 0.25%	HBSS, CMF
119	Wood	CNS cells	Trypsin: 0.25%	EBSS
116	Morrison	Sciatic nerves	Trypsin: 0.025% Collagenase Type 3: 0.1%	L-15 medium See Reference
120	Brewer	Hippocampal neurons	Papain: 0.2%	HibernateA/B27
121	Hu Hong-Zhen	Dorsal root ganglion neurons	Collagenase Type 4: 0.125% Trypsin: 0.05%	DMEM/Ham's F12
122	Lin CR	Spinal progenitor cells	PDS kit: with modifications	Neurobasal medium
123	Connor	Trigeminal neurons	Papain: 20 u/ml Collagenase: 0.3%	CMF Hanks
124	Lacroix-Fralish	astrocytes	PDS kit: per instructions	DMEM
125	Buchhalter	Pyramidal neurons Nonpyramidal neurons	Trypsin: 0.027%	HEPES
126	Sarthy PV	Retina	Trypsin: 0.25%	Ham's F-12
127	Raff	Neurons and glial	Trypsin: 0.25%	MEM See Reference
128	Obradovic Darja	Hippocampal neurons	PDS kit: per instructions	Neurobasal A
129	Sakisaka	Superior cervical ganglion	Collagenase: 0.05%	L-15
130	Moriya-Ito K	Vomeronasal receptor neurons	Collagenase/Dispase: 0.1% Papain: 0.5 u/ml	DMEM/F12
131	Rayport	Postnatal dopamine neurons	Trypsin: 0.035%	See Reference
132	Brockes	Schwann	Trypsin: 0.25%	DMEM
133	Schafer	Myenteric ganglia	Trypsin: 0.05%	MEM-HEPES
134	Neuhoff	Neurons, hippocampal	Papain: 20 u/ml	EBSS
135	Johansson	Spinal cord	Trypsin: 0.133%	HBSS and PIPES
136	Allen	Basal forebrain neurons	Trypsin: 0.125%	Gey's BSS

Species: Salamander

Table 24.15: **Salamander**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
137	Bader	Photoreceptors, retina	Papain: 0.05%	See Reference
138	Townes-Anderson	Retina	Papain: 14 u/ml	Saline

Species: Shellfish

Table 24.16: **Shellfish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
139	Camardo	LUQ cells RUQ cells	Protease: 1%	L15 medium
140	Cohan	Buccal ganglia	Trypsin: 0.2%	L-15 medium
141	Lee	Neurons	Neutral Protease: 1.0%	L-15-ASW
142	Schacher	Neurons LUQ cells	Protease: 1%	L15 medium
143	Haydon	Somata, buccal ganglia	Trypsin: 0.2%	Antibiotic saline, Leibowitz 50%
144	Zoran	Buccal ganglia; SLT muscle	Trypsin: 0.2%	DMEM

Species: Turtle

Table 24.17: **Turtle**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
145	Lam	Retinal	Papain: 0.1% (13.5 u/mg)	Kreb's Ringer

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25. Pancreas (Tissue Dissociation)

Species: Bovine

Table 25.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Cotton	Duct epithelial	Collagenase: 0.1%	HEPES
2	Stiles	Platelets	Trypsin:	See Reference
3	Sato	Ductal	Neutral Protease: 0.05%	EBSS

Species: Canine

Table 25.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Zoran	Buccal ganglia; SLT muscle	Trypsin: 0.2%	DMEM
5	Noel	Islets	Collagenase Type 4: 600-1100 u/ml Deoxyribonuclease I: 10 ug/ml	RPMI 1640

Species: Fish

Table 25.3: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
6	Schrezenmeir	Islets	Collagenase: 0.12 - 0.46 u/ml	RPMI 1640

Species: Guinea-Pig

Table 25.4: **Guinea-Pig**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Schultz	Acinar	Collagenase Type 3: 60 u/ml	Kreb's Ringer
8	Amsterdam	Exocrine	Hyaluronidase: 0.15% - 0.2%	Kreb's Ringer
9	Gardner	Acinar	Soybean Trypsin Inhibitor: 0.01%	Kreb's Ringer

Species: Hamster

Table 25.5: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
10	Feldman	Islets	Collagenase Type 4: 1.3% - 2.0%	HBSS

Species: Human

Table 25.6: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
11	Cane	Acinar	CLSPA: 200 u/ml Soybean Trypsin Inhibitor: 0.01%	See Reference
12	Li	Pancreatic cancer stem cells	Collagenase Type 4: 200 u/ml	medium 199
13	Watt	Islets	Collagenase: 0.2% Deoxyribonuclease I: 200 u/ml	Eurocollins solution
14	Izumi	Islets	Collagenase Type 4: 0.8%	HBSS
15	Gray	Islets	Collagenase (1 or 4): 0.60%	HBSS
16	Contractor	Islets	Collagenase: 0.4%	HBSS
17	Warnock	Islets	Collagenase: 0.6%	Eurocollins solution
18	Sutherland	Islets	Collagenase: 170-210 u/ml	HBSS

Species: Monkey

Table 25.7: **Monkey**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
19	Scharp	Islets	Hyaluronidase: 0.05%	HBSS

Species: Mouse

Table 25.8: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
20	Koh	Islets	Collagenase: 0.1-0.25%	HBSS
21	Bertera	Islets	Collagenase: 0.2%	RPMI 1640
22	Li	Islets	Collagenase: 1,000 u/ml	RPMI 1640
23	Carter	Islets	Collagenase: 0.14%	RPMI 1640
24	Szot	Islets	Collagenase: 0.03-0.08%	RPMI 1640
25	Haefliger Jacques-Antoine	Pancreatic islet	Collagenase Type 4: 0.2%	HBSS
26	Wu Yulian	Islets	Collagenase Type 4: 0.2%	HBSS
27	Toivola	Acinar cells and acini	Collagenase Type 1: See Reference CLSPA: See Reference	See Reference
28	Strowski M	Pancreatic islets	Collagenase Type 4: 0.4%	Gey's BSS
29	Koster	Islets	Collagenase Type 2: 0.2%	CF Medium
30	Githens	Duct	Papain: 25 u/ml	DMEM /F-12
31	Jauch	Acinar	Collagenase: 100 u/ml	HEPES
32	Dalpe-Scott	Islets	Hyaluronidase: 0.5%	Kreb's Ringer bicarbonate buffer
33	Kobayashi	Islets	Collagenase: 0.2%	RPMI 1640
34	Astrof	Islets	Collagenase Type 4: 0.2%	RPMI
35	Huang	Islets	Collagenase Type 4: 0.2%	RPMI 1540
36	Wang	Pancreatic ductal	CLSPA: 50 u/ml Hyaluronidase: 400 u/ml Soybean Trypsin Inhibitor: 0.02%	DMEM
37	Taguchi	Islets	Collagenase Type 4: 0.1%	HBSS
38	Yesil	Islets	Collagenase: See Reference	DMEM
39	Huch	Pancreas organoid	Neutral Protease: 0.012% Collagenase: 0.012%	DMEM
40	Voronina	Acinar	CLSPA: 200 u/ml	See Reference

41	Ji	Ancinar	CLSPA: See Reference Soybean Trypsin Inhibitor: 0.001%	DMEM
42	Fogarty	Acinar	CLSPA: See Reference	See Reference
43	Kurup	Acinar	Collagenase: 0.1%	Waymouth's MB
44	Greggio	Pancreatic progenitor	Neutral Protease: 0.125%	DMEM
45	Burnham DB	Acinar	CLSPA: 70-90 u/ml Soybean Trypsin Inhibitor: 0.01%	Krebs-Henseleit

Species: Porcine

Table 25.9: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
46	Johnson	Islets	Collagenase: 0.1%-0.2%	HBSS
47	Iwatsuki	Acinar	Collagenase: 100 u/ml	Saline
48	Korbutt	Islets	Collagenase: 0.25%	HBSS
49	Ricordi	Islets	Collagenase: 0.2%	HBSS
50	Zhao	Acinar	Collagenase Type 3: 200 u/ml	RPMI 1640
51	Heiser	Islets	Collagenase: 0.1%	HBSS
52	Brandhorst	Islets	Collagenase: 0.1%	HBSS
53	Van der Burg Michael P M	Islets	Collagenase: See Reference	Univ of Wisconsin solution

Species: Rabbit

Table 25.10: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
10	Feldman	Islets	Collagenase Type 4: 1.3% - 2.0%	HBSS
54	Renckens	Acinar	Hyaluronidase: 0.2%	Kreb's Ringer bicarbonate buffer

Species: Rat

Table 25.11: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
55	Williams	Acinar	CLSPA: 100 u/ml	DMEM
56	MacGregor	Islets	Collagenase Type 1: 450 u/ml	RPMI 1640
57	Blinman TA	Pancreatic acini	CLSPA: 30 u/ml Collagenase Type 4: 30 u/ml Soybean Trypsin Inhibitor: 0.01%	M199
58	Verspohl	Islets	Collagenase: 126 - 196 u/ml	HBSS
30	Githens	Duct	Papain: 25 u/ml	DMEM /F-12
59	Takaki	Islets	Collagenase: 0.5 - 0.9%	HBSS
60	Schulz	Exocrine	Hyaluronidase: 0.9%	Kreb's Ringer
61	Kondo	Exocrine	Hyaluronidase: 0.15%	Krebs
62	Essner	Ascites hepatoma	Trypsin: 0.1%	Phosphate buffer See Reference
63	Gotoh	Islets	Collagenase Type 4: 0.2%	HBSS
25	Haefliger Jacques-Antoine	Pancreatic islet	Collagenase Type 4: 0.2%	HBSS
64	Williams	Islets	Collagenase Type 1: 450 u/ml	Leibowitz L-15
65	Sutton	Islets	Collagenase Type 1: 0.3%	Hank's solution
66	Tsao	Epithelial	Trypsin: 0.1%	Ham's F-12/HBSS See Reference
67	Ballinger	Islets	Collagenase: 0.5%	HBSS
68	Getty-Kaushik	Islets	Collagenase Type 4: See Reference	RPMI 1640
69	Lacy	Islets	Collagenase: 0.5%	Hanks solution
70	Leonard	Islets	Trypsin: 0.05%	Puck's saline buffered w/ EDTA 0.02%
71	Ohzato	Islets	Collagenase: 0.1% - 0.2%	HBSS

72	Githens	Duct	Trypsin: 0.01%	HBSS
73	Mangos	Acinar, parotid	Trypsin: 0.01% Collagenase: 40-50 u/ml Hyaluronidase: 0.10%	HBSS CMF
74	Githens	Interlobular ducts	Papain: 25 u/ml	DMEM/Ham's F-12
75	Ji B	Ancinar	CLSPA: See Reference Soybean Trypsin Inhibitor: 0.01%	DMEM
76	Yeh	Parotid acinar	Trypsin: 0.001%	F12 medium
77	Menozzi	Acinar	Soybean Trypsin Inhibitor: 0.01%	HEPES
78	Williams	Acinar	Hyaluronidase: 0.18%	Kreb's Henseleit bicarbonate buffer
79	Hirschi	Acinar	Hyaluronidase: 462 u/ml	Ham's F12
80	Quissell	Acinar, submandibular gland	Hyaluronidase: 0.1 %	HBSS, CF
81	Oliver	Acinar Exorbital lacrimal, parotid, pancreas	Trypsin: 0.01%	HBSS, CMF
82	Brannon	Acinar	Hyaluronidase: 0.1%	HBSS See Reference
83	Verga Falzacappa	Islets	Collagenase Type 4: 0.2%	CMRL 1066
84	Lacy	Islets	Collagenase Type 4: 1.0% - 1.2%	HBSS
85	Foskett	Parotid acinar	Trypsin: 0.02%	Solution B (See reference)
86	Katada	Islets	Collagenase Type 4: 1%	Medium 199
87	Shibata	Islets	Collagenase Type 4: 0.5%	HBSS
88	Melvin	Acinar, parotid	Hyaluronidase: 0.015%	Earle's MEM
89	Tian XH	Pancreatic islets	Collagenase: 0.75%	RPMI 1640
90	Wolters	Islets	Collagenase: 0.2%	Kreb's Ringer bicarbonate buffer
91 See Reference	Braaten	Islets	Collagenase Type 4: 0.63%	EBSS

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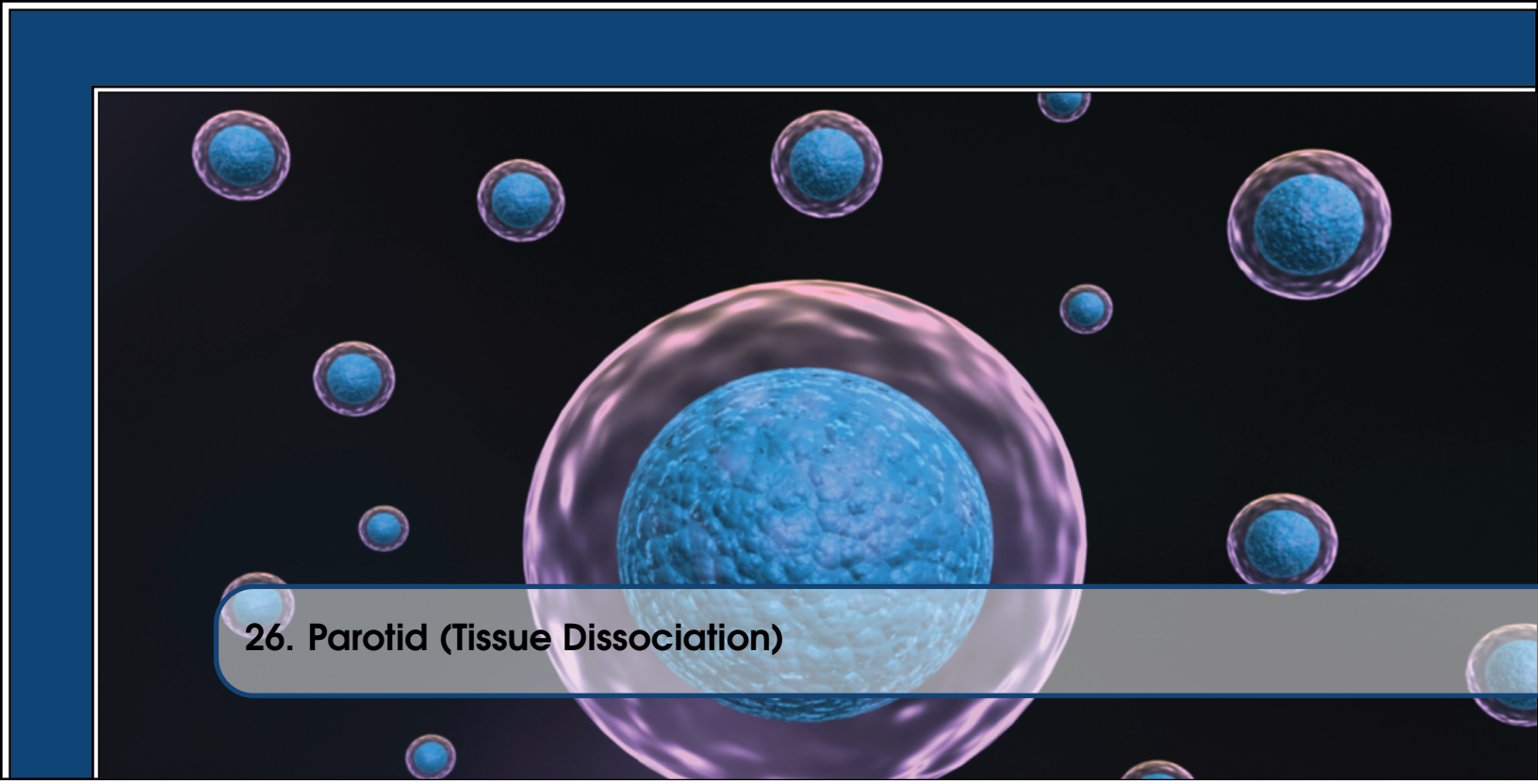

26. Parotid (Tissue Dissociation)

Species: Mouse

Table 26.1: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Ogawa	Parotid and sublingual glandular	Neutral Protease: 50 u/ml Collagenase Type 1: 100 u/ml	PBS

Species: Rat

Table 26.2: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Takuma	Parotid	Hyaluronidase: 0.025%	HBSS with 20mM HEPES
3	D'Silva NJ	Parotid acinar cells	Collagenase Type 2: 90 u/ml	Krebs-Henseleit Bicarbonate
4	Mangos	Acinar, parotid	Trypsin: 0.01% Collagenase: 40-50 u/ml Hyaluronidase: 0.10%	HBSS CMF
5	Prasad	Epithelial	Collagenase: 50-75 u/ml Hyaluronidase: 0.1%	HBSS CF
6	Yeh	Parotid acinar	Trypsin: 0.001%	F12 medium
7	Oliver	Acinar Exorbital lacrimal, parotid, pancreas	Trypsin: 0.01%	HBSS, CMF
8	Foskett	Parotid acinar	Trypsin: 0.02%	Solution B (See reference)
9	Looms	Acinar	Collagenase: 75 u/ml Hyaluronidase: 153 u/ml	RPMI 1640
10	Melvin	Acinar, parotid	Hyaluronidase: 0.015%	Earle's MEM

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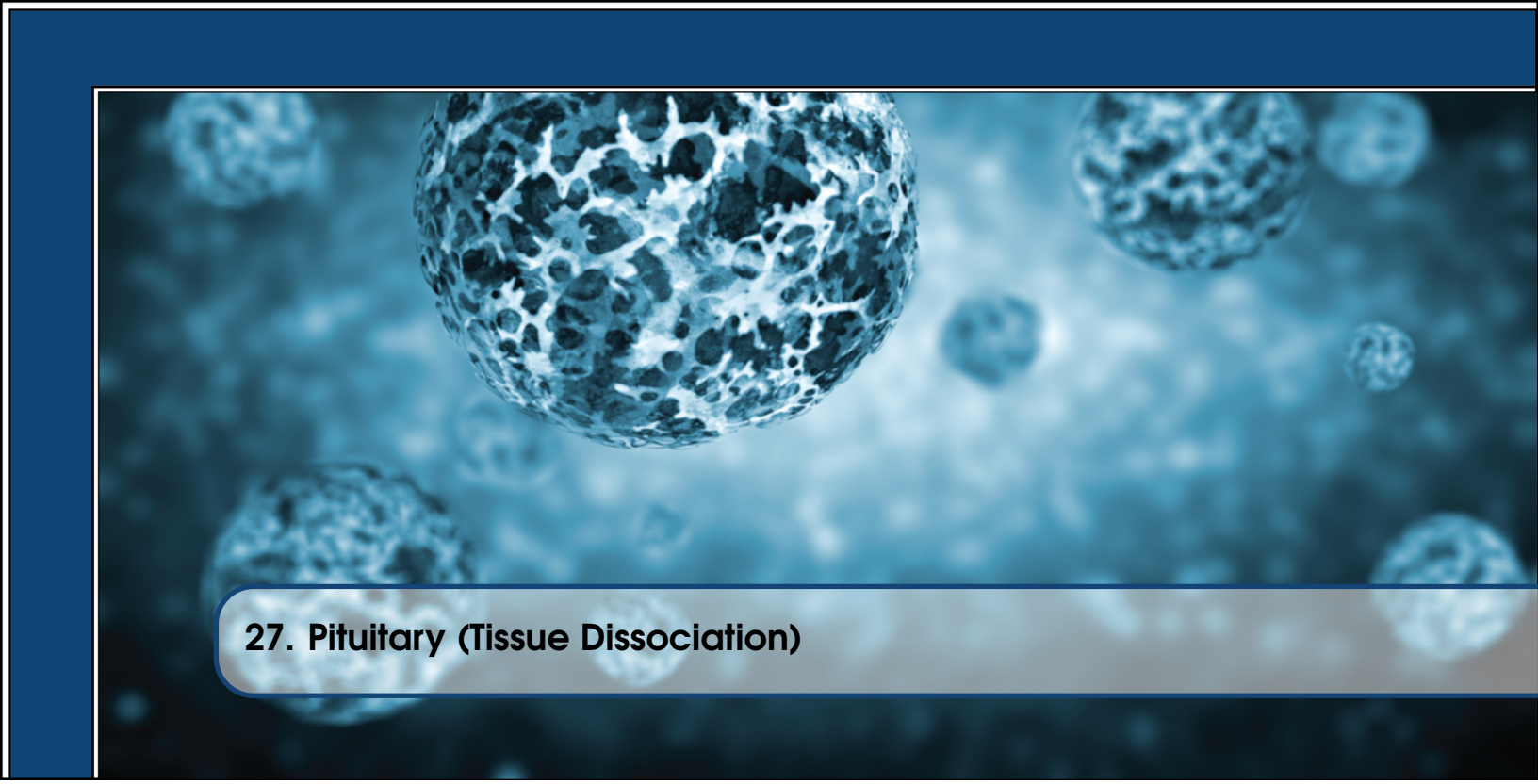

27. Pituitary (Tissue Dissociation)

Species: Bovine

Table 27.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Mason	Pituitary	Collagenase: 0.1%	EBSS, CMF
2	Ferrara	Follicular, anterior pituitary and pars tuberalis	Deoxyribonuclease I: 200 μg/ml	HBSS, CMF
3	Ridgway	Pitituary	Hyaluronidase: 0.1%	DMEM
4	Hassan	Pituitary	Collagenase: 0.3%	DMEM

Species: Mouse

Table 27.2: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
5	Steveson Tami C	Pituitary	Collagenase: 0.4% Hyaluronidase: 0.1% Trypsin: 0.3%	DMEM/Han's F12

Species: Ovine

Table 27.3: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
6	Xu Ruwei	Somatotropes	Collagenase Type 1: 0.3% Hyaluronidase:	Medium 199

Species: Rat

Table 27.4: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Wilfinger	Pituitary	Trypsin: 0.3 %	HEPES
8	Akieda-Asai	Pituitary	Collagenase Type 2: 0.4% Deoxyribonuclease I: 0.04%	DMEM
9	D'Emden	Anterior pituitary gland	Trypsin: 0.1%	EBSS, CMF
10	Portanova	Anterior pituitary	Trypsin: 0.25%	Krebs
11	Zhou	Pituitary	Trypsin: 0.1%	DMEM

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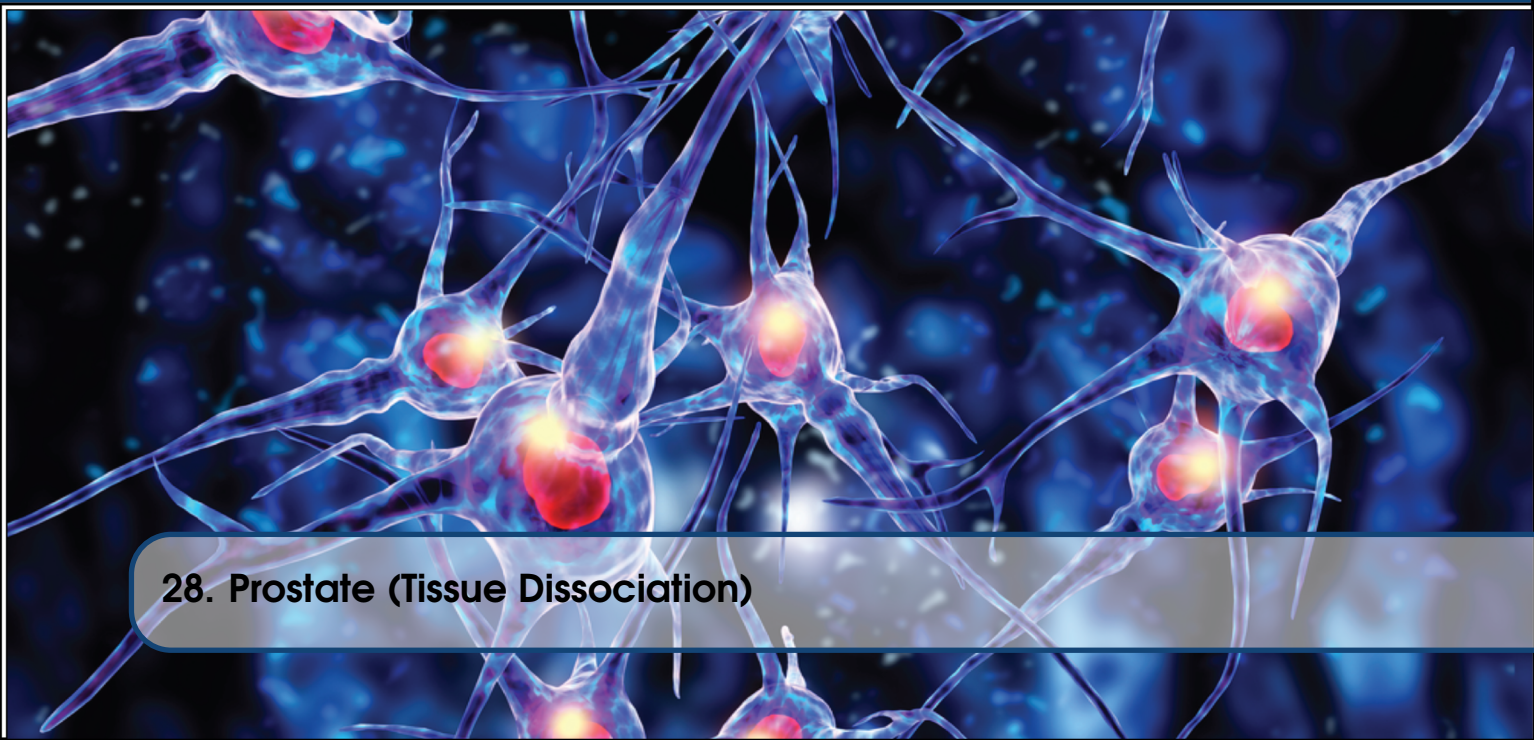

28. Prostate (Tissue Dissociation)

Species: Human

Table 28.1: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Taylor	Prostatic cancer fibroblasts	Collagenase: 225 u/ml Hyaluronidase: 125 u/ml	RPMI 1640
2	Nakashiro Koh-Ichi	Prostate stromal cells	Collagenase Type 1: 0.1%	RPMI 1640
3	Le Hanh	Prostatic stromal cells	Collagenase Type 1: 0.2%	DMEM/F-12
4	Levine AC	Prostatic fibroblasts	Collagenase Type 1: 0.125%	DMEM/F12

Species: Mouse

Table 28.2: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
5	Imamov Otabek	Prostatic epithelial	Collagenase Type 3: 170 u/ml	DMEM
6	Burger	Prostate epithelial/stem	Collagenase Type 2: 0.5% Trypsin: 0.05%	HBSS
7	Dubey P	Prostatic stem	Collagenase Type 1: 170 u/ml	DMEM

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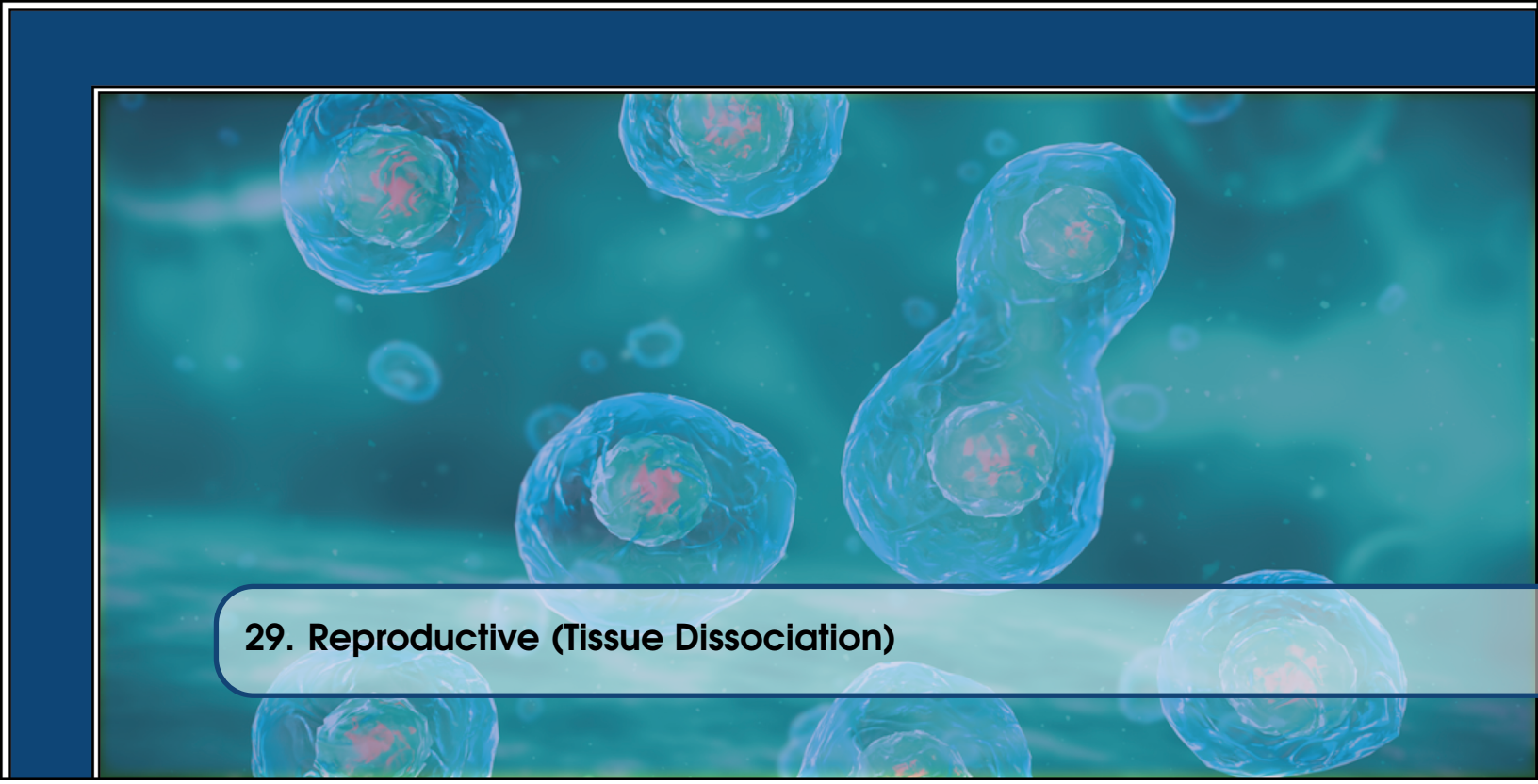

29. Reproductive (Tissue Dissociation)

Species: Bovine

Table 29.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Levy N	Corpus leuteal cells	Collagenase Type 4: 420 u/ml	M-199
2	Munson	Epithelial Endometrial	Collagenase Type 2: 0.1%	DMEM/EBSS
3	Marcus	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%	Moscona's BSS
4	Tsang PC	Leuteal	Collagenase Type 1: 0.2%	Ham's F-12
5	Coplen	Fibroblasts	Collagenase: 0.1%	Medium 199

Species: Canine

Table 29.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
6	Park	Amniotic membrane MSC	Trypsin: 0.25% Collagenase Type 1: 0.2%	LG-DMEM

Species: Chicken

Table 29.3: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Du Meihong	Primary follicles	Trypsin: 0.15% Collagenase Type 1: 0.125%	Dulbecco's phosphate buffered saline

Species: Frog

Table 29.4: **Frog**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Alagem	Oocytes	Collagenase Type 1: 0.2%	CF Medium
9	Karkanias	Oocytes	Collagenase: 0.1%	Barth's solution, CF
10	Moriarty	Oocytes	Collagenase: 0.2%	CF Medium
11	Tian	Oocytes	Collagenase Type 1: 1%	See Reference
12	Chatzidaki	Oocytes	Collagenase: 0.2%	Barth's solution
13	Cohen	Oocytes	Collagenase: 0.5%	Barth's solution, CF
14	Mruk	Oocytes	Collagenase: 0.2%	See Reference
15	Pannaccione Anna	Oocytes	Collagenase Type 1: 0.2%	See Reference

Species: Hamster

Table 29.5: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
16	Wallis	Ovary	Trypsin: 5%	Dialyzed fetal calf serum, 10% and 0.5M Methotrexate

Species: Human

Table 29.6: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
17	Patel	Endothelial colony forming	Collagenase Type 1: 0.1% Deoxyribonuclease I: 0.1% Neutral Protease: 0.075%	HBSS
18	Patel	Uterine epithelial	Pancreatin: 0.34% Hyaluronidase: 0.01% Collagenase: 0.16%	HBSS
19	Shalini	Mesenchymal stem	Collagenase Type 1: 0.4% Deoxyribonuclease I: 0.01%	DMEM/F12
20	Steigman	Mesenchymal stem	Collagenase Type 2: 10% Neutral Protease: See Reference	CMF-DPBS
21	Spessotto	Extravillanous Trophoblasts and Decidual Stromal	Trypsin: 0.1-0.25% Collagenase Type 1: 0.1% Deoxyribonuclease I: 0.02-0.05%	HBSS RPMI
22	Teng Yan	Sertoli cells	Trypsin: 2.5% Collagenase Type 1: 2% Hyaluronidase: 1%	DMEM/F-12
23	Wang Xin	Endothelial placental	Collagenase Type 1: 0.2% Trypsin: 0.2% Deoxyribonuclease I: 0.1%	DMEM
24	Sun Kang	Amnion epithelial and fibroblast	Trypsin: 0.125% Collagenase: 0.1% Deoxyribonuclease I: 0.02%	PBS
25	Fahey John V	Uterine epithelial cells	Pancreatin: 0.34% Collagenase: 0.16% Hyaluronidase: 0.16%	HBSS
26	Yusuf RZ	Chorionic villi	Trypsin: See Reference Collagenase Type 3: 100 u/ml	HBSS
27	Zhang	Stem, embryonic	Neutral Protease: 0.01% - 0.02%	DMEM
28	Nikitenko LL	Endometrial endothelial cells	Collagenase Type 1: 0.2%	McCoys medium
29	Friden BE	Corpus luteum cells	Collagenase Type 2: 0.25% Deoxyribonuclease I: .005%	PBS
30	Runesson E	Theca cells	Collagenase Type 1: 0.3% Deoxyribonuclease I: 0.0005% Hyaluronidase: 0.1%	PBS
31	Zhang J	Stromal endometrial	Collagenase Type 3: 45 u/ml Deoxyribonuclease I: .00035%	DMEM/Ham's F12

32	Bradbury.	Placental	Deoxyribonuclease I: 0.04% Collagenase Type 2: 0.1%	PSS
33	Takeuchi	Epithelial, fallopian tube	Collagenase Type 1: 1%	Medium 199
34	Branchaud	Trophoblasts, placental	Trypsin: 0.25%	EBSS, CMF
35	Jie	Trophoblasts, placental	Trypsin: 0.25%	PBS
36	Egan	Chorionic, placental	Deoxyribonuclease I: 0.003%	HBSS
37	Morrish	Placental	Trypsin: 0.25%	DMEM
38	Siegfried	Epithelial Stromal	Collagenase: 0.25%	See reference
39	Kirk	Epithelial Stromal	Collagenase Type 1: 180 u/ml	DMEM
3	Marcus	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%	Moscona's BSS
40	Arnold	Endometrium epithelial and stromal cells	Collagenase Type 1: 0.2%	HBSS
41	Oberlin	Endothelial Hematopoietic Stromal	Collagenase Type 1/2/4: 0.1%	DMEM
42	Lockwood	Decidual	Collagenase: 0.25% Deoxyribonuclease I: 6.25 u/ml	DMEM/F12
43	Meter	Uterine epithelial	Pancreatin: 0.34% Hyaluronidase: 0.01% Collagenase: 0.16%	HBSS
44	Witz Craig A	Mesothelial	Collagenase Type 1: 0.1% Deoxyribonuclease I: 0.05%	Eagle's MEM
45	Zhang J	Endometrial epithelial cells	Collagenase Type 3: 45 u/ml Deoxyribonuclease I: .00035%	DMEM/F-12
46	Huang JC	Endometrial stromal cells	Collagenase: 4000 u/ml	DMEM/F-12
47	Rinehart	Endometrial	Collagenase: 2%	RPMI 1640
48	Gargett CE	Microvascular endothelial cells	Collagenase Type 2: 0.2% Deoxyribonuclease I: 0.0015% Trypsin: 0.05%	PBS
49	Hovatta O	Follicles	Collagenase Type 2: 0.025-0.1%	EBSS
50	Rifas	Smooth muscle, uterine	Trypsin: 0.05%	EBSS
51	Auersperg	Epithelial Ovary	Trypsin: 0.125%	HBSS, CMF
52	Chan Rachel W S	Endometrial epithelial and stromal cells	Collagenase Type 3: 0.03% Deoxyribonuclease I: 0.004%	DMEM/F-12
53	Friden BE	Luteal cells	Collagenase Type 2: 0.25% Deoxyribonuclease I: 0.005%	PBS
54	Lechner	Epithelial Prostate	Trypsin: 0.1%	HBSS

Species: Insect

Table 29.7: **Insect**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
55	Ma	Ovarian	Collagenase Type 2: 0.1%	Grace's
56	Salmand	Cardiac differentiating	Collagenase Type 1: 20 u/ml Trypsin: 0.25% Deoxyribonuclease I: 4 u/ml	Schneider

Species: Mouse

Table 29.8: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
57	Venkataraman	Vaginal smooth muscle	Collagenase Type 2: 175 u/ml Elastase: 0.025%	DMEM/F12
58	White	Ovarian	Collagenase Type 4: 800 u/ml	HBSS
59	Chen	Uterine stomal	Neutral Protease: 0.6% Pancreatin: 0.25% Collagenase Type 3: 0.05%	DMEM/F12
60	Breault	Germ cells	Collagenase Type 1: 100 u/ml	HBSS
61	Iguchi	Epithelial, vagina	Collagenase Type 3: 0.1%	HBSS
62	Turner	Epithelial, prostate gland	Hyaluronidase: 0.1%	Medium 199
63	Thompson	Prostate	Trypsin: 1.0%	HBSS/ DMEM
64	Nalbandian Angele	Sertoli	Collagenase Type 2: 500 u/ml Hyaluronidase: 0.1% Deoxyribonuclease I: .0005%	DMEM
65	Eppig JJ	Oocyte-granulosa	Collagenase Type 1: 0.1% Deoxyribonuclease I: 0.02%	Waymouth
66	Martin	Spermatogonial stem	Trypsin: 0.05% Collagenase Type 1: 0.03% Deoxyribonuclease I: 80 u/ml	DMEM
67	O'Shaughnessy PJ	Testicular cells	Collagenase Type 1: 0.1%	DMEM/F-12
68	Bigsby	Epithelial Mesencymal	Trypsin: 1%	DMEM
69	Cooke	Epithelial	Trypsin: 1%	Medium 199
70	Spindle	Cumulus, one-cell embryos	Hyaluronidase: 0.1%	PBS, CMF
71	Gekas	Hematopoietic stem cells	Collagenase: 0.1%	PBS
72	Jiang	Lymphocytes	Collagenase: 450 u/ml	RPMI
73	Tsai	vaginal epithelial	Collagenase Type 3: 38 u/ml	DMEM
74	Ghosh	Uterine	Trypsin: 0.2%	HBSS
75	Getun	Testis, meiotic	Collagenase Type 1: 120 u/ml Deoxyribonuclease I: 0.001% Trypsin: 0.1%	Gey's BSS
76	Stalvcey	Leydig Testis	Deoxyribonuclease I: 0.001%	Medium 199 w/ BSA
77	Lin	Seminiferous tubules	Trypsin: 0.05%	DMEM
78	Faldikova	Leydig	Collagenase: 0.06%	Medium E 199
79	Tomooka	Epithelial	Trypsin: 0.5%	Medium 199

Species: Ovine

Table 29.9: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
80	Salamonsen	Epithelial	Collagenase: 125 - 190 u/ml	DMEM

Species: Porcine

Table 29.10: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Marcus	Interna & corpus luteum Endometrium Ovarian Uterine	Pronase: 0.1%	Moscona's BSS
81	Mather	Leydig Testis	Trypsin: 0.0003%	Lebovitz L-15 Medium
82	Ciereszko	Corpus Leuteum	Collagenase Type 4: 600 u/ml	Medium 199
83	Dirami G	Seminiferous epithelial cells	Collagenase: 0.15% Deoxyribonuclease I: .0001% Hyaluronidase: 0.15% Trypsin: 0.05%	DMEM/F12

Species: Rabbit

Table 29.11: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
84	Mulholland	Endometrial epithelial	Collagenase Type 1: 0.005%	DMEM
85	Nicosia	Mesothelial and surface epithelial Ovaries	Trypsin: 0.125%-0.5%	Medium 199
86	Setrakian	Ovarian Mesothelial	Collagenase Type 1: 300 u/ml	HBSS
87	Phillippe	Myocytes, uterine	Deoxyribonuclease I: 200 μg/ml	HBSS-HEPES buffer
88	Boulet	Myometrial	Trypsin: 0.02%, 0.03%, 0.0375%	HBSS
89	Kubota	Testicular germ	Collagenase Type 1: 0.1% Trypsin: 0.25% Deoxyribonuclease I: 0.7%	HBSS
90	Piquette	Ovarian surface epithelial and peritoneal mesothelial	Collagenase Type 1: 300 IU/ml (280 IU/mg)	HBSS, CMF

Species: Rat

Table 29.12: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
91	Leckie	Testes	Trypsin: 0.1%	Medium 199 w/ Hank's salts
92	Kassis	Uterine	Deoxyribonuclease I: 0.025%	DMEM buffered with HEPES
93	Rajan	Luteal, ovaries	Collagenase: 0.3%	McCoy's
94	Skinner	Sertoli	Trypsin: 0.15%	See Reference
95	Branham	Uterine	Trypsin: 1%	HBSS
96	Hadley MA	Sertoli cells	Collagenase Type 2: 500 u/ml Deoxyribonuclease I: 0.0005% Hyaluronidase: 0.1%	PBS
97	Duleba AJ	Ovarian theca-interstitial	Collagenase Type 1: 0.5% Deoxyribonuclease I: 0.02%	Medium 199
98	Sharma RS	Leydig cells	Collagenase: .05-0.1%	Medium 199
99	Rich	Sertolli, seminiforous tubules	Collagenase: 0.03%	Serum-free medium
100	Abou-Haila	Seminiferous tubules	Trypsin: 0.05%	Krebs-Ringer bicarbonate buffer See Reference
101	Hsueh	Testicular	Deoxyribonuclease I: 10 μg/ml	HEPES
102	Glasser	Luminal epithelial	Trypsin: 0.5%	HBSS
103	Rajan	Luteal, ovaries	Deoxyribonuclease I: 0.0004%	McCoy's
104	Azhar	Luteal	Deoxyribonuclease I: 0.0004%	Medium 199
105	Tellieria	Corpus luteum	Neutral Protease: 2.4 u/ml Deoxyribonuclease: 200u/ml	Serum-free medium See Reference
106	Rajendran	Luteal, ovaries	Hyaluronidase: 0.1%	EBSS
107	Ando	Ovary	Collagenase Type 1: 144 u/ml	McCoy's 5a

108	Abayasekara	Leydig	Trypsin: 0.02%	DMEM
109	Ramachandran	Leydig	Collagenase: 0.1%	Krebs Ringer bicarbonate buffer
110	Hadley	Sertoli	Trypsin: 0.025%	DMEM
111	Ng	Leydig Adrenal	Collagenase Type 2: 0.03% (adrenal)	Krebs Ringer bicarbonate buffer
112	Onoda	Sertoli	Hyaluronidase: 0.1%	DMEM
113	Pampfer	Uterine	Trypsin: 0.5%	PBS
114	Conti	Vaginal epithelial	Trypsin: 0.5%	PBS

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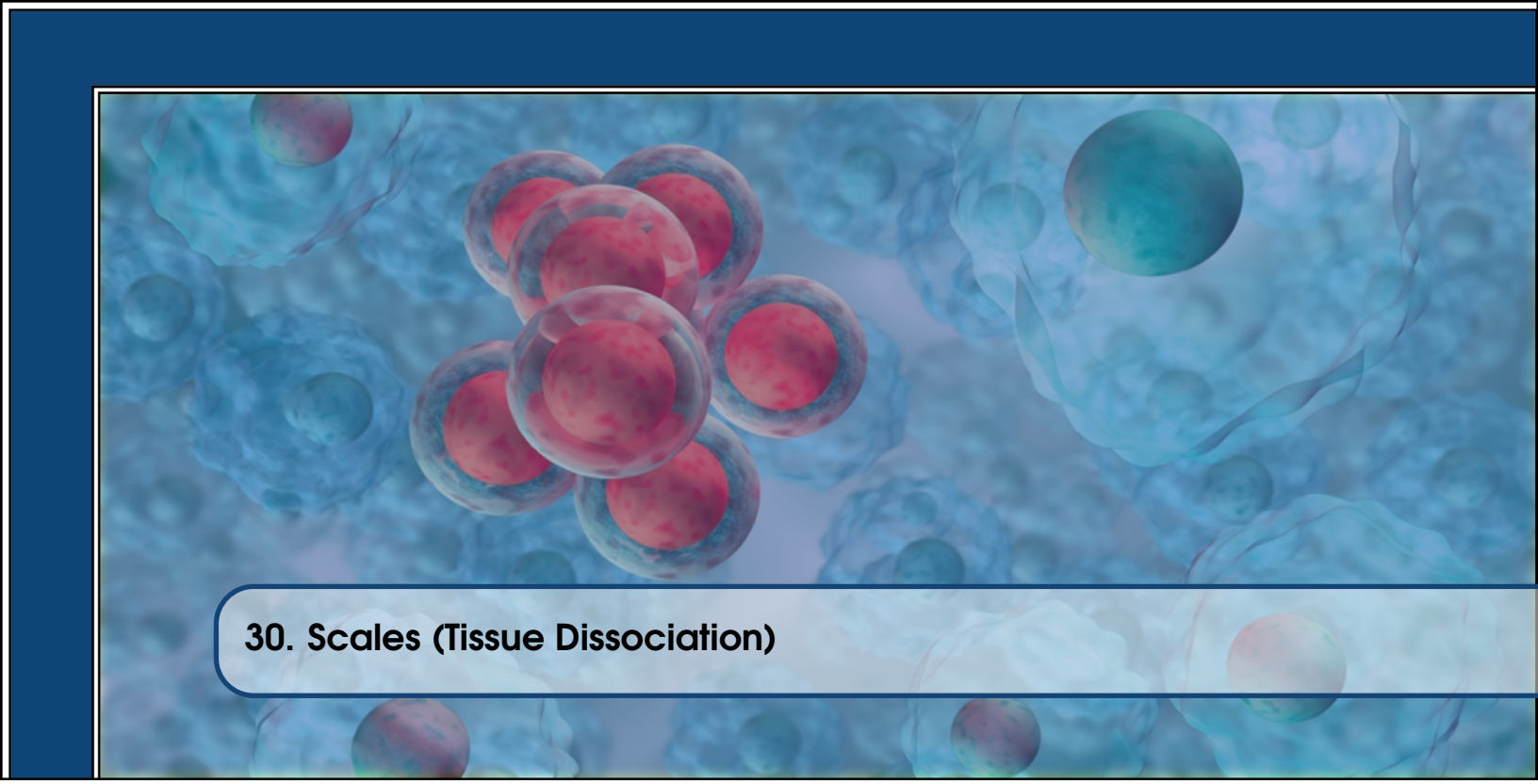

30. Scales (Tissue Dissociation)

Species: Fish

Table 30.1: **Fish**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Lo	Pigment, xanthopores	Deoxyribonuclease I: 0.005%	Medium 199 w/BSA

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31. Skin (Tissue Dissociation)

Species: Canine

Table 31.1: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Zheng	Epidermal	Collagenase: 0.35% Neutral Protease: 0.1%	DMEM

Species: Frog

Table 31.2: **Frog**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Nishikawa	Epidermal	Trypsin: 0.18%	Barth's solution, CMF

Species: Goat

Table 31.3: **Goat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Deng	Dermis isolated aggrecan sensitive	Neutral Protease: 0.5% Collagenase Type 2: 200 u/ml	DMEM

Species: Human

Table 31.4: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Cheuk	Skin	Neutral Protease: 5 u/ml Collagenase Type 3: 0.3% Deoxyribonuclease I: 0.005%	RPMI 1640
5	Karumbayaram	Fibroblasts	Collagenase animal free: 2%	HBSS
1	Zheng	Epidermal	Collagenase: 0.35% Neutral Protease: 0.1%	DMEM
6	Zhang	skin and keloid progenitor cells	Collagenase Type 1: 0.4% Neutral Protease: 0.3%	PBS

7	Tuan	Fibroblasts	Collagenase Type 1: 100-320 u/ml	DMEM
8	Yu Hong	Hair follicular epithelial	Neutral Protease: 1.25% Trypsin: 0.25%	DMEM/F-12
9	Clark	fibroblasts	Collagenase: 0.5% Trypsin: 0.25%	DMEM/F12
10	Babina Magda	Mast cells	Neutral Protease: 0.05% Collagenase Type 4: 1.0%	RPMI
11	Tuan Tai-Lan	Fibroblasts	Trypsin: 0.25%	DMEM
12	Supp Dorothy M	Keratinocytes, fibroblasts, endothelial	Neutral Protease: See Reference Collagenase: See Reference	See Reference
13	Hybbinette S	Keratinocytes	Neutral Protease: 0.25% Trypsin: 0.17% Thermolysin: 0.05%	See Reference
14	Grutzkau A	Human skin mast cells	Neutral Protease: 0.1% Collagenase Type 1: See Reference	PBS
15	Reece J C	Epidermal	Collagenase Type 2: 0.1% Neutral Protease: 0.5-1.0%	PBS
16	Harley	Epidermis plus dermis Abdomen or inner forearm	Trypsin: 0.125%	DMEM, M199
17	Judd	Keratinocytes	Trypsin: 0.05%	Dulbecco's PBS
18	Regnier	Keratinocytes	Trypsin: 0.25%	DMEM
19	Limat	Fibroblasts	Trypsin: 0.25%	CMF solution
20	Krouse	Sweat gland	Collagenase Type 2: 0.015%	See Reference
21	Peacocke	Melanocytes, skin/ foreskin	Trypsin: 0.25%	PBS
22	Lee	Human sweat glands	Collagenase: 0.2%	HBSS
23	Dover	Keratinocytes	Trypsin: 0.25%	DMEM
24	Rheinwald	Keratinocytes	Trypsin: 0.25%	See Reference
25	McCoy	Fibroblasts	Trypsin: 0.1%	HBSS
26	Bell	Sweat duct	Collagenase: 0.03%	MEM
27	Chen	Dermal fibroblasts	Neutral Protease: 0.1% Collagenase Type 1: 0.1%	DMEM
28	Nagel	Epidermal stem cells	Neutral Protease: 2 u/ml Trypsin: 2.5%	DMEM
29	Wang	Dermal fibroblasts	Collagenase: 0.5-1.0%	DMEM
30	Baudoux	Keratinocytes	Neutral Protease: 0.25%	PBS
31	Alitalo K	Epidermal keratinocytes	Trypsin: 0.25% Collagenase: 0.2% Deoxyribonuclease I: 0.001%	Eagle's MEM
32	Hirel	Keratinocytes	Trypsin: 0.25%	PBS
33	Pedersen	Human sweat duct	Collagenase Type 2: 0.2%	RPMI 1640 See Reference
34	Huschtscha	Fibroblasts	Trypsin: 0.2%	DMEM
35	Davies	Smooth muscle, fibroblasts	Trypsin: 0.055%	DMEM
36	Li	Keratinocytes	Neutral Protease: 0.4% Collagenase: 0.3%	DMEM
37	Hansbrough	Fibroblasts	Trypsin: 0.25%	DMEM
38	Liu	Keratinocytes	Trypsin: 0.3%	DMEM

Species: Mouse

Table 31.5: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
39	Gu	Epidermal	Neutral Protease: 0.5% Collagenase Type 4: 0.1%	DMEM
40	Scheibye-Knudsen	Dermal fibroblasts	Collagenase: 400 u/ml	AminoMAX II
41	King	Epidermal and dermal	Collagenase Type 4: 0.18% Collagenase/Dispase: 0.18%	PBS

42	Eidsmo	Dermal	Collagenase Type 3: 0.3% Deoxyribonuclease I: 0.0005%	HBSS
43	Baxter Ruth M	Dermal fibroblasts	Trypsin: 0.25% Collagenase Type 1: 0.25%	DMEM
44	Takanami-Ohnishi Yoko	Ear epidermal	Trypsin: 0.1% Collagenase: 0.2%	PBS
45	Farina	Dermal fibroblasts	Collagenase Type 2: 0.04% Trypsin: 0.025%	DME
46	Bradshaw AD	Fibroblasts, mesangial, smooth muscle	Trypsin: 0.25% Collagenase: See Reference Soybean Trypsin Inhibitor: .05%	DMEM
47	Montanaro F	Skin side population	Collagenase Type 4: 0.2% Neutral Protease: 1.2 u/ml	PBS
48	Crigler Lauren	Dermal	Collagenase: 0.35% Deoxyribonuclease I: See Reference	PBS
49	Cha	Microvascular endothelial	Neutral Protease: 0.005% Collagenase Type 1: 4%	DMEM

Species: Porcine

Table 31.6: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
50	Regauer S	Keratinocytes	Neutral Protease: 0.25%	Dulbecco-Vogt MEM
51	Ando	Synovial membrane and skin stem cells	Collagenase: 0.2%	DMEM

Species: Rat

Table 31.7: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
52	Vaughan	Keratinocytes	Trypsin: 1%	EBSS
53	Acheson	Fibroblasts	Trypsin: 0.2%	HEPES buffered DMEM
54	Laurent	Sebaceous	Trypsin: 0.2%	DMEM with FBS
PBS				
55	Sugihara	Dermal fibroblasts and keratinocytes	Trypsin: 0.25%	Ham's F-12

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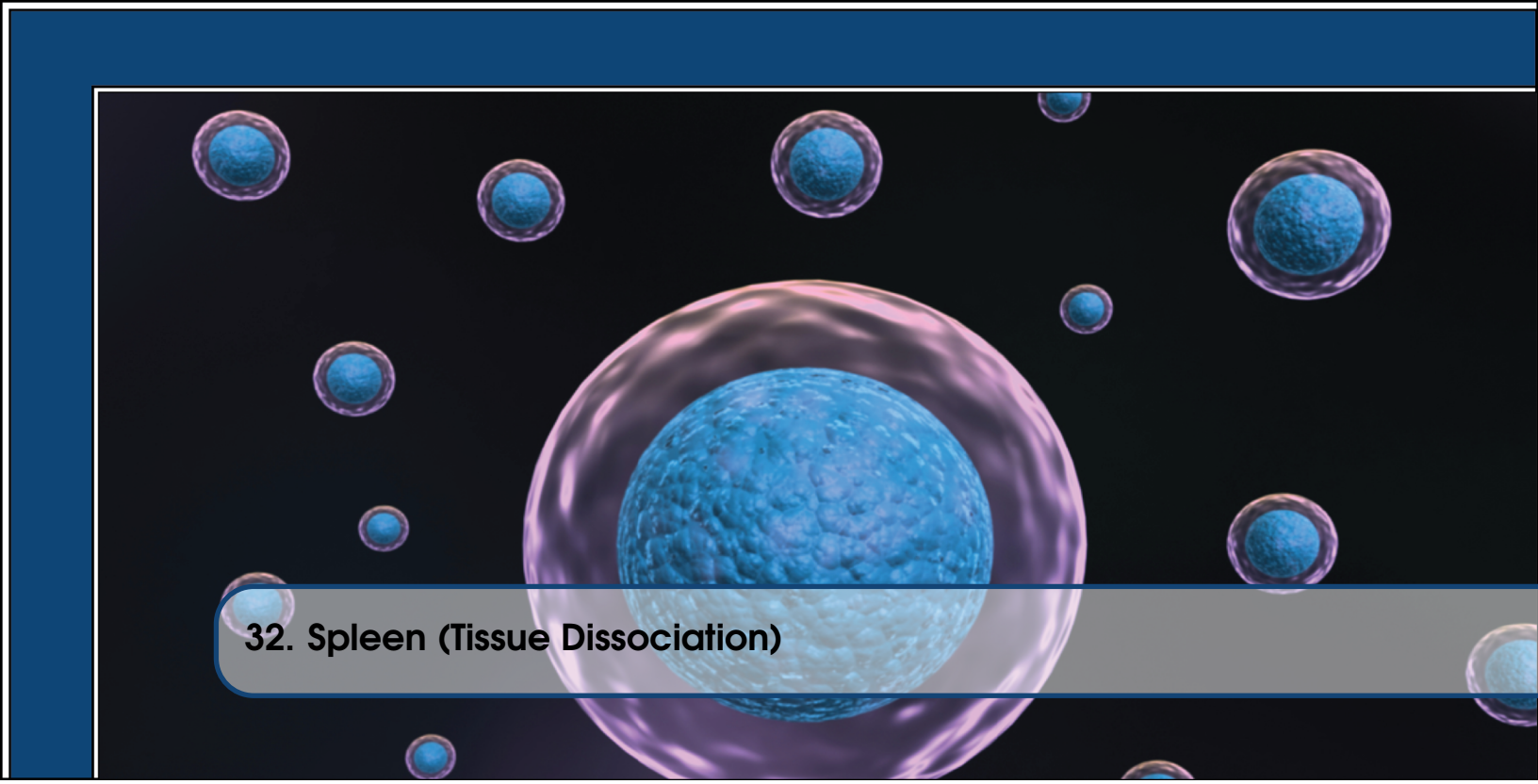

32. Spleen (Tissue Dissociation)

Species: Mouse

Table 32.1: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Yi	Splenocytes	Collagenase Type 2: 0.16% Deoxyribonuclease I: 0.002%	PBS
2	Klebanoff	Splenic	Collagenase Type 2: 0.1%	PBS
3	Shi	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml	PBS
4	Flano	Dendritic	Collagenase Type 1: 0.5%	HBSS
5	Abe	Dentritic	Collagenase: 300 u/ml Deoxyribonuclease I: 0.002%	RPMI 1640
6	Mueller	Splenic	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.1%	RPMI
7	Benedict Chris A	Splenic stromal	Collagenase Type 3: 100-400 u/ml	HBSS
8	Siragam	Leukocytes	Collagenase Type 4: 43 u/ml	RPMI 1640
9	Schiavoni	Dentritic	Collagenase Type 3: 0.1% Deoxyribonuclease I: 325 u/ml	RPMI 1640
10	Brasel K	Dentritic	Collagenase: 100 u/ml	HBSS
11	McLellan AlexanderD	Dentritic	Collagenase Type 1: 0.1% Deoxyribonuclease I: 0.001%	RPMI-1640
12	Abou Fakher	Dendritic	Collagenase Type 4: 0.05%	RPMI 1640

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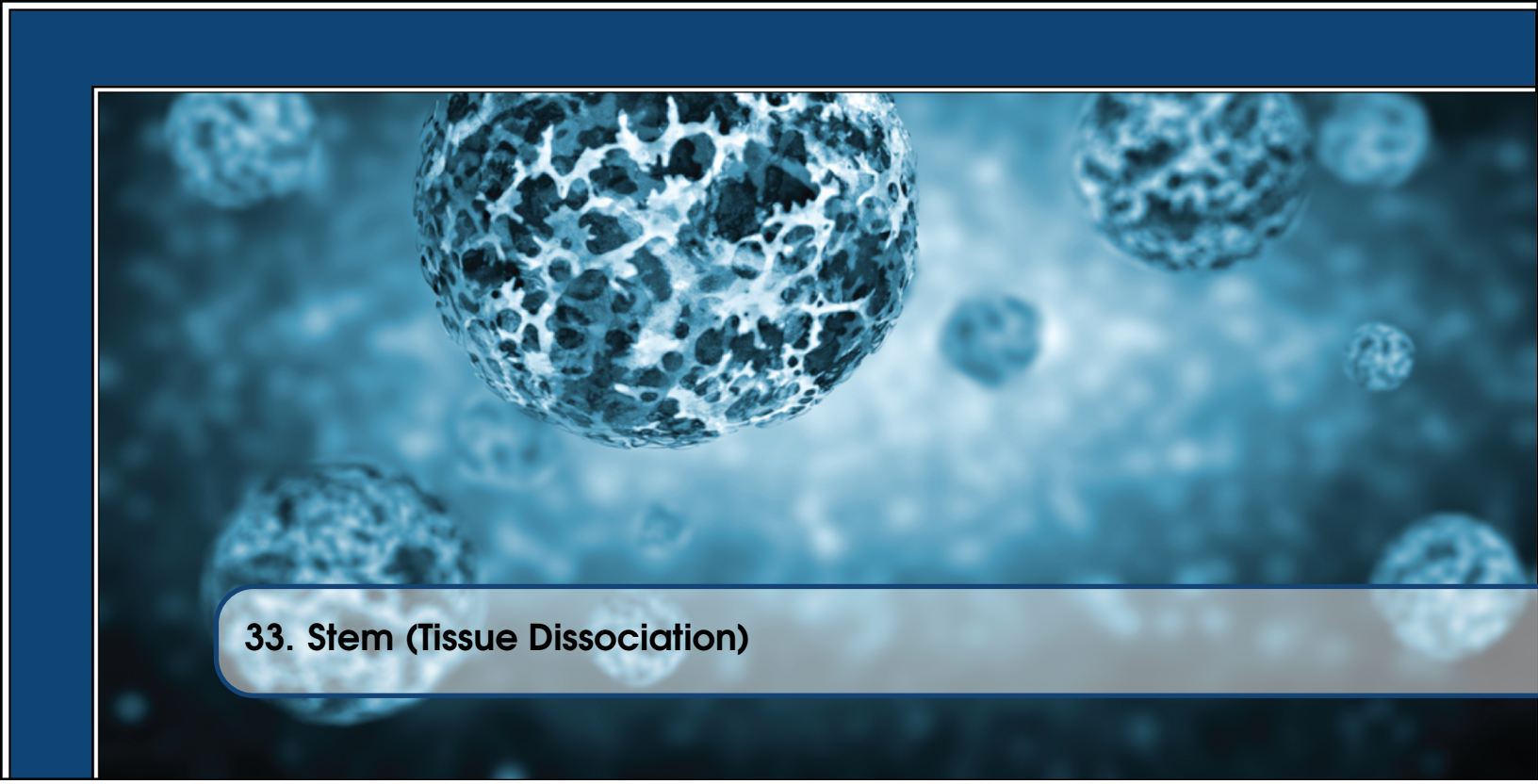

33. Stem (Tissue Dissociation)

Species: Avian

Table 33.1: **Avian**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Tandon	Adipose derived stem cells	Collagenase Type 1: 0.1%	DMEM

Species: Canine

Table 33.2: **Canine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Park	Amniotic membrane MSC	Trypsin: 0.25% Collagenase Type 1: 0.2%	LG-DMEM
3	Fischer	Adipose stem cells	Collagenase: See Reference	Media-199

Species: Equine

Table 33.3: **Equine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Vidal	Adipose derived stem cells	Collagenase Type 1: 0.1%	PBS
5	Vidal	Adipose derived stem cells	Collagenase Type 1: 0.1%	PBS

Species: Human

Table 33.4: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
6	Choudhery	Mesenchymal Stromal Cells	Collagenase Type 4: 0.2%	PBS
7	Satish	Adipose derived stem cells	Collagenase Type 2: 0.1%	HBSS
8	Wu	Dental mesenchymal stem	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM
9	Hang	Umbilical mesenchymal stem	Collagenase Type 2: 0.1% Trypsin: 0.25%	DMEM
10	Bonnamain	Dental pulp stem	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM/F12

11	Carvalho	Adipose stromal stem	Collagenase animal free: 200 u/ml	DMEM/Hams F-12
12	Sakai	Dental pulp derived stem cell	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM
13	Cervelli	Adipose-derived stem cells	Collagenase Type 1: 0.1%	PBS
14	Salehinejad	Wharton jelly mesenchymal stem	Collagenase Type 2: 0.1% Trypsin: 0.25%	DMEM
15	Shalini	Mesenchymal stem	Collagenase Type 1: 0.4% Deoxyribonuclease I: 0.01%	DMEM/F12
16	Farias	Umbilical cord stromal stem	Collagenase Type 4: 0.08% Neutral Protease: 0.138% Hyaluronidase: 0.02%	DMEM
17	Klein	Vascular wall-resident multipotent stem cells	Collagenase Type 2: 0.2% Elastase: 5 u/ml	See Reference
18	Yu	Adipose derived stem	Collagenase Type 1: 0.1%	DMEM/Ham's F-12
19	Tan	Adipose tissue-derived stem	Collagenase Type 2: 1.0%	DMEM/F12
20	Zeddou	Umbilical cord mesenchymal	Hyaluronidase: 0.05% Collagenase: 0.08%	DMEM
21	Du	Corneal stromal stem	Neutral Protease: 1.2 u/ml Collagenase: 0.1%	DMEM
22	Hjelmeland	Glioma stem cells	PDS kit: per instructions	neurobasal medium
23	Huang	Dental pulp and apical papilla stem cells	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	MEM
24	Hareendran	Adipose stem cells	Collagenase Type 1: 0.1%	DMEM
25	Varghese	Filum terminale neural progenitor	Trypsin: See Reference	DMEM/F-12
26	Zhang	skin and keloid progenitor cells	Collagenase Type 1: 0.4% Neutral Protease: 0.3%	PBS
27	Jeong	Adipose derived stem cells	Collagenase Type 1: 0.075%	DMEM
28	Wauthier	Hepatic stem cells and heptoblasts	Collagenase Type 4: 0.014-0.06%	various
29	Secco	Umbilical mesenchymal stem	Collagenase: 0.1%	DMEM
30	Nesti	Muscle derived multiprogenitor cells	Collagenase Type 2: 0.05%	DMEM
31	Pilgaard	Stem Cells	Collagenase: 280 u/ml	D-PBS
32	Li	Pancreatic cancer stem cells	Collagenase Type 4: 200 u/ml	medium 199
33	Welter	Bone marrow derived MSC	Trypsin: 0.05% Papain: 0.0025%	DMEM
34	Kern Susanne	Mesenchymal stem	Collagenase Type 1: 0.075%	DMEM
35	Boquest Andrew C	Stromal stem cells	Collagenase Type 1: 0.2%	HBSS
36	Portmann-Lanz CBettina	Placental mesenchymal stem	Collagenase Type 2: 270 u/ml Neutral Protease: 2.4 u/ml	MEM
37	Devireddy	Adult stem cells	Collagenase Type 1: 0.1%	PBS
38	Covas DT	Umbilical vein mesenchymal stem cells	Collagenase: 1%	PBS
39	Uchida	Central nervous system stem	Collagenase: 0.1% Hyaluronidase: 0.1%	HBSS
40	Thomson JA	Embryonic stem	Neutral Protease: 1% Collagenase Type 4: 0.1%	DMEM
41	Alessandri Giulio	Muscle-derived stem cells	Trypsin: 0.25%	DMEM/F12
42	Sun	Adult human adipose stem cells	Collagenase Type 2: 0.075%	DMEM
43	Miura	Stem cells Human exfoliated deciduous teeth	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	See Reference
44	Bi Yanming	Tendon stem/progenitor	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM

45	Al-Saqi	Adipose derived mesenchymal stem	Collagenase Type 2: 0.1%	See Reference
46	Koellensperger	Adipose derived stem cells	Collagenase Type 1: 0.15%	DMEM
47	Nagel	Epidermal stem cells	Neutral Protease: 2 u/ml Trypsin: 2.5%	DMEM
48	Mitchell James B	Adipose-derived adult stem	Collagenase Type 1: 0.1%	DMEM/F-12 Ham's
49	Kambe	Human skin mast cells	Collagenase Type 2: 0.15% Hyaluronidase: 0.07% Deoxyribonuclease I: 0.03%	HBSS
50	Papini S	Human epidermal keratinocyte stem cells	Neutral Protease: 0.5%	DMEM
51	Guilak	Adipose derived adult stem cells	Collagenase Type 1: 0.1%	DMEM/F-12
52	Aust	Adipose derived adult stem cells	Collagenase Type 1: 0.1%	DMEM-Ham's F-12
53	Malhi	Epithelial progenitor	Collagenase: 0.03%	DMEM
54	Kossack	Spermatogonial stem cells	Collagenase: 1% Deoxyribonuclease I: 0.22% Trypsin: 0.4%	DMEM
55	Lei	Adipose derived adult stem cells	Collagenase Type 1: 0.1%	DMEM
56	Blasi	Adipose derived stem cells	Collagenase: 0.25% Deoxyribonuclease I: 0.002%	PBS

Species: Monkey

Table 33.5: **Monkey**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
57	Nagano	Primate spermatogonial	Collagenase Type 2: 0.1% Trypsin: 0.05% Deoxyribonuclease I: 0.1%	DMEM
58	Chen	Embryonic stem	Collagenase Type 4: 0.08%	DMEM

Species: Mouse

Table 33.6: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
59	Shi	Spleen, bone marrow endothelial	Collagenase Type 4: 0.3-1.0% Deoxyribonuclease I: 20 u/ml	PBS
60	Han	Stem and progenitor	Collagenase Type 2: 0.2%	HBSS
61	Cho	Adipose tissue-derived stem	Collagenase Type 1: 0.075%	Modified Eagles
62	Hutton	Neural progenitor cell	Papain: 10 ul/ml	PBS
63	Breault	Germ cells	Collagenase Type 1: 100 u/ml	HBSS
64	Meletis	Neural stem cells	Papain: See Reference	DMEM/F-12
65	Li Wen-Lin	Liver epithelial progenitor cells	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.05%	DMEM
66	Bertoncello	Bone marrow	Collagenase Type 1: 0.15% Neutral Protease: 0.15%	PBS
67	Seaberg	Neural progenitor	PDS kit: per instructions	See Reference
68	Futami	Synovial mesenchymal	Collagenase: 0.1% Deoxyribonuclease I: 0.005%	DMEM
69	Gritti	Neural stem cells	Papain: 0.1%	DMEM/F-12
70	Wang	Cardiac progenitor	Collagenase Type 1: 0.1%	DMEM
71	Staszkiewicz	Ear mesenchymal stem	Collagenase Type 1: 0.2%	DMEM/F12
72	Howell	Pluripotent stem cells	Collagenase Type 1: 220 u/ml Neutral Protease: 33 u/ml	MEM
73	Gritti A	Neural subventricular zone	Trypsin: 0.13% Hyaluronidase: 0.067%	DMEM/F12
74	Di Rocco Giuliana	Adipose mesenchymal stem	Collagenase: 0.2%	PBS
75	Jackson	Muscle hematopoietic stem cells	Collagenase: 0.2% Trypsin: 0.1%	DMEM

76	Bracko	Neural stem cells	Papain: 0.01% Neutral Protease: 0.1% Deoxyribonuclease I: 0.01%	DMEM/F12
77	Xu	Bone marrow mesenchymal stem	Collagenase Type 1: 0.25%	RPMI 1640
78	Burger	Prostate epithelial/stem	Collagenase Type 2: 0.5% Trypsin: 0.05%	HBSS
44	Bi Yanming	Tendon stem/progenitor	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM
79	Chow	Lung mesenchymal stem	Collagenase Type 2: 0.2%	HBSS
80	Sugii	Adipose derived stem	Collagenase Type 1: 0.025%	HBSS
81	Kotton	Liver derived stem cells	Collagenase Type 1: 0.1% Neutral Protease: 2.4 u/ml	HBSS
82	Deleyrolle	Adult neural stem	Trypsin: 0.05%	DMEM
83	Lee	Cerebellar stem cells	Papain: 10u/ml Deoxyribonuclease I: 250 u/ml	Dulbecco's PBS
84	Lu Shi-Jiang	HES-BC cells	Trypsin: 0.05%	DMEM
85	Schatten Gerald	Embryonic fibroblast feeder cells	Collagenase Type 4: 0.1%	DMEM
86	Estivill-Torrus	Cortical progenitors	PDS kit: per instructions	Serum free medium
87	Janebodin	Dental Pulp Stem Cells	Collagenase Type 4: 0.2% Neutral Protease: 1.2 u/ml	PBS
88	Hotta	Enteric neural crest progenitors	Neutral Protease: 0.5% Collagenase animal free: 0.05%	DMEM/F12

Species: Porcine

Table 33.7: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
89	Ando	Synovial membrane and skin stem cells	Collagenase: 0.2%	DMEM
90	Williams	Adipose mesenchymal stem	Collagenase Type 1: 0.1%	DMEM

Species: Rabbit

Table 33.8: **Rabbit**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
91	Zhang	Tenocytes and tendon stem cells	Collagenase Type 1: 0.3% Neutral Protease: 0.4%	DMEM

Species: Rat

Table 33.9: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
92	Emre	Mesenchymal Stem Cells	Collagenase Type 1: 0.075%	DMEM
93	Wei	Adipose Derived Stem	Collagenase Type 1: 0.1%	DMEM
94	Ray	Central nervous system stem cells	Papain: 0.01% Neutral Protease: 0.1%	DMEM/F-12
95	Palmer	Neural stem cells	Papain: 2.5 u/ml Deoxyribonuclease I: 250 u/ml Neutral Protease: 1 u/ml	DMEM/F-12
96	Maric	Neurons and progenitor	Papain: 20 u/ml	EBSS
97	Gobbel GT	Neural stem cells	Papain: 0.09% Deoxyribonuclease I: 0.1%	EBSS
98	Kruger	Gut Neural Crest Stem Cells	Collagenase Type 4: 0.1% Trypsin: 0.025%	HBSS
99	Zeng Yuan-Shan	Neutral stem cells, Schwann cells	Trypsin: 0.25% Collagenase: 0.16%	DMEM/F12

100	Veronesi	Mesenchymal Stromal Cells	Collagenase Type 2: 0.075%	DMEM

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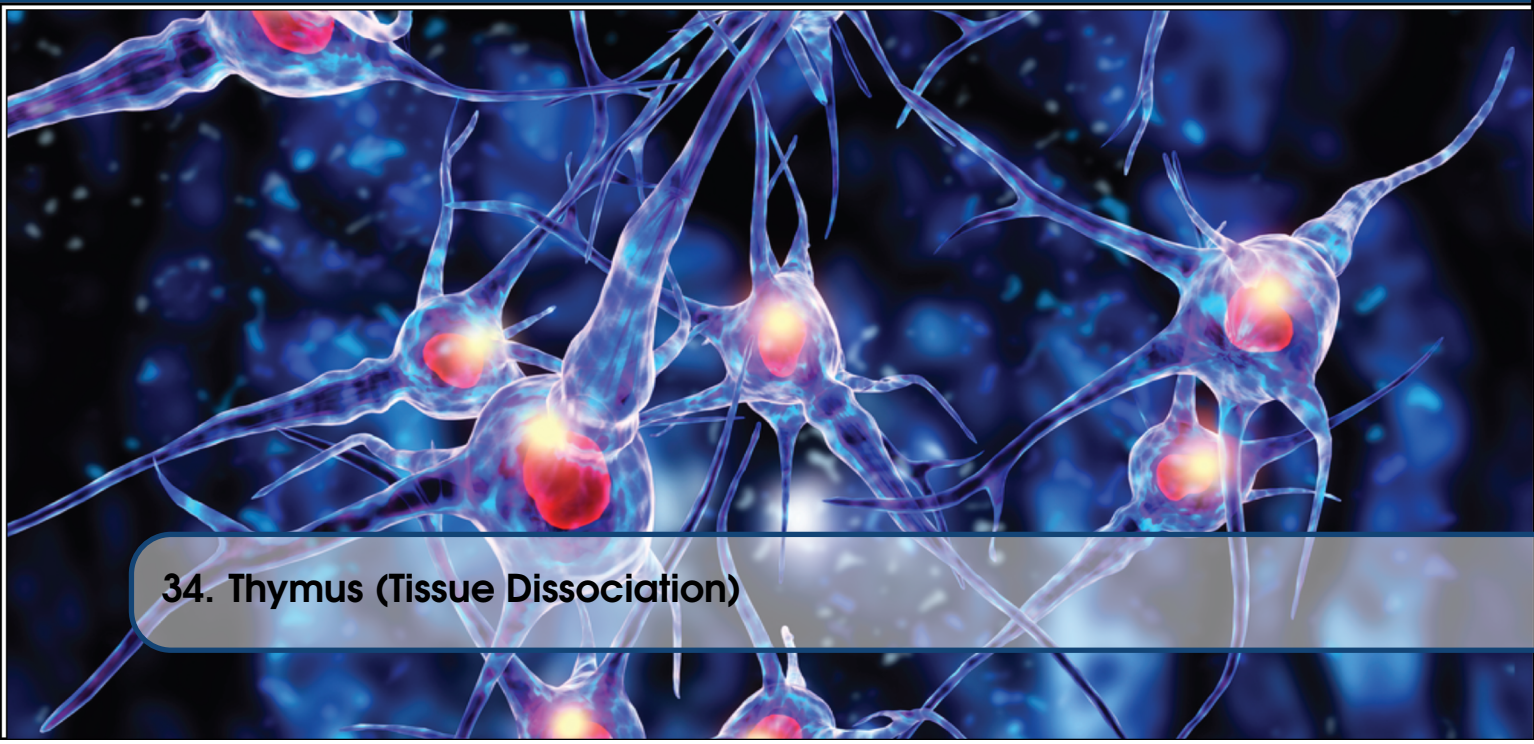

34. Thymus (Tissue Dissociation)

Species: Human

Table 34.1: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Vandenabeele S	Dentritic	Collagenase Type 2: 0.1% Deoxyribonuclease I: 0.002%	RPMI 1640

Species: Mouse

Table 34.2: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
2	Phillips	Stromal	Collagenase Type 3: 0.2% Hyaluronidase: 0.1%	RPMI 1640
3	Schiavoni	Dentritic	Collagenase Type 3: 0.1% Deoxyribonuclease I: 325 u/ml	RPMI 1640
4	Ropke	Epithelial, thymus	Neutral Protease: 1.5 μg/ml	DMEM
5	Smith KM	Thymic	Collagenase Type 3: 100-400 u/ml	HBSS
6	Ehmann	Epithelial	Collagenase Type 3: 0.1%	DMEM
7	Jones	Thymus	Collagenase Type 3: 150 u/ml	DMEM

Species: Rat

Table 34.3: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
8	Masuda	Thymic epithelial	Collagenase Type 3: 0.1%	DMEM
9	Masuda	Epthelial	Collagenase Type 3: 0.1%	Eagle's MEM Serum-free
10	Bonfanti	Thymic	Trypsin: 0.05%	HBSS

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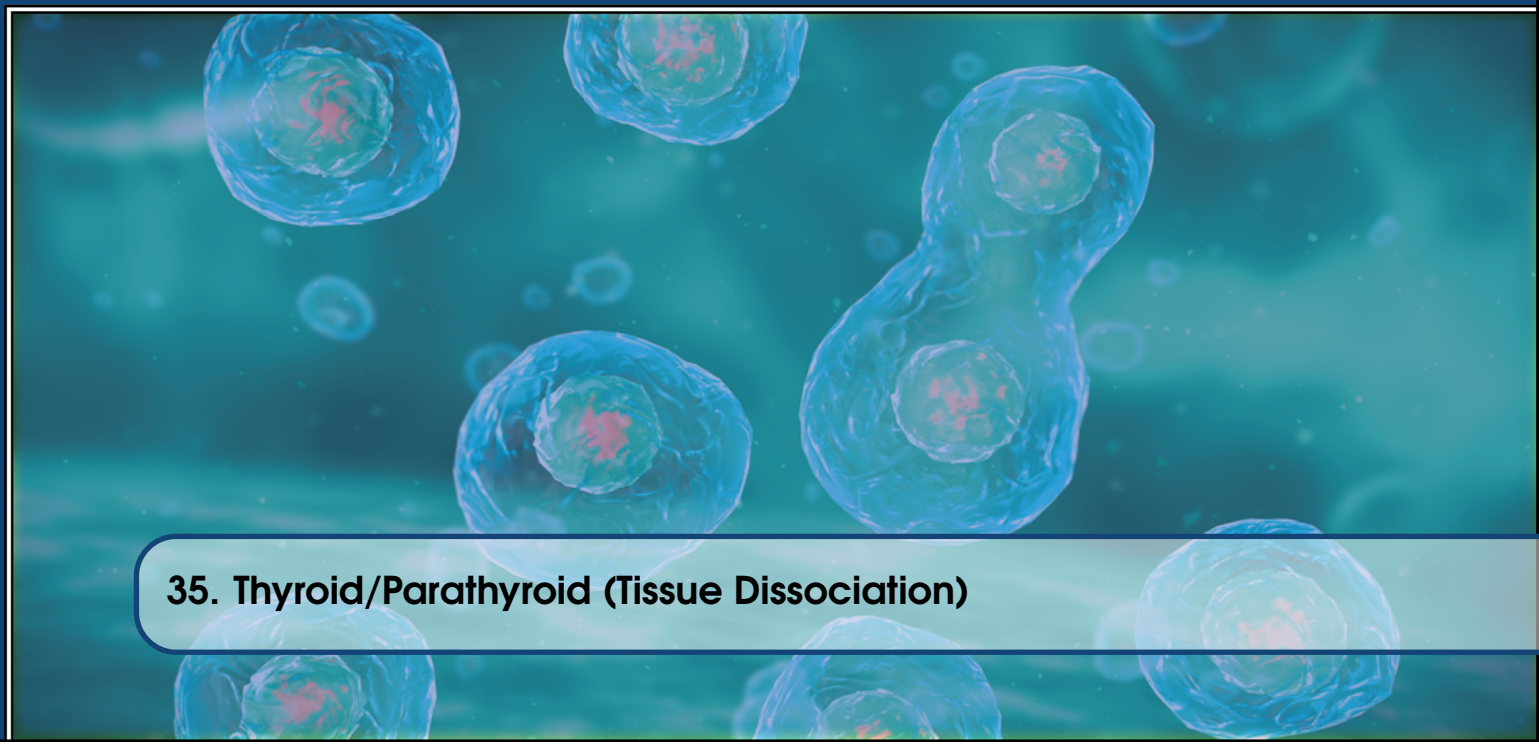

35. Thyroid/Parathyroid (Tissue Dissociation)

Species: Bovine

Table 35.1: **Bovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Wallace	Parathyroid glands	Deoxyribonuclease I: 0.0075%	HEPES buffer
2	Brown	Parathyroid	Deoxyribonuclease I: 0.004%	Eagle's #2 medium without bicarbonate
3	Nygren	Parathyroid	Deoxyribonuclease I: 0.005%,	HEPES Ham's F10
4	Tong	Thyroid	Trypsin: 0.004%	EBSS

Species: Chicken

Table 35.2: **Chicken**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
5	Hilfer	Thyroid Muscle Heart	Collagenase: 0.25%	Tyrode's saline, potassium free
6	Spooner	Thyroid follicular	Collagenase: 0.2%	Tyrode's solution, CMF

Species: Human

Table 35.3: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
7	Patel	Endothelial	Collagenase Type 2: 0.1%	DMEM
8	Gianoukakis	Thyrocytes	Collagenase Type 1: 130 u/ml Neutral Protease: 0.5 u/ml	HBSS
9	Howie	Thyrocytes	Neutral Protease: 0.5% Trypsin: 0.25% Collagenase: 0.1%	EBSS
10	Miller	Thyroid	Collagenase: 300 u/ml	Ham's F-12/MEM

Species: Mouse

Table 35.4: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
11	Martin	Thyroid	Neutral Protease: 0.0012 u/ml Collagenase Type 2: 0.25 u/ml	RPMI 1640

Species: Ovine

Table 35.5: **Ovine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
12	Kerkof	Thyroid	Collagenase: 0.2%	Puck's Saline F

Species: Porcine

Table 35.6: **Porcine**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
4	Tong	Thyroid	Trypsin: 0.004%	EBSS

Species: Rat

Table 35.7: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
13	Arauchi	Thyroid	Collagenase Type 2: 0.15% Collagenase Type 4: 0.15%	DMEM

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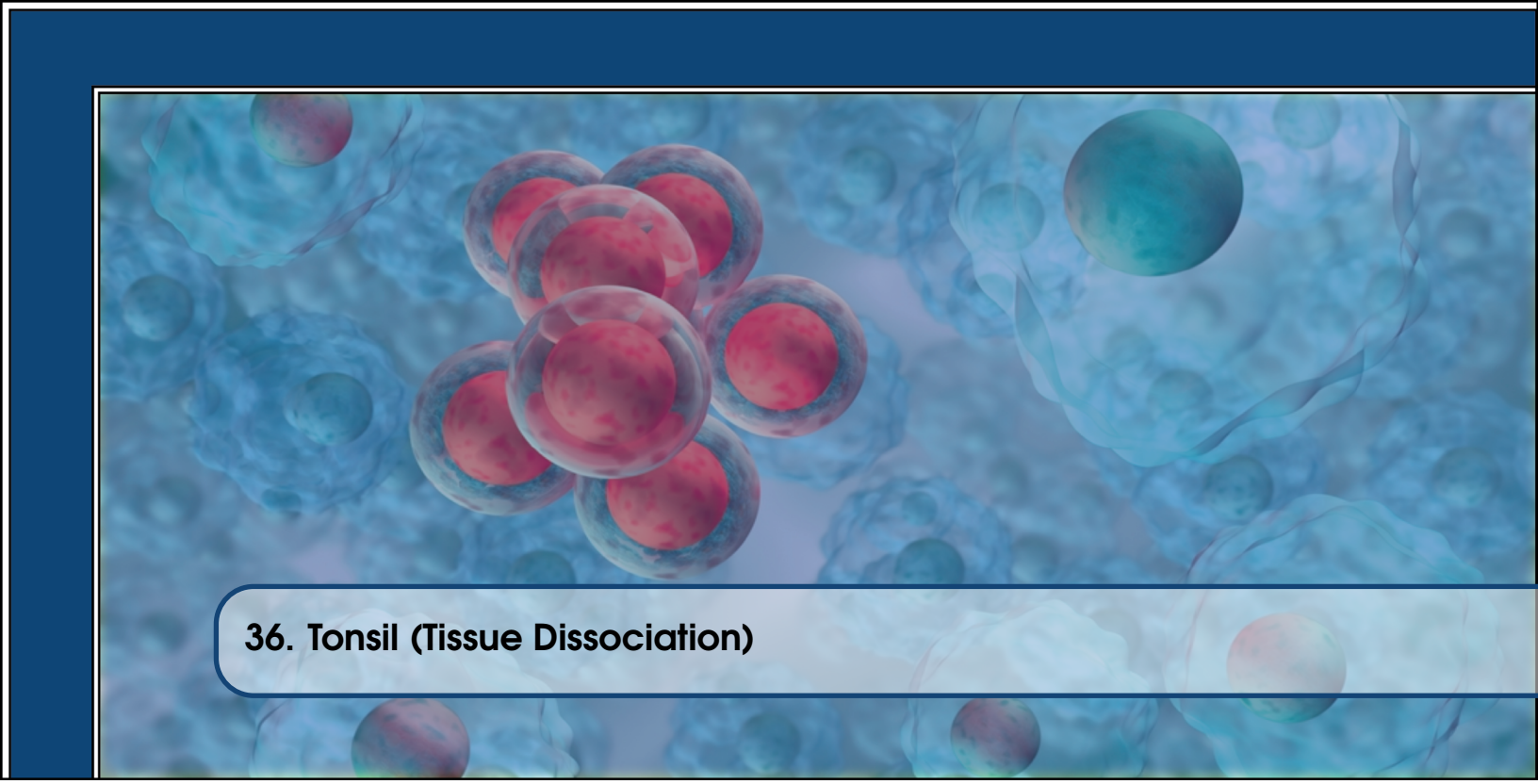

36. Tonsil (Tissue Dissociation)

Species: Human

Table 36.1: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Grammer AC	Tonsillar mononuclear cells	Collagenase Type 1: 210 u/ml Deoxyribonuclease I: 90 u/ml	RPMI
2	Grammer Amrie C	Tonsillar mononuclear cells	Collagenase Type 1: 210 u/ml Deoxyribonuclease I: 90 u/ml	RPMI

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37. Tumor (Tissue Dissociation)

Species: Hamster

Table 37.1: **Hamster**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
1	Min	Buccal pouch	Neutral Protease: 0.24%	CMF HBSS
2	Gonzalez	Tumor	Hyaluronidase: 0.1%	Waymouth's MB

Species: Human

Table 37.2: **Human**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
3	Liang	Non-small cell lung tumor	Collagenase Type 1: 0.1% Collagenase Type 2: 0.25%	RPMI-1640
4	Welte	Melanoma	Collagenase Type 4: 0.1% Deoxyribonuclease I: 0.01%	PBS
5	Chou	Tumor	Collagenase Type 1: 0.1% Hyaluronidase: 0.01% Deoxyribonuclease I: 0.01%	DMEM/F12
6	Zhou	Colorectal cancer	Collagenase Type 4: 2% Deoxyribonuclease I: 0.1%	RPMI 1640
7	Quintana	Tumorigenic melanoma	Collagenase Type 4: 200 u/ml Trypsin: 0.05% Deoxyribonuclease I: 50-100 u/ml	PBS
8	Kim	Pancreatic tumor	Collagenase Type 4: 200 u/ml	RPMI-1640
9	Sauvageot	Tumor	Collagenase Type 4: 0.1% Hyaluronidase: 0.07% Deoxyribonuclease I: 0.04%	See Reference
10	Varnat	Colon cancer	Collagenase Type 1: 300 u/ml Hyaluronidase: 100 u/ml	DMEM/F12
11	Liu	Breast epithelial	Collagenase Type 3: 200 u/ml	HBSS
12	Nakashiro Koh-Ichi	Prostate stromal cells	Collagenase Type 1: 0.1%	RPMI 1640

13	Emenaker N	Colonocytes	Collagenase:	DMEM/F12
14	MacLeod	Epithelial, fibroblasts	Trypsin: 0.25%	Ham's F-12
15	Hague	Colon adenocarcinoma	Hyaluronidase: 100 u/ml	DMEM
16	Beaupain	Tumor, breast	Hyaluronidase: 100 u/ml	DMEM
17	Kruse	Glioma	Hyaluronidase: 0.01%	HBSS
18	Emerman	Epithelial	Collagenase: 2.0%	DMEM/Ham's F-12
19	Boyd	Tumor	Neutral Protease: 0.24%	DMEM/Ham's F-12
20	Sacks	Tumor	Trypsin: 0.05%	DMEM
21	Brattain	Tumor, colon	Trypsin: 0.25%	McCoy's
22	Leung	Tumor, breast	Neuraminidase: 0.8 u/ml	HBSS
23	Friedman	Epthelial and tumor Colon	Collagenase: 300 u/ml	PBS medium 199 or medium F 12
24	Creasey	Melanoma Metastatic tumors	Collagenase Type 3: 0.10%	DMEM
25	Lasfargues	Mammary tumors, hard	Collagenase: 0.10%	RPMI-1640 w/ 5% Fetal Calf Serum
26	Sheela S	Neurofibroma	Neutral Protease: 1.25 u/ml Collagenase Type 1: 0.05% Hyaluronidase: 0.1%	L-15
27	Nishio Jun	Human synovial sarcoma	Collagenase Type 2: 200 u/ml	DMEM/F-12

Species: Mouse

Table 37.3: **Mouse**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
28	Liu	Mammary tumor	Collagenase: 0.15% Hyaluronidase: 0.020%	DMEM/F12
29	Chen	Rhabdomycosarcoma tumor	Trypsin: 0.012% Collagenase Type 2: 0.1%	DMEM
30	Kazerounian	Tumor endothelial	Collagenase Type 1: 0.2%	HBSS
31	Sharon	Fibroblasts	Collagenase Type 2: 0.25% Collagenase Type 4: 0.25% Deoxyribonuclease I: 0.05%	DMEM
32	Mazzoleni	Prostate tumor	Collagenase Type 4: 1,600 u/ml	DMEM/F12
33	Vaughan	Lung tumor	Neutral Protease: 50 u/ml Collagenase: 400 u/ml Deoxyribonuclease I: 50 u/ml	DMEM
34	Rasheed	Pancreatic Cancer Stem	Collagenase Type 4: 200 u/ml Neutral Protease: 0.6 u/ml	DMEM
35	Kwong	Tumor infiltration lymphocytes	Collagenase Type 1: 0.25% Collagenase Type 2: 0.15% Collagenase Type 4: 0.1% Hyaluronidase: 0.025%	RPMI 1640
36	Prince	Tumor	Collagenase Type 3: 200 u/ml	RPMI-1640
37	Oliver	Granule cell precursors, pre-neoplastic and tumor cells	Papain: 10 u/ml Deoxyribonuclease I: 250 u/ml	Neurobasal/B27
38	Bergers Gabriele	Pancreatic tumor	Collagenase Type 2: 0.5% Collagenase Type 4: 0.5% Deoxyribonuclease I: 0.2%	PBS
39	Uekusa Yasuhiro	Tumor-infiltrating lymphocyte	Collagenase: 200 u/ml	RPMI 1640
40	Yang	Mammary tumors Epithelial	Collagenase: 1.0%	HBSS
41	Berger	Tumor endothelial	Collagenase Type 4: 500 u/ml Collagenase Type 2: 550 u/ml Deoxyribonuclease I: 3 u/ml	PBS
8	Kim	Pancreatic tumor	Collagenase Type 4: 200 u/ml	RPMI-1640
42	Hida Kyoko	Tumor associated endothelial cells	Collagenase Type 2:	See Reference
43	Hosick	Neoplastic Epithelial tumor	Trypsin:	DMEM

44	Kopelovich	Mammary	Trypsin NF 1:250: 0.25%	HBSS
45	Watkins	Dendritic, macrophages	Collagenase (1 or 4): 100-200 u/ml Deoxyribonuclease I: 0.01%	RPMI
46	Arbiser JL	Melanoma tumor cells	Collagenase Type 2: 0.5%	DMEM

Species: Rat

Table 37.4: **Rat**

Ref. #	1st Author	Cell(s)	Enzyme(s)	Medium
47	Brennan	Yolk sac tumor	Trypsin: 0.01%	DMEM
48	Gazdar	Tumor, islet	Trypsin: 0.05%	Medium 199
49	Essner	Ascites hepatoma	Trypsin: 0.1%	Phosphate buffer See Reference
50	Masuda	Epthelial	Collagenase Type 3: 0.1%	Eagle's MEM Serum-free
51	Duarte	Tumor	Collagenase Type 1: 0.16% Hyaluronidase: 0.002% Deoxyribonuclease I: 0.006%	DMEM/F12
52	Sharma N	Sponge infiltrating cells	Collagenase Type 4: 0.15% Deoxyribonuclease I: 0.02%	RPMI 1640
53	Cohen	Epithelial, cancer and tumor	Collagenase: 0.1%	Eagles's MEM

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38. Tissue Culture Glossary

38.1 Tissue Culture Glossary

- **Adventitious:** Developing from unusual points of origin, such as shoots or root tissues from callus or embryos from sources other than zygotes. This term can also be used to describe agents which contaminate cell cultures.
- **Anchorage-dependent cells or cultures:** Cells, or cultures derived from them, which will grow, survive, or maintain function only when attached to a surface such as glass or plastic. The use of this term does not imply that the cells are normal or that they are or are not neoplastically transformed.
- **Aneuploid:** The situation which exists when the nucleus of a cell does not contain an exact multiple of the haploid number of chromosomes; one or more chromosomes being present in greater or lesser number than the rest. The chromosomes may or may not show rearrangements.
- **Asepsis:** Without infection or contaminating microorganisms.
- **Aseptic technique:** Procedures used to prevent the introduction of fungi, bacteria, viruses, mycoplasma or other microorganisms into cell, tissue and organ culture. Although these procedures are used to prevent microbial contamination of cultures, they also prevent cross contamination of cell cultures as well. These procedures may or may not exclude the introduction of infectious molecules.
- **Attachment efficiency:** The percentage of cells plated (seeded, inoculated) which attach to the surface of the culture vessel within a specified period of time. The conditions under which such a determination is made should always be stated.
- **Autocrine cell:** In animals, a cell which produces hormones, growth factors or other signaling substances for which it also expresses the corresponding receptors. (See also Endocrine and Paracrine.)
- **Axenic culture:** A culture without foreign or undesired life forms. An axenic culture may

include the purposeful cocultivation of different types of cells, tissues or organisms.

- **Callus:** An unorganized, proliferative mass of differentiated plant cells; a wound response.
- **Cell culture:** Term used to denote the maintenance or cultivation of cells *in vitro* including the culture of single cells. In cell cultures, the cells are no longer organized into tissues.
- **Cell generation time:** The interval between consecutive divisions of a cell. This interval can best be determined, at present, with the aid of cinephotomicrography. This term is not synonymous with "population doubling time".
- **Cell hydridization:** The fusion of two or more dissimilar cells leading to the formation of a synkaryon.
- **Cell line:** A cell line arises from a primary culture at the time of the first successful subculture. The term "cell line" implies that cultures from it consist of lineages of cells originally present in the primary culture. The terms finite or continuous are used as prefixes if the status of the culture is known. If not, the term line will suffice. The term "continuous line" replaces the term "established line". In any published description of a culture, one must make every attempt to publish the characterization or history of the culture. If such has already been published, a reference to the original publication must be made. In obtaining a culture, as originally named and described, must be maintained and any deviations in cultivation from the original must be reported in any publication.
- **Cell strain:** A cell strain is derived either from a primary culture or a cell line by the selection or cloning of cells having specific properties or markers. In describing a cell strain, its specific features must be defined. The terms finite or continuous are to be used as prefixes if the status of the culture is known. If not, the term strain will suffice. In any published description of a cell strain, one must make every attempt to publish the characterization or history of the strain. If such has already been published, a reference to the original publication must be made. In obtaining a culture from another laboratory, the proper designation of the culture, as originally named and described, must be maintained and any deviations in cultivation from the original must be reported in any publication.
- **Chemically defined medium:** A nutritive solution for culturing cells in which each component is specifiable and ideally, is of known chemical structure.
- **Clonal propagation:** Asexual reproduction of plants that are considered to be genetically uniform and originated from a single individual or explant.
- **Clone:** In animal cell culture terminology a population of cells derived from a single cell by mitoses. A clone is not necessarily homogeneous and , therefore, the terms clone and cloned do not indicate homogeneity in a cell population, genetic or otherwise. In plant culture terminology, the term may refer to a culture derived as above or it may refer to a group of plants propagated only be vegetative and asexual means, all members of which have been derived by repeated propagation from a single individual.
- **Cloning efficiency:** The percentage of cells plated (seeded, inoculated) that form a clone. One must be certain that the colonies formed arose from single cells in order to properly use this term. (See Colony forming efficiency)
- **Colony forming efficiency:** The percentage of cells plated (seeded, inoculated) that form a colony.
- **Complementation:** The ability of two different genetic defects to compensate for one another.
- **Contact inhibition of locomotion:** A phenomenon characterizing certain cells in which two cells meet, locomotory activity diminishes, and the forward motion of one cell over the surface of the other is stopped.

- **Continuous cell culture:** A culture which is apparently capable of an unlimited number of population doublings; often referred to an as immortal cell culture. Such cells may or may not express the characteristics of *in vitro* neoplastic or malignant transformation. (See also Immortalization)
- **Crisis:** A stage of the *in vitro* transformation of cells. It is characterized by reduced proliferation of the culture, abnormal mitotic figures, detachment of cells from the culture substrate, and the formation of multinucleated or giant cells. During this massive cultural degeneration, a small number of colonies usually, but not always, survive and give rise to a culture with an apparent unlimited *in vitro* lifespan. This process was first described in human cells following infection with an oncogenic virus (SV40). See also Cell line, *In vitro* transformation and *In vitro* senescence.
- **Cryopreservation:** Ultra-low temperature storage of cells, tissues, embryos or seeds. This storage is usually carried out using temperatures below -100°C.
- **Cumulative population doublings:** See Population doubling level.
- **Cybrid:** The viable cell resulting from the fusion of a cytoplast with a whole cell, thus creating a cytoplasmic hybrid.
- **Cytoplast:** The intact cytoplasm remaining following the enucleation of a cell.
- **Cytoplasmic hybrid:** Synonymous with "cybrid"
- **Cytoplasmic inheritance:** Inheritance attributable to extranuclear genes; for example genes in cytoplasmic organelles such as mitochondria or chloroplasts, or in plasmids, etc.
- **Density-dependent inhibition of growth:** Mitotic inhibition correlated with increased cell density.
- **Differentiated:** Cells that maintain, in culture, all or much of the specialized structure and function typical of the cell type *in vivo*.
- **Diploid:** The state of the cell in which all chromosomes, except sex chromosomes, are two in number and are structurally identical with those of the species from which the culture was derived. Where there is a Commission Report available, the experimenter should adhere to the convention for reporting the karyotype of the donor. Commission Reports have been published for mouse1, human2, and rat3. In defining a diploid culture, one should present a graph depicting the chromosome number distribution leading to the modal number determination along with representative karyotypes.
- **Electroporation:** Creation , by means of an electrical current, of transient pores in the plasmalemma usually for the purpose of introducing exogenous material, especially DNA, from the medium.
- **Embryo culture:** *In vitro* development or maintenance of isolated mature or immature embryos.
- **Embryogenesis:** The process of embryo initiation and development.
- **Endocrine cell:** In animals, a cell which produces hormones, growth factors or other signaling substances for which target cells, expressing the corresponding receptors, are located at a distance. (See also Autocrine and Paracrine)
- **Epigenetic event:** Any change in a phenotype which does not result from an alteration in DNA sequence. This change may be stable and heritable and includes alteration in DNA methylation, transcriptional activation, translational control and posttranslational modifications
- **Epigenetic variation:** Phenotypic variability which has a nongenetic basis.
- **Epithelial-like:** Resembling or characteristic of, having the form or appearance of epithelial cells. In order to define a cell as an epithelial cell, it must possess characteristics typical of epithelial cells. Often one can be certain of the histologic origin and/or function of the cells

placed into culture and, under these conditions, one can be reasonably confident in designating the cells as epithelial. It is incumbent upon the individual reporting on such cells to use as many parameters as possible in assigning this term to a culture. Until such time as a rigorous definition is possible, it would be most correct to use the term "epithelial-like".

- **Euploid:** The situation which exists when the nucleus of a cell contains exact multiples of the haploid number of chromosomes.
- **Explant:** Tissue taken from its original site and transferred to an artificial medium for growth or maintenance.
- **Explant culture:** The maintenance or growth of an explant in culture.
- **Feeder layer:** A layer of cells (usually lethally irradiated for animal cell culture) upon which are cultured a fastidious cell type. (See also Nurse culture)
- **Fibroblast-like:** Resembling or characteristic of, having the form or appearance of fibroblast cells. In order to define a cell as a fibroblast cell, it must possess characteristics typical of fibroblast cells. Often one can be certain of the histologic origin and/or function of the cells placed into culture and, under these conditions, one can be reasonably confident in designating the cells as fibroblast. It is incumbent upon the individual reporting on such cells to use as many parameters as possible in assigning this term to a culture. Until such time as a rigorous definition is possible, it would be most correct to use the term "fibroblast-like."
- **Finite cell culture:** A culture which is capable of only a limited number of population doubling after which the culture ceases proliferation. (See *In vitro* senescence)
- **Friability:** A term indicating the tendency for plant cells to separate from one another.
- **Gametoclonal variation:** Variation in phenotype, either genetic or epigenetic in origin, expressed by gametoclones.
- **Gametoclone:** Plants regenerated from cell cultures derived from meiospores, gametes or gametophytes.
- **Habituation:** The acquired ability of a population of cells to grow and divide independently of exogenously supplied growth regulators.
- **Heterokaryon:** A cell possessing two or more genetically different nuclei in a common cytoplasm, usually derived as a result of cell-to-cell fusion.
- **Heteroploid:** The term given to a cell culture when the cells comprising the culture possess nuclei containing chromosome numbers other than the diploid number. This is a term used only to describe a culture and is not used to describe individual cells. Thus, a heteroploid culture would be one which contains aneuploid cells.
- **Histiotypic:** The *in vitro* resemblance of cells in culture to a tissue in form or function or both. For example, a suspension of fibroblast-like cells may secrete a glycosaminoglycan-collagen matrix and the result is a structure resembling fibrous connective tissue, which is, therefore, histiotypic. This term is not meant to be used along with the word "culture." Thus, a tissue culture system demonstrating form and function typical of cells *in vivo* would be said to be histiotypic.
- **Homokaryon:** A cell possessing two or more genetically identical nuclei in a common cytoplasm, derived as a result of cell-to-cell fusion.
- **Hybrid cell:** The term used to describe the mononucleate cell which results from the fusion of two different cells, leading to a formation of a synkaryon.
- **Hybridoma:** The cell which results form the fusion of an antibody producing tumor cell (myeloma) and an antigenically-stimulated normal plasma cell. Such cells are constructed because they produce a single antibody directed against the antigen epitope which stimulated the plasma cell. This antibody is referred to as a monoclonal antibody.

- **Immortalization:** The attainment by a finite cell culture, whether by perturbation or intrinsically, of the attributes of a continuous cell line. An immortalized cell is not necessarily one which is neoplastically or malignantly transformed.
- **Immortal cell culture:** See Continuous cell culture.
- **Induction:** Initiation of a structure, organ or process *in vitro*.
- ***In vitro* neoplastic transformation:** The acquisition, by cultured cells, of the property to form neoplasms, benign or malignant, when inoculated into animals. Many transformed cell populations which arise *in vitro* intrinsically or through deliberate manipulation by the investigator, produce only benign tumors which show no local invasion or metastasis following animal inoculation. If there is supporting evidence, the term "*in vitro* malignant neoplastic transformation" or "*in vitro* malignant transformation" can be used to indicate than an injected cell line does, indeed, invade or metastasize.
- ***In vitro* propagation:** Propagation of plants in a controlled, artificial environment, using plastic or glass culture vessels, aseptic techniques and a defined growing medium.
- ***In vitro* senescence:** In vertebrate cell cultures, the property attributable to finite cell cultures; namely, their inability to grow beyond a finite number of population doublings. Neither invertebrate nor plant cell cultures exhibit this property.
- ***In vitro* transformation:** A heritable change, occurring in cells in culture, either intrinsically or from treatment with chemical carcinogens, oncogenic viruses, irradiation, transfection with oncogenes, etc. and leading to the acquisition of altered morphological, antigenic, neoplastic, proliferative or other properties. This expression is distinguished form "*in vitro* neoplastic transformation" in that the alterations occurring in the cell population may not always include the ability of the cells to produce tumors in appropriate hosts. The type of transformation should always be specified in any description.
- **Juvenile:** A phase in the sexual cycle of a plant characterized by differences in a appearance from the adult and which lacks the ability to respond to flower-inducing stimuli.
- **Karyoplast:** A cell nucleus, obtained from the cell by enucleation, surrounded by a narrow rim of cytoplasm and a plasma membrane.
- **Line:** See Cell line.
- **Liposome:** A closed lipid vesicle surrounding an aqueous interior; may be used to encapsulate exogenous materials for ultimate delivery of these into cells by fusion with the cell.
- **Meristem culture:** *In vitro* culture of a generally shiny, dome-like structure measuring less than 0.1 mm in length when excised, most often excised form the shoot apex.
- **Microcell:** A cell fragment, containing one to a few chromosomes, which is formed by the enucleation or disruption of a micronucleated cell.
- **Micronucleated cell:** A cell which has been mitotically arrested and in which small groups of chromosomes function as foci for the reassembly of the nuclear membrane thus forming miconuclei the maximum of which could be equal to the total number of chromosomes.
- **Micropropagation:** *In vitro* clonal propagation of plants from shoot tips or nodal explants, usually with an accelerated proliferation of shoots during subcultures.
- **Morphogenesis:** (a) The evolution of a structure from an undifferentiated to a differentiated state. (b) The process of growth and development of differentiated structures.
- **Mutant:** A phenotypic variant resulting from a changed or new gene.
- **Nurse culture:** In the culture of plant cells, the growth of a cell or cells on a contiguous culture of different origin which in turn is in contact with the tissue culture medium. The cultured cell or tissue may be separated from the feeder layer by a porous matrix such as filter paper or

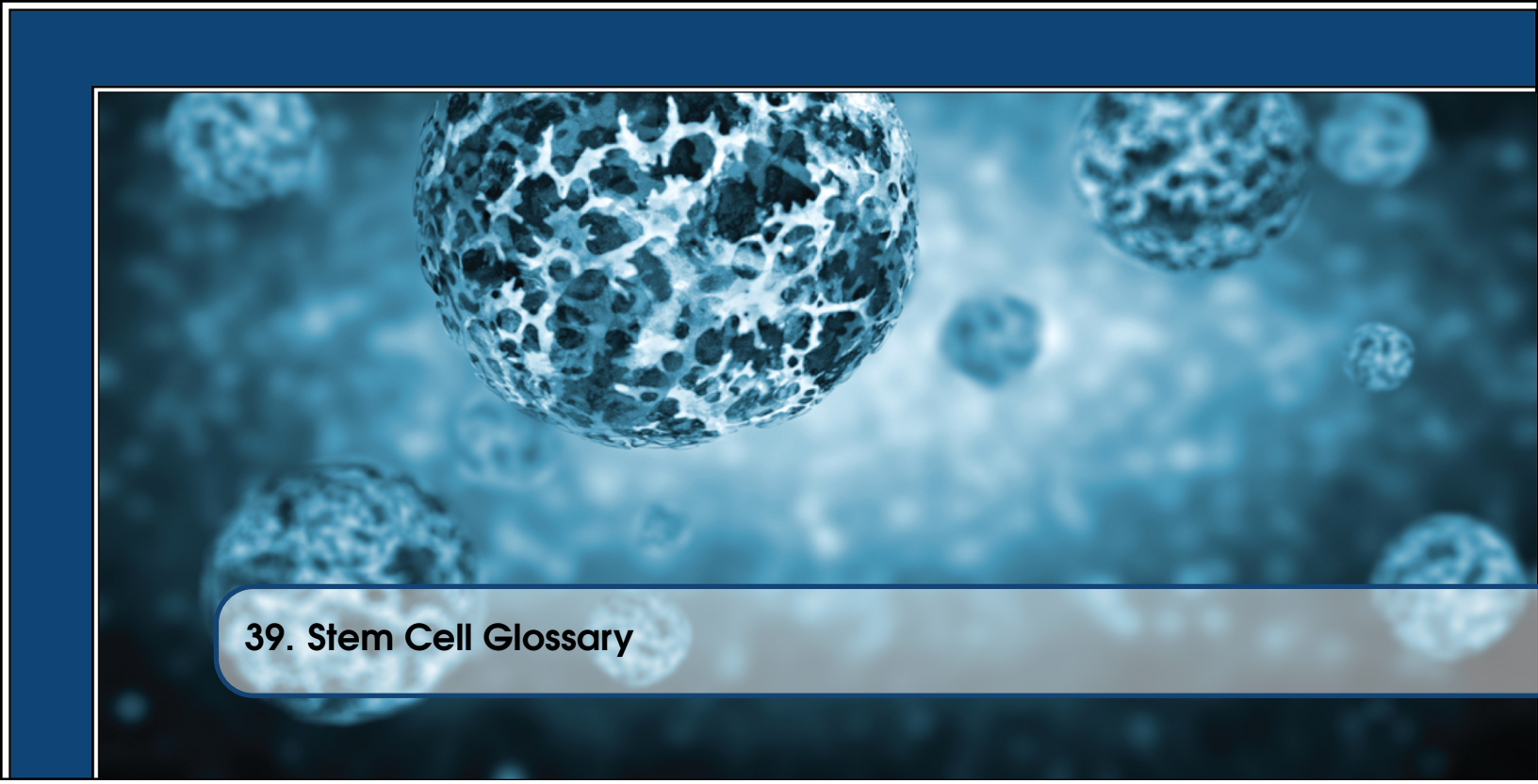
membranous filters. (See also Feeder layer)

- **Organ culture:** The maintenance or growth of organ primordia or the whole or parts of an organ *in vitro* in a way that may allow differentiation and preservation of the architecture and/or function.
- **Organized:** Arranged into definite structures.
- **Organogenesis:** The evolution, from dissociated cells, of a structure which shows natural organ form or function or both.
- **Organotypic:** Resembling an organ *in vivo* in three dimensional form or function or both. For example, a rudimentary organ in culture may differentiate in an organotypic manner, or a population of dispersed cells may become rearranged into an organotypic structure and may also function in an organotypic manner. This term is not meant to be used along with the word " culture" but is meant to be used as a descriptive term.
- **Paracrine:** In animals, a cell which produces hormones, growth factors or other signaling substances for which the target cells, expressing the corresponding receptors, are located in its vicinity, or in a group adjacent to it. (See also Autocrine and Endocrine)
- **Passage:** The transfer or transplantation of cell, with or without dilution, from one culture vessel to another. It is understood that nay time cells are transferred from one vessel to another, a certain portion of the cells may be lost and, therefore, dilution of cells, whether deliberate or not, may occur. This term is synonymous with the term "subculture".
- **Passage number:** The number of times the cells in the culture have been subcultured or passaged. In descriptions of this process, the ration or dilution of the cells should be stated so that the relative cultural age can be ascertained.
- **Pathogen free:** Free from specific organisms based on specific tests for the designated organisms.
- **Plant tissue culture:** The growth or maintenance of plant cells, tissues, organs or whole plants *in vitro*.
- **Plating efficiency:** This is a term which originally encompasses the terms "Attachment ("Seeding") efficiency", Cloning efficiency", and "colony forming efficiency" and which is now better described by using one or more of them in its place as the term "plating" is not sufficiently descriptive of what is taking place. (See Attachment, Cloning, Colony forming efficiency)
- **Population density:** The number of cells per unit area or volume of a culture vessel. Also the number of cells per unit volume of medium in a suspension culture.
- **Population doubling level:** The total number of population doubling of a cell line or strain since its initiation *in vitro*. A formula to use for the calculation of "population doublings" in a singe passage is: Number of population doublings=$Log_10(\frac{N}{N_0})$ x 3.33 where: N = number of cells in the growth vessel at the end of a period of growth. N_0= number of cells plated in the growth vessel. It is best to use the number of viable cells or number of attached cells for this determination. Population doubling level is synonymous with "cumulative population doublings."
- **Population doubling time:** The interval, calculated during the logarithmic phase of growth in which, for example, 1.0 x 10^6 cells increase to 2.0 x 10^6 cells. This term is not synonymous with "cumulative population doublings".
- **Primary culture:** A culture started from cells, tissues or organs taken directly from organisms. A primary culture may be regarded as such until it is successfully subcultured for the first time. It then becomes a "cell line".
- **Protoplast:** A cell from which the entire cell wall has been removed. This term is used to describe such plant, bacterial or fungal cells. (See Spheroplast for comparison.)
- **Protoplast fusion:** Technique in which protoplasts are fused into a single cell.

- **Pseudodiploid:** This describes the condition where the number of chromosomes in a cell is diploid but, as a result of chromosomal rearrangements, the karyotype is abnormal and linkage relationships may be disrupted.
- **Recon:** The viable cell reconstructed by the fusion of a karyoplast with a cytoplast.
- **Reconstituted cell:** Synonymous with "Recon".
- **Reculture:** The process by which a cell monolayer or a plant explant is transferred, without subdivision, into fresh medium. (See also Passage)
- **Regeneration:** In plant cultures, a morphogenetic response to a stimulus that results in the production of organs, embryos or whole plants.
- **Saturation density:** The maximum cell number attainable, under specified culture conditions, in a culture vessel. This term is usually expressed as the number of cells per square centimeter in a monolayer culture or the number of cells per cubic centimeter in a suspension culture.
- **Seeding efficiency:** (See Attachment efficiency)
- **Senescence:** (See *In vitro* senescence)
- **Shoot apical meristem:** Undifferentiated tissue, located within the shoot tip, generally appearing as a shiny dome-like structure distal to the youngest leaf primordium and measuring less than 0.1 mm in length when excised.
- **Shoot tip (apex) culture:** A structure consisting of the shoot apical meristem plus one to several primordial leaves, usually measuring from 0.101.0 mm in length; in instances where more mature leaves are included, the structure can measure up to several centimeters in length. Somaclonal variation: Phenotypic variation, either genetic or epigenetic in origin, displayed among somaclones.
- **Somaclone:** Plants derived from any form of cell culture involving the use of somatic plant cells.
- **Somatic cell hybrid:** The cell or plant resulting from the fusion of animal cells or plant protoplasts respectively, derived from somatic cells which differ genetically.
- **Somatic cell genetics:** The study of genetic phenomena of somatic cells. The cells under study are most often cells grown in culture.
- **Somatic cell hybridization:** The *in vitro* fusion of animal cells or plant protoplasts derived from somatic cells which differ genetically.
- **Somatic embryogenesis:** In plant culture, the process of embryo initiation and development from vegetative or nongametic cells.
- **Spheroplast:** A cell from which most of the cell wall has been removed. (See Protoplasts for comparison)
- **Stage I:** A step in *in vitro* propagation characterized by the establishment of an aseptic tissue culture of a plant.
- **Stage II:** A step in *in vitro* plant propagation characterized by the rapid numerical increase of organs other structures
- **Stage III:** A step in *in vitro* plant propagation characterized by preparation of propagules for successful transfer to soil, a process involving rooting of shoot cuttings, hardening of plants and initiating the change from the heterotrophic to the autotrophic state.
- **Stage IV:** A step in *in vitro* plant propagation characterized by the establishment in soil of a tissue culture derived plant, either after undergoing a Stage III pretransplant treatment or, in certain species, after the direct transfer of plants from Stage II into soil.
- **Sterile:** (a) Without Life. (b) Inability of an organism to produce functional gametes.
- **Strain:** See Cell strain.
- **Subculture:** See Passage. With plant cultures, this is the process by which the tissue or explant

is first subdivided, then transferred into fresh culture medium.

- **Substrain:** A substrain can be derived from a strain by isolation a single cell or groups of cells having properties or markers not shared by all cells of the parent strain.
- **Surface or substrate dependent cells or cultures:** See Anchorage dependent cells.
- **Suspension culture:** A type of culture in which cells, or aggregates of cells, multiply while suspended in liquid medium.
- **Synkaryon:** A hybrid cell which results from the fusion of the nuclei it carries.
- **Tissue culture:** The maintenance or growth of tissues, *in vitro*, in a way that may allow differentiation and preservation of their architecture and/or function.
- **Totipotency:** A cell characteristic in which the potential for forming all the cell types in the adult organism is retained.
- **Transfection:** The transfer, for the purposed of genomic integration, of naked, foreign DNA into cells in culture. The traditional microbiological usage of this term implied that the DNA being transferred was derived from a virus. The definition as stated here is that which is in use to describe the general transfer of DNA irrespective of its source. (See also Transformation)
- **Transformation:** In plant cell culture, the introduction and stable genomic integration of foreign DNA into a plant cell by any means, resulting in a genetic modification. This definition is the traditional microbiological definition. For animal cell culture, see *In vitro* transformation, *In vitro* neoplastic transformation and Transfection.
- **Type I callus:** A type of adventive embryogenesis found with gramineous monocots, which has been induced on an explant where the somatic embryos are arrested at the coleptilar or scutellar stage of embryogeny. The embryos are often fused together especially at the coleorhizal end of the embryo axis. This tissue can be subcultured and maintain this morphology.
- **Type II callus:** A type of adventive embryogenesis found with gramineous monocots, which has been induced on an explant where the somatic embryos are arrested at the globular stage of embryogeny. The globular embryos often arise individually from a common base. The tissue can be subcultured and maintain this morphology.
- **Variant:** A culture exhibition a stable phenotypic change whether genetic or epigenetic in origin.
- **Vegetative propagation:** Reproduction of plants using a nonsexual process involving the culture of plant parts such as stem and leaf cuttings.
- **Undifferentiated:** With plant cells, existing in a state of cell development characterized by isodiametric cell shape, very little or no vacuole, and a large nucleus, and exemplified by cells comprising an apical meristem or embryo. with animal cells, this is the state wherein the cell in culture lacks the specialized structure and/or function of the cell type *in vivo*.
- **Virus-free:** Free from specified viruses based on tests designed to detect the presence of the organisms in question.



39. Stem Cell Glossary

39.1 Stem Cell Glossary

- **Adult stem cell:** See somatic stem cell.
- **Astrocyte:** A type of supporting (glial) cell found in the nervous system.
- **Blastocoel:** The fluid-filled cavity inside the blastocyst, an early, preimplantation stage of the developing embryo.
- **Blastocyst:** A preimplantation embryo of about 150 cells produced by cell division following fertilization. The blastocyst is a sphere made up of an outer layer of cells (the trophoblast), a fluid-filled cavity (the blastocoel), and a cluster of cells on the interior (the inner cell mass).
- **Bone marrow stromal cells:** A population of cells found in bone marrow that are different from blood cells.
- **Bone marrow stromal stem cells (skeletal stem cells):** A multipotent subset of bone marrow stromal cells able to form bone, cartilage, stromal cells that support blood formation, fat, and fibrous tissue.
- **Cell-based therapies:** Treatment in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cells or tissues.
- **Cell culture:** Growth of cells in vitro in an artificial medium for research or medical treatment.
- **Cell division:** Method by which a single cell divides to create two cells. There are two main types of cell division depending on what happens to the chromosomes: mitosis and meiosis.
- **Chromosome:** A structure consisting of DNA and regulatory proteins found in the nucleus of the cell. The DNA in the nucleus is usually divided up among several chromosomes.The number of chromosomes in the nucleus varies depending on the species of the organism. Humans have 46 chromosomes.
- **Clone:** (v) To generate identical copies of a region of a DNA molecule or to generate genetically identical copies of a cell, or organism;

(n) The identical molecule, cell, or organism that results from the cloning process.
1. In reference to DNA: To clone a gene, one finds the region where the gene resides on the DNA and copies that section of the DNA using laboratory techniques.
2. In reference to cells grown in a tissue culture dish: a clone is a line of cells that is genetically identical to the originating cell. This cloned line is produced by cell division (mitosis) of the original cell.
3. In reference to organisms: Many natural clones are produced by plants and (mostly invertebrate) animals. The term clone may also be used to refer to an animal produced by somatic cell nuclear transfer (SCNT) or parthenogenesis.

- **Cloning:** See Clone.
- **Cord blood stem cells:** See Umbilical cord blood stem cells.
- **Culture medium:** The liquid that covers cells in a culture dish and contains nutrients to nourish and support the cells. Culture medium may also include growth factors added to produce desired changes in the cells.
- **Differentiation:** The process whereby an unspecialized embryonic cell acquires the features of a specialized cell such as a heart, liver, or muscle cell. Differentiation is controlled by the interaction of a cell's genes with the physical and chemical conditions outside the cell, usually through signaling pathways involving proteins embedded in the cell surface.
- **Directed differentiation:** The manipulation of stem cell culture conditions to induce differentiation into a particular cell type.
- **DNA:** Deoxyribonucleic acid, a chemical found primarily in the nucleus of cells. DNA carries the instructions or blueprint for making all the structures and materials the body needs to function. DNA consists of both genes and non-gene DNA in between the genes.
- **Ectoderm:** The outermost germ layer of cells derived from the inner cell mass of the blastocyst; gives rise to the nervous system, sensory organs, skin, and related structures.
- **Embryo:** In humans, the developing organism from the time of fertilization until the end of the eighth week of gestation, when it is called a fetus.
- **Embryoid bodies:** Rounded collections of cells that arise when embryonic stem cells are cultured in suspension. Embryoid bodies contain cell types derived from all 3 germ layers.
- **Embryonic germ cells:** Pluripotent stem cells that are derived from early germ cells (those that would become sperm and eggs). Embryonic germ cells (EG cells) are thought to have properties similar to embryonic stem cells.
- **Embryonic stem cells:** Primitive (undifferentiated) cells that are derived from preimplantation-stage embryos, are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.
- **Embryonic stem cell line:** Embryonic stem cells, which have been cultured under in vitro conditions that allow proliferation without differentiation for months to years.
- **Endoderm:** The innermost layer of the cells derived from the inner cell mass of the blastocyst; it gives rise to lungs, other respiratory structures, and digestive organs, or generally "the gut."
- **Enucleated:** Having had its nucleus removed.
- **Epigenetic:** Having to do with the process by which regulatory proteins can turn genes on or off in a way that can be passed on during cell division.
- **Feeder layer:** Cells used in co-culture to maintain pluripotent stem cells. For human embryonic stem cell culture, typical feeder layers include mouse embryonic fibroblasts (MEFs) or human embryonic fibroblasts that have been treated to prevent them from dividing.
- **Fertilization:** The joining of the male gamete (sperm) and the female gamete (egg).

- **Fetus:** In humans, the developing human from approximately eight weeks after conception until the time of its birth.
- **Gamete:** An egg (in the female) or sperm (in the male) cell. See also Somatic cell.
- **Gastrulation:** The process in which cells proliferate and migrate within the embryo to transform the inner cell mass of the blastocyst stage into an embryo containing all three primary germ layers.
- **Gene:** A functional unit of heredity that is a segment of DNA found on chromosomes in the nucleus of a cell. Genes direct the formation of an enzyme or other protein.
- **Germ layers:** After the blastocyst stage of embryonic development, the inner cell mass of the blastocyst goes through gastrulation, a period when the inner cell mass becomes organized into three distinct cell layers, called germ layers. The three layers are the ectoderm, the mesoderm, and the endoderm.
- **Hematopoietic stem cell:** A stem cell that gives rise to all red and white blood cells and platelets.
- **Human embryonic stem cell (hESC):** A type of pluripotent stem cells derived from early stage human embryos, up to and including the blastocyst stage, that are capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.
- **Induced pluripotent stem cell (iPSC):** A type of pluripotent stem cell, similar to an embryonic stem cell, formed by the introduction of certain embryonic genes into a somatic cell.
- ***In vitro*:** Latin for "in glass"; in a laboratory dish or test tube; an artificial environment.
- ***In vitro* fertilization:** A technique that unites the egg and sperm in a laboratory instead of inside the female body.
- **Inner cell mass (ICM):** The cluster of cells inside the blastocyst. These cells give rise to the embryo and ultimately the fetus. The ICM may be used to generate embryonic stem cells.
- **Long-term self-renewal:** The ability of stem cells to replicate themselves by dividing into the same non-specialized cell type over long periods (many months to years) depending on the specific type of stem cell.
- **Mesenchymal stem cells:** A term that is currently used to define non-blood adult stem cells from a variety of tissues, although it is not clear that mesenchymal stem cells from different tissues are the same.
- **Meiosis:** The type of cell division a diploid germ cell undergoes to produce gametes (sperm or eggs) that will carry half the normal chromosome number. This is to ensure that when fertilization occurs, the fertilized egg will carry the normal number of chromosomes rather than causing aneuploidy (an abnormal number of chromosomes).
- **Mesoderm:** Middle layer of a group of cells derived from the inner cell mass of the blastocyst; it gives rise to bone, muscle, connective tissue, kidneys, and related structures.
- **Microenvironment:** The molecules and compounds such as nutrients and growth factors in the fluid surrounding a cell in an organism or in the laboratory, which play an important role in determining the characteristics of the cell.
- **Mitosis:** The type of cell division that allows a population of cells to increase its numbers or to maintain its numbers. The number of chromosomes remains the same in this type of cell division.
- **Multipotent:** Having the ability to develop into more than one cell type of the body. See also pluripotent and totipotent.
- **Neural stem cell:** A stem cell found in adult neural tissue that can give rise to neurons and glial (supporting) cells. Examples of glial cells include astrocytes and oligodendrocytes.
- **Neurons:** Nerve cells, the principal functional units of the nervous system. A neuron consists of

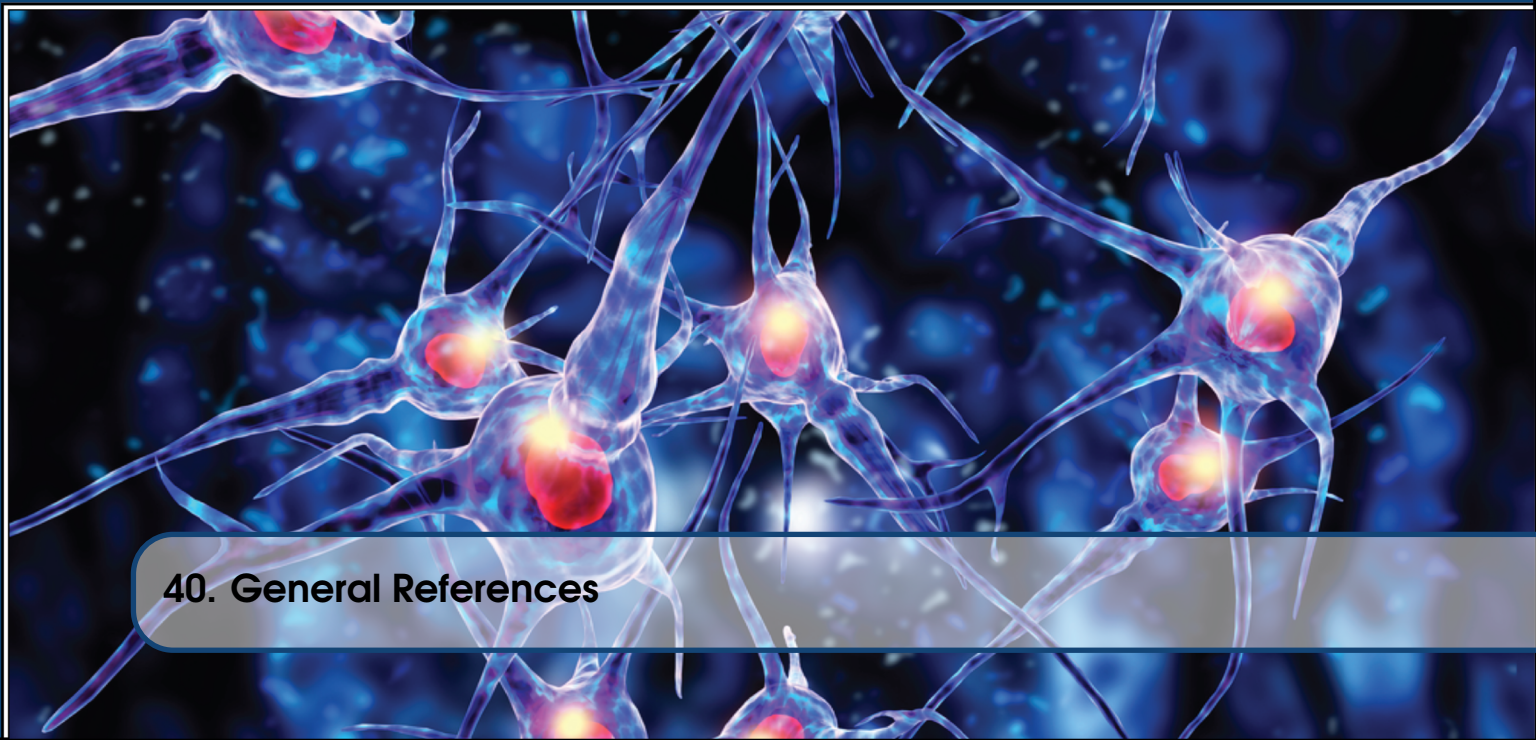
a cell body and its processes—an axon and one or more dendrites. Neurons transmit information to other neurons or cells by releasing neurotransmitters at synapses.

- **Oligodendrocyte:** A supporting cell that provides insulation to nerve cells by forming a myelin sheath (a fatty layer) around axons.
- **Parthenogenesis:** The artificial activation of an egg in the absence of a sperm; the egg begins to divide as if it has been fertilized.
- **Passage:** In cell culture, the process in which cells are disassociated, washed, and seeded into new culture vessels after a round of cell growth and proliferation. The number of passages a line of cultured cells has gone through is an indication of its age and expected stability.
- **Pluripotent:** The state of a single cell that is capable of differentiating into all tissues of an organism, but not alone capable of sustaining full organismal development. Scientists demonstrate pluripotency by providing evidence of stable developmental potential, even after prolonged culture, to form derivatives of all three embryonic teratoma after injection into an immunosuppressed mouse.
- **Polar Body:** A polar body is a structure produced when an early egg cell, or oogonium, undergoes meiosis. In the first meiosis, the oogonium divides its chromosomes evenly between the two cells but divides its cytoplasm unequally. One cell retains most of the cytoplasm, while the other gets almost none, leaving it very small.
  This smaller cell is called the first polar body. The first polar body usually degenerates. The ovum, or larger cell, then divides again, producing a second polar body with half the amount of chromosomes but almost no cytoplasm. The second polar body splits off and remains adjacent to the large cell, or oocyte, until it (the second polar body) degenerates. Only one large functional oocyte, or egg, is produced at the end of meiosis.
- **Preimplantation:** With regard to an embryo, preimplantation means that the embryo has not yet implanted in the wall of the uterus. Human embryonic stem cells are derived from preimplantation-stage embryos fertilized outside a woman's body (*in vitro*).
- **Proliferation:** Expansion of the number of cells by the continuous division of single cells into two identical daughter cells.
- **Regenerative medicine:** A field of medicine devoted to treatments in which stem cells are induced to differentiate into the specific cell type required to repair damaged or destroyed cell populations or tissues. (See also cell-based therapies).
- **Reproductive cloning:** The process of using somatic cell nuclear transfer (SCNT) to produce a normal, full grown organism (e.g., animal) genetically identical to the organism (animal) that donated the somatic cell nucleus. In mammals, this would require implanting the resulting embryo in a uterus where it would undergo normal development to become a live independent being. The first mammal to be created by reproductive cloning was Dolly the sheep, born at the Roslin Institute in Scotland in 1996. See also Somatic cell nuclear transfer (SCNT).
- **Sigals:** Internal and external factors that control changes in cell structure and function. They can be chemical or physical in nature.
- **Somatic cell:** Any body cell other than gametes (egg or sperm); sometimes referred to as "adult" cells. See also Gamete.
- **Somatic cell nuclear transfer (SCNT):** A technique that combines an enucleated egg and the nucleus of a somatic cell to make an embryo. SCNT can be used for therapeutic or reproductive purposes, but the initial stage that combines an enucleated egg and a somatic cell nucleus is the same. See also therapeutic cloning and reproductive cloning.
- **Somatic (adult) stem cells:** A relatively rare undifferentiated cell found in many organs and

differentiated tissues with a limited capacity for both self renewal (in the laboratory) and differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin. This is an active area of investigation.

- **Stem cells:** Cells with the ability to divide for indefinite periods in culture and to give rise to specialized cells.
- **Stromal cells:** Connective tissue cells found in virtually every organ. In bone marrow, stromal cells support blood formation.
- **Subculturing:** Transferring cultured cells, with or without dilution, from one culture vessel to another.
- **Surface markers:** Proteins on the outside surface of a cell that are unique to certain cell types and that can be visualized using antibodies or other detection methods.
- **Telomere:** The end of a chromosome, associated with a characteristic DNA sequence that is replicated in a special way. A telomere counteracts the tendency of the chromosome to shorten with each round of replication.
- **Teratoma:** A multi-layered benign tumor that grows from pluripotent cells injected into mice with a dysfunctional immune system. Scientists test whether they have established a human embryonic stem cell (hESC) line by injecting putative stem cells into such mice and verifying that the resulting teratomas contain cells derived from all three embryonic germ layers.
- **Tetraploid complementation assay:** An assay that can be used to test a stem cell's potency. Scientists studying mouse chimeras (mixing cells of two different animals) noted that fusing two 8-cell embryos produces cells with 4 sets of chromosomes (tetraploid cells) that are biased toward developing into extra-embryonic tissues such as the placenta. The tetraploid cells do not generate the embryo itself; the embryo proper develops from injected diploid stem cells. This tendency has been exploited to test the potency of a stem cell. Scientists begin with a tetraploid embryo. Next, they inject the stem cells to be tested. If the injected cells are pluripotent, then an embryo develops. If no embryo develops, or if the resultant embryo cannot survive until birth, the scientists conclude that the cells were not truly pluripotent.
- **Therapeutic cloning:** The process of using somatic cell nuclear transfer (SCNT) to produce cells that exactly match a patient. By combining a patient's somatic cell nucleus and an enucleated egg, a scientist may harvest embryonic stem cells from the resulting embryo that can be used to generate tissues that match a patient's body. This means the tissues created are unlikely to be rejected by the patient's immune system. See also Somatic cell nuclear transfer (SCNT).
- **Totipotent:** Having the ability to give rise to all the cell types of the body plus all of the cell types that make up the extraembryonic tissues such as the placenta. (See also Pluripotent and Multipotent).
- **Transdifferentiation:** The process by which stem cells from one tissue differentiate into cells of another tissue.
- **Trophectoderm:** The outer layer of the preimplantation embryo in mice. It contains trophoblast cells.
- **Trophoblast:** The outer cell layer of the blastocyst. It is responsible for implantation and develops into the extraembryonic tissues, including the placenta, and controls the exchange of oxygen and metabolites between mother and embryo.
- **Umbilical cord blood stem cells:** Stem cells collected from the umbilical cord at birth that can produce all of the blood cells in the body (hematopoietic). Cord blood is currently used to treat patients who have undergone chemotherapy to destroy their bone marrow due to cancer or other blood-related disorders.

- **Undifferentiated:** A cell that has not yet developed into a specialized cell type.



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Worthington Biochemical Tissue Dissociation Guide

- **Using the Hepatocyte Isolation System:** Hepatocyte Isolation System, *in Worthington Biochemical Corporation Tissue Dissociation Guide*, (Santangelo, C., Ed.), 13 (2008).
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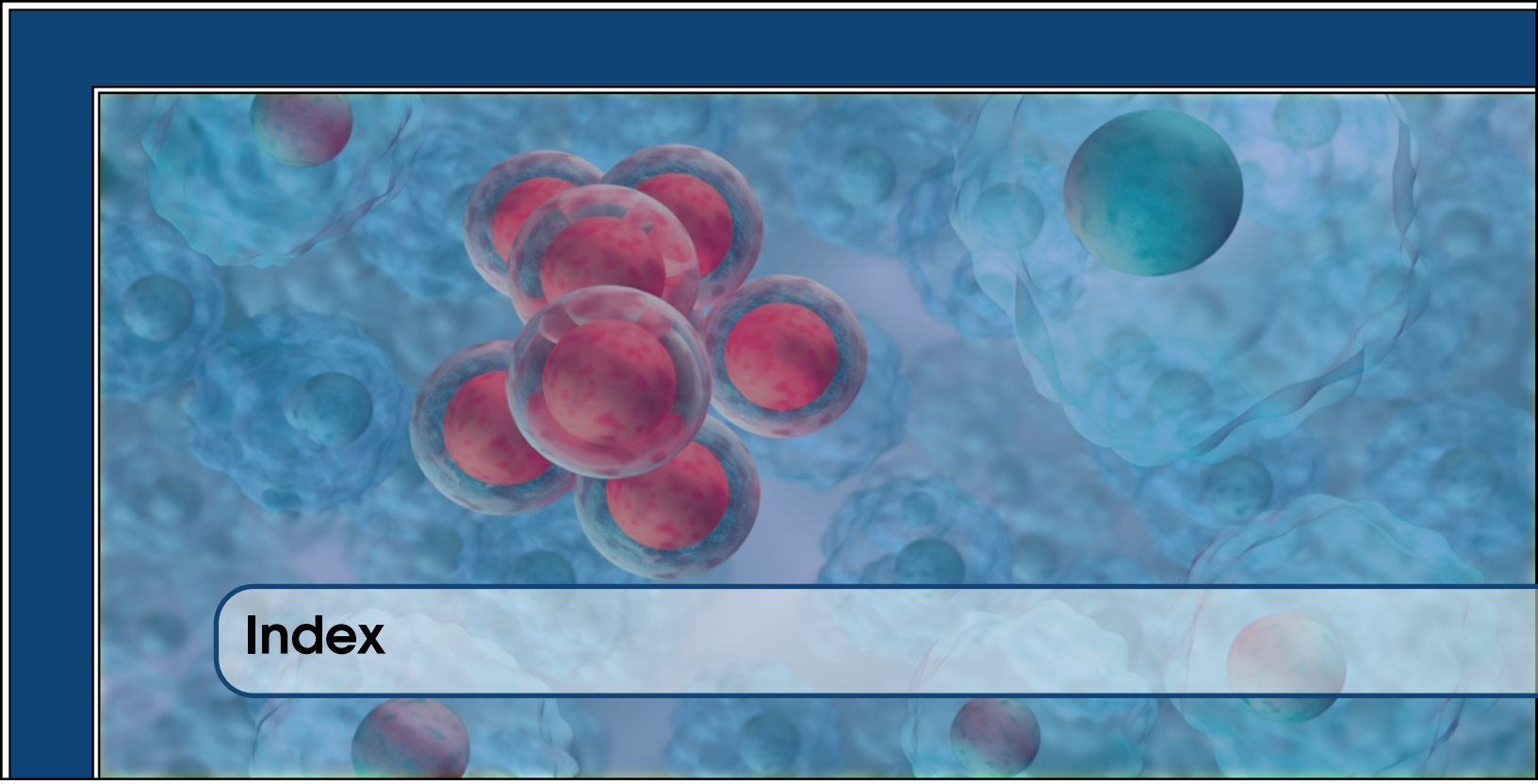

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