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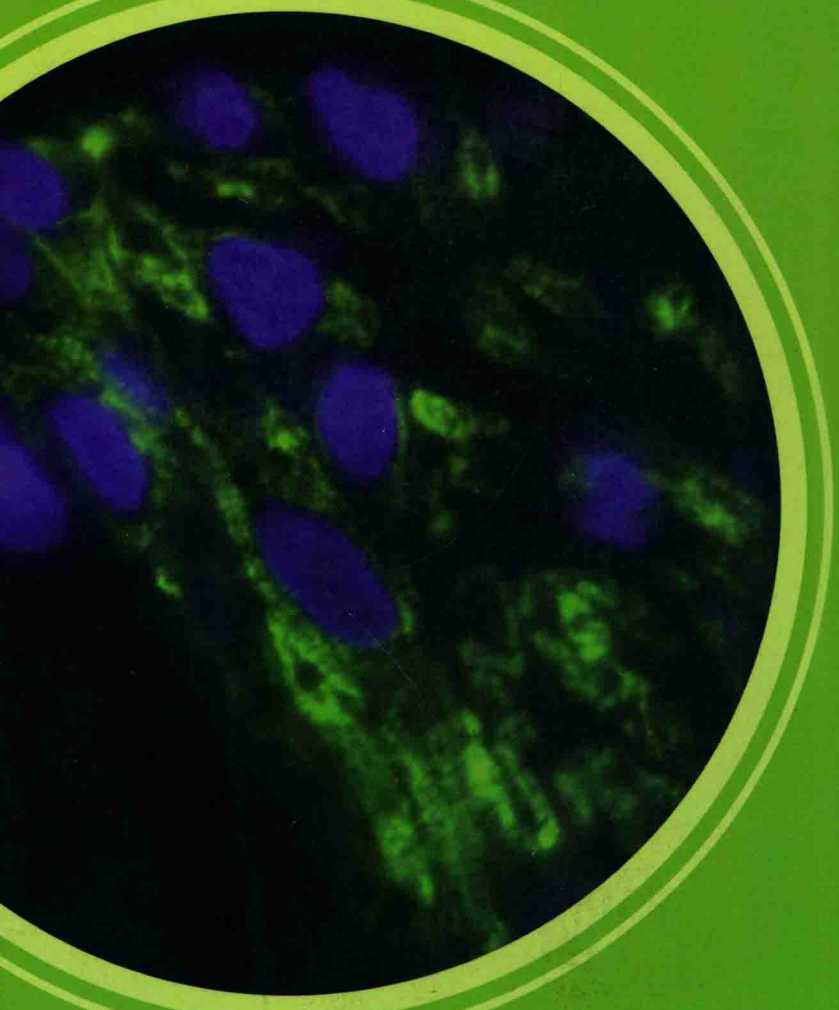
CULTURE of SPECIALIZED CELLS

Culture of Cells for Tissue Engineering



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CULTURE OF CELLS FOR TISSUE ENGINEERING

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Culture of Specialized Cells

Series Editor

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Gordana Vunjak-Novakovic and R. Ian Freshney, Editors

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Preface

Culture of Cells for Tissue Engineering is a new volume in the John Wiley series *Culture of Specialized Cells*, with focus on procedures for obtaining, manipulating, and using cell sources for tissue engineering. The book has been designed to follow the successful tradition of other Wiley books from the same series, by selecting a limited number of diverse, important, and successful tissue engineering systems and providing both the general background and the detailed protocols for each tissue engineering system. It addresses a long-standing need to describe the procedures for cell sourcing and utilization for tissue engineering in one single book that combines key principles with detailed step-to-step procedures in a manner most useful to students, scientists, engineers, and clinicians. Examples are used to the maximum possible extent, and case studies are provided whenever appropriate. We first talked about the possible outline of this book in 2002, at the World Congress of in vitro Biology, encouraged by the keen interest of John Wiley and inspired by discussions with our colleagues.

We made every effort to provide a user-friendly reference for sourcing, characterization, and use of cells for tissue engineering, for researchers with a variety of backgrounds (including basic science, engineering, medical and veterinary sciences). We hope that this volume can also be a convenient textbook or supplementary reading for regular and advanced courses of cell culture and tissue engineering. To limit the volume of the book, we selected a limited number of cells and tissues that are representative of the state of the art in the field and can serve as paradigms for engineering clinically useful tissues. To offer an in-depth approach, each cell type or tissue engineering system is covered by a combination of the key principles, step-by-step protocols for representative established methods, and extensions to other cell types and tissue engineering applications. To make the book easy to use and internally consistent, all chapters are edited to follow the same format, have complementary contents and be written in a single voice.

The book is divided into two parts and contains fifteen chapters, all of which are written by leading experts in the field. ***Part I*** describes procedures currently

used for the in vitro cultivation of selected major types of cells used for tissue engineering, and contains five chapters. *Chapter 1* (by Ian Freshney) reviews basic considerations of cell culture relevant to all cell types under consideration in this book. This chapter also provides a link to the Wiley classic *Culture of Animal Cells*, now in its *Fifth Edition. Chapter 2* (by Donald Lennon and Arnold Caplan) covers mesenchymal stem cells and their current use in tissue engineering. *Chapter 3* (by Shulamit Levenberg, Ali Khademhosseini, Mara Macdonald, Jason Fuller, and Robert Langer) covers another important source of cells: embryonic human stem cells. *Chapter 4* (by Brian Johnstone, Jung Yoo, and Matthew Stewart) deals with various cell sources for tissue engineering of cartilage. *Chapter 5* (by Henning Madry) discusses the methods of gene transfer, using chondrocytes and cartilage tissue engineering as a specific example of application.

Part II deals with selected tissue engineering applications by first describing key methods and then focusing on selected case studies. *Chapter 6* (by Gordana Vunjak-Novakovic) reviews basic principles of tissue engineering, and provides a link to tissue engineering literature. *Chapter 7* (by Koichi Masuda and Robert Sah) reviews tissue engineering of articular cartilage, by using cells cultured on biomaterial scaffolds. *Chapter 8* (by Jingsong Chen, Gregory H. Altman, Jodie Moreau, Rebecca Horan, Adam Collette, Diah Bramano, Vladimir Volloch, John Richmond, Gordana Vunjak-Novakovic, and David L. Kaplan) reviews tissue engineering of ligaments, by biophysical regulation of cells cultured on scaffolds in bioreactors. *Chapter 9* (by Jennifer Elisseeff, Melanie Ruffner, Tae-Gyun Kim, and Christopher Williams) reviews microencapsulation of differentiated and stem cells in photopolymerizing hydrogels. *Chapter 10* (by Janet Shansky, Paulette Ferland, Sharon McGuire, Courtney Powell, Michael DelTatto, Martin Nackman, James Hennessey, and Herman Vandenburgh) focuses on tissue engineering of human skeletal muscle, an example of clinically useful tissue obtained by a combination of cell culture and gene transfer methods. *Chapter 11* (by Thomas Eschenhagen and Wolfgang H. Zimmermann) describes tissue engineering of functional heart tissue and its multidimensional characterization, in vitro and in vivo. *Chapter 12* (by Rebecca Y. Klinger and Laura Niklason) describes tissue engineering of functional blood vessels and their characterization in vitro and in vivo. *Chapter 13* (by Sandra Hofmann, David Kaplan, GordanaVunjak-Novakovic, and Lorenz Meinel) describes in vitro cultivation of engineered bone, starting from human mesenchymal stem cells and protein scaffolds. *Chapter 14* (by Peter I. Lelkes, Brian R. Unsworth, Samuel Saporta, Don F. Cameron, and Gianluca Gallo) reviews tissue engineering based on neuroendocrinal and neuronal cells. *Chapter 15* (by Gregory H. Underhill, Jennifer Felix, Jared W. Allen, Valerie Liu Tsang, Salman R. Khetani, and Sangeeta N. Bhatia) reviews tissue engineering of the liver in the overall context of micropatterned cell culture.

We expect that the combination of key concepts, well-established methods described in detail, and case studies, brought together for a limited number of interesting

and distinctly different tissue engineering applications, will be of interest for the further growth of the exciting field of tissue engineering. We also hope that the book will be equally useful to a well-established scientist and a novice to a field. We greatly look forward to further advances in the scientific basis and clinical application of tissue engineering.

Gordana Vunjak-Novakovic
R. Ian Freshney

List of Abbreviations

AAF	athymic animal facility
ACL	anterior cruciate ligament
ACLF	human ACL fibroblasts
AIM	adipogenic induction medium
AMP	2-amino-2-methylpropanol
ARC	alginate-recovered-chondrocyte
BAMs	bioartificial muscles
BDM	2,3-butanedione monoxime
bFGF	basic fibroblast growth factor (FGF-1)
BPG	β-glycerophosphate
BDNF	brain-derived growth factor
BSA	bovine serum albumin
BSS	balanced salt solution
CAT	chloramphenicol-acetyl transferase
CBFHH	calcium and bicarbonate-free Hanks' BSS with HEPES
CLSM	confocal light scanning microscopy
CM	cell-associated matrix
CMPM	cardiomyocyte-populated matrices
DA	dopamine
DBH	dopamine-β-hydroxylase
Dex	dexamethasone
DMEM	Dulbecco's modification of Eagle's medium
DMEM-10FB	DMEM with 10% fetal bovine serum
DMEM-HG	DMEM with high glucose, 4.5 g/L
DMEM-LG	DMEM with low glucose, 1 g/L
DMMB	dimethylmethylene blue
DMSO	dimethyl sulfoxide
%dw	percentage by dry weight
E	epinephrine (adrenaline)
EB	embryoid bodies

EC	endothelial cell
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
EGFP	enhanced green fluorescent protein
EHT	engineered heart tissue
ELISA	enzyme-linked immunosorbent assay
EMA	ethidium monoazide bromide
ES	embryonal stem (cells)
FACS	fluorescence-activated cell sorting
FBS	fetal bovine serum
FM	freezing medium
FRM	further removed matrix
GAG	glycosaminoglycan
GRGDS	glycine-arginine-glycine-aspartate-serine
HARV	high aspect ratio vessel
HBAMs	human bioartificial muscles
HBSS	Hanks' balanced salt solution
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
hES	human embryonal stem (cells)
HIV	human immunodeficiency virus
HMEC	human microvascular endothelial cell
hMSC	human mesenchymal stem cell
HPLC	high-performance liquid chromatography
HUVEC	human umbilical vein endothelial cell
IBMX	isobutylmethylxanthine
ID	internal diameter
IM	incubation medium
IP	intraperitoneal
LAD	ligament augmentation devices
L_{max}	length at which EHTs develop maximal active force
MEF	mouse embryo fibroblasts
MRI	magnetic resonance imaging
MSC	mesenchymal stem cell
MSCGM	mesenchymal stem cell growth medium
NASA	National Aeronautics and Space Administration
NE	norepinephrine (noradrenaline)
NGF	nerve growth factor
NT2	NTera-2/clone D1 teratocarcinoma cell line
NT2M	NT2 medium
NT2N	Terminally differentiated NT2
OD	optical density
OD	outer or external diameter
OP-1	osteogenic protein 1 (BMP-7)
PAEC	porcine aortic endothelial cells

PBSA	Dulbecco's phosphate-buffered saline without Ca^{2+} and Mg^{2+}
PECAM	platelet endothelial cell adhesion molecule (CD31)
PEG	polyethylene glycol
PEGDA	polyethylene glycol diacrylate
Pen/strep	penicillin-streptomycin mixture, usually stocked at 10,000 U and 10 mg/ml, respectively
PEO	polyethylene oxide
PET	polyethylene terephthalate
PGA	polyglycolic acid
PITC	phenylisothiocyanate
PLA	poly-L-lactic acid
PLGA	polylactic-co-glycolic acid
PNMT	phenylethanolamine-*N*-methyl-transferase
RCCS	Rotatory Cell Culture Systems™
RGD	arginine-glycine-aspartic acid
RWV	rotating wall vessel bioreactors
SA	sympathoadrenal
SC	Sertoli cells
SDS-PAGE	polyacrylamide gel electrophoresis in the presence of sodium dodecyl (lauryl) sulfate
SMC	smooth muscle cell
SNAC	Sertoli-NT2N-aggregated-cell
SR	sarcoplasmic reticulum
SSEA-3 and 4	stage-specific embryonic antigens 3 and 4
STLV	slow turning lateral vessel (NASA derived)
SZP	superficial zone protein
TBSS	Tyrode's balanced salt solution
TH	tyrosine hydroxylase
TJA	total joint arthroplasty
TRITC	tetramethylrhodamine isothiocyanate
TT	twitch tension
Tween 20	polyoxyethylene-sorbitan mono-laurate
UTS	ultimate tensile strength

Contents

Part I

Cell Culture

1

Basic Principles of Cell Culture

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Culture of Cells for Tissue Engineering, edited by Gordana Vunjak-Novakovic and R. Ian Freshney

1. INTRODUCTION

The bulk of the material presented in this book assumes background knowledge of the principles and basic procedures of cell and tissue culture. However, it is recognized that people enter a specialized field, such as tissue engineering, from many different disciplines and, for this reason, may not have had any formal training in cell culture. The objective of this chapter is to highlight those principles and procedures that have particular relevance to the use of cell culture in tissue engineering. Detailed protocols for most of these basic procedures are already published [Freshney, 2005] and will not be presented here; the emphasis will be more on underlying principles and their application to three-dimensional culture. Protocols specific to individual tissue types will be presented in subsequent chapters.

2. TYPES OF CELL CULTURE

2.1. Primary Explantation Versus Disaggregation

When cells are isolated from donor tissue, they may be maintained in a number of different ways. A simple small fragment of tissue that adheres to the growth surface, either spontaneously or aided by mechanical means, a plasma clot, or an extracellular matrix constituent, such as collagen, will usually give rise to an outgrowth of cells. This type of culture is known as a *primary explant*, and the cells migrating out are known as the *outgrowth* (Figs. 1.1, 1.2, See Color Plate 1). Cells in the outgrowth are selected, in the first instance, by their ability to migrate from the explant and subsequently, if subcultured, by their ability to proliferate. When a tissue sample is disaggregated, either mechanically or enzymatically (See Fig. 1.1), the suspension of cells and small aggregates that is generated will contain a proportion of cells capable of attachment to a solid substrate, forming a *monolayer*. Those cells within the monolayer that are capable of proliferation will then be selected at the first subculture and, as with the outgrowth from a primary explant, may give rise to a *cell line*. Tissue disaggregation is capable of generating larger cultures more rapidly than explant culture, but explant culture may still be preferable where only small fragments of tissue are available or the fragility of the cells precludes survival after disaggregation.,

2.2. Proliferation Versus Differentiation

Generally, the differentiated cells in a tissue have limited ability to proliferate. Therefore, differentiated cells do not contribute to the formation of a primary culture, unless special conditions are used to promote their attachment and preserve their differentiated status. Usually it is the proliferating committed precursor compartment of a tissue (Fig. 1.3), such as fibroblasts of the dermis or the basal epithelial layer of the epidermis, that gives rise to the bulk of the cells in a