

WILEY-VCH

Edited by Ricardo A. Pires, Iva Pashkuleva,  
and Rui L. Reis

# Multifunctional Hydrogels for Biomedical Applications



# **Multifunctional Hydrogels for Biomedical Applications**

*Edited by*

*Ricardo A. Pires*

*Iva Pashkuleva*

*Rui L. Reis*

**WILEY-VCH**

**Editors****Dr. Ricardo A. Pires**

University of Minho  
3B's Research Group  
Parque de Ciência e Tecnologia  
Zona Industrial da Gandra  
4805-017 Barco, Guimarães  
Portugal

**Dr. Iva Pashkuleva**

University of Minho  
3B's Research Group  
Parque de Ciência e Tecnol  
4805-017 Barco, Guimarães  
Portugal

**Prof. Rui L. Reis**

University of Minho  
3B's Research Group  
Parque de Ciência e Tecnologia  
4805-017 Barco, Guimarães  
Portugal

**Cover Image:** © NANOCLOUDERING/Getty Images

All books published by **WILEY-VCH** are carefully produced. Nevertheless, authors, editors, and publisher do not warrant the information contained in these books, including this book, to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

**Library of Congress Card No.:** applied for

**British Library Cataloguing-in-Publication Data**

A catalogue record for this book is available from the British Library.

**Bibliographic information published by the**

**Deutsche Nationalbibliothek** The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at <<http://dnb.d-nb.de>>.

© 2022 WILEY-VCH GmbH, Boschstr. 12,  
69469 Weinheim, Germany

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

**Print ISBN:** 978-3-527-34716-2

**ePDF ISBN:** 978-3-527-82581-3

**ePub ISBN:** 978-3-527-82583-7

**oBook ISBN:** 978-3-527-82582-0

**Typesetting** Straive, Chennai, India

Printed on acid-free paper

10 9 8 7 6 5 4 3 2 1

## Contents

### Preface *xiii*

<b>1</b>	<b>Extracellular Matrix Hydrogels from Decellularized Tissues for Biological and Biomedical Applications</b>	<b>1</b>
	<i>Brendan C. Jones, Nicola Elvassore, Paolo De Coppi, and Giovanni G. Giobbe</i>	
1.1	Introduction to Hydrogels	1
1.1.1	Definition and Use of Hydrogels in Biomedical Applications	1
1.1.2	Classification and Properties of Hydrogels	2
1.1.2.1	Synthetic Hydrogels	3
1.1.2.2	Natural Hydrogels	5
1.2	Key Features and Functions of the Extracellular Matrix in Homeostasis and Development	6
1.3	Extracellular Matrix-Based Hydrogels Derived from Decellularization of Organs	8
1.3.1	Production of ECM Hydrogels	8
1.3.2	Characterization of ECM Hydrogels	10
1.3.3	Pancreatic ECM-Derived Hydrogels	11
1.3.4	ECM Hydrogels Derived from Liver	12
1.3.5	Lung ECM Hydrogels	13
1.3.6	Hydrogels Derived from Decellularized Colon	14
1.3.7	ECM-Derived Hydrogels from Small Intestine	15
1.3.8	Cellular Responses to ECM Hydrogels	17
1.4	Commercially Available Products	18
	References	19
<b>2</b>	<b>Collagen-Based Systems to Mimic the Extracellular Environment</b>	<b>23</b>
	<i>Umber Cheema and Vivek Mudera</i>	
2.1	Cells in Tissues	23
2.2	Collagen in Tissues	24
2.2.1	Structure of Collagen	25
2.2.2	Collagen Sources	25
2.3	Controlling Collagen Architecture	26

2.3.1	Direction: Collagen Orientation	26
2.3.2	Diameter: Collagen Fibril Diameter	27
2.3.3	Density: Fibril Packing and Cross-Linking	28
2.4	Engineering Collagen Scaffolds	29
2.4.1	Collagen Cross-Linking	29
2.4.2	Diffusion of Nutrients and Oxygen Through Collagen Scaffolds	29
2.4.3	Proliferation of Cells in 3D	30
2.4.4	Mechanical Stimulation and Bioreactors	31
2.4.5	Growth Factors	32
2.4.6	Drug-Loaded Scaffolds	32
2.5	Conclusions	33
	References	33
<b>3</b>	<b>Designing Elastin-Like Recombinamers for Therapeutic and Regenerative Purposes</b>	<b>37</b>
	<i>José Carlos Rodríguez-Cabello, Sara Escalera, Diana Juanes-Gusano, Mercedes Santos, and Alessandra Girotti</i>	
3.1	Introduction	37
3.2	ELR-Based Hydrogels in Tissue Engineering	39
3.2.1	Hydrogels in Musculoskeletal Tissue Regeneration	42
3.2.2	Hydrogels in Cardiovascular Tissue Regeneration	44
3.2.3	Hydrogels in Skin Tissue Regeneration	46
3.2.4	Hydrogels in Neural Tissue Regeneration	47
3.3	ELR-Based Hydrogels for Drug Delivery	48
3.3.1	Physically Cross-Linked Hydrogels	48
3.3.2	Chemically Cross-Linked Hydrogels	52
3.4	Future Remarks	56
	References	56
<b>4</b>	<b>Enzyme-Assisted Hydrogel Formation for Tissue Engineering Applications</b>	<b>63</b>
	<i>Silvia Pérez-Rafael, Eva Ramon, and Tzanko Tzanov</i>	
4.1	Introduction	63
4.2	Enzymatically Cross-Linked Hydrogels	66
4.2.1	Oxidoreductases	67
4.2.1.1	Peroxidases – HRP	67
4.2.1.2	Tyrosinase	72
4.2.1.3	Laccase	72
4.2.2	Transferases: Transglutaminase	73
4.3	Supramolecular Enzyme-Driven Hydrogelation	75
4.3.1	Hydrolases	75
4.3.1.1	Phosphatases	75
4.3.1.2	Metalloproteinases	80
4.3.1.3	Thermolysin	80
4.3.1.4	$\beta$ -Lactamases	80

# 3

## Designing Elastin-Like Recombinamers for Therapeutic and Regenerative Purposes

*José Carlos Rodríguez-Cabello, Sara Escalera<sup>☆</sup>, Diana Juanes-Gusano<sup>☆</sup>, Mercedes Santos, and Alessandra Girotti*

*University of Valladolid, BIOFORGE lab (Group for Advanced Materials and Nanobiotechnology) CIBER-BBN, Edificio Lucia, Paseo de Belén 19, Valladolid 47011, Spain*

### 3.1 Introduction

The field of biomedicine relies on the development of advanced systems that mimic the extracellular matrix (ECM) to allow *in vitro* studies of cell–matrix interactions and subsequent implementation *in vivo*. The principal matrices for biomedical applications are hydrogels, which are hydrophilic polymer networks that can absorb a large volume of water in resemblance to natural tissues (see Chapter 1). The materials used to obtain these biomimetic scaffolds include a large variety of synthetic polymers such as polyethylene glycol (PEG) [1], as well as biopolymers, mostly proteins from animal tissues such as collagen [2] (see Chapter 2). Combinations of natural and synthetic polymers have also been tested to improve the properties of hydrogels [3].

Essential characteristics for the development of hydrogels for general biomedical applications include (i) an ability to provide a structural support to the surrounding cells, thus promoting natural and adequate cell growth that helps complete integration of the scaffold into the natural surrounding tissue and provides mechanical stability, (ii) an ability to mimic the ECM topography of tissues, (iii) an ability to mimic the natural environment so that cells can develop their normal functions and help restore damaged tissue, (iv) an ability to absorb and retain large quantities of water while maintaining their structures, thereby maintaining the hydration levels found in most tissues, (v) an ability to modulate their structures to match the shape and the size of defects, (vi) an ability to be easily manipulated, and, particularly, (vii) biocompatibility and biodegradability [4–7]. Depending on the final application, hydrogels for use in regenerative medicine will need specific requirements in order to simulate the tissue to be repaired, such as cell adhesion or growth factors, which could also be included in the scaffold.

<sup>☆</sup> equal contribution

**87** Thomas, D., Fontana, G., Chen, X. et al. (2014). A shape-controlled tuneable microgel platform to modulate angiogenic paracrine responses in stem cells. *Biomaterials* 35 (31): 8757–8766.

**88** Cao, R., Bråkenhielm, E., Pawliuk, R. et al. (2003). Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. *Nat. Med.* 9 (5): 604.

**89** Dash, B.C., Thomas, D., Monaghan, M. et al. (2015). An injectable elastin-based gene delivery platform for dose-dependent modulation of angiogenesis and inflammation for critical limb ischemia. *Biomaterials* 65: 126–139.

**90** Asai, D., Xu, D., Liu, W. et al. (2012). Protein polymer hydrogels by in situ, rapid and reversible self-gelation. *Biomaterials* 33 (21): 5451–5458.

**91** Zhang, Y.N., Avery, R.K., Vallmajó-Martin, Q. et al. (2015). A highly elastic and rapidly crosslinkable elastin-like polypeptide-based hydrogel for biomedical applications. *Adv. Funct. Mater.* 25 (30): 4814–4826.

**92** Mukerji, R., Schaal, J., Li, X. et al. (2016). Spatiotemporally photoradiation-controlled intratumoral depot for combination of brachytherapy and photodynamic therapy for solid tumor. *Biomaterials* 79: 79–87.

**93** Asai, D., Kanamoto, T., Takenaga, M., and Nakashima, H. (2017). In situ depot formation of anti-HIV fusion-inhibitor peptide in recombinant protein polymer hydrogel. *Acta Biomater.* 64: 116–125.

**94** Asai, D., Fukuda, T., Morokuma, K. et al. (2019). Injectable polypeptide hydrogel depot system for assessment of the immune response-inducing efficacy of sustained antigen release alone. *Macromol. Biosci.* 19: 1900167.

**95** Wang, H., Paul, A., Nguyen, D. et al. (2018). Tunable control of hydrogel microstructure by kinetic competition between self-assembly and crosslinking of elastin-like proteins. *ACS Appl. Mater. Interfaces* 10 (26): 21808–21815.