

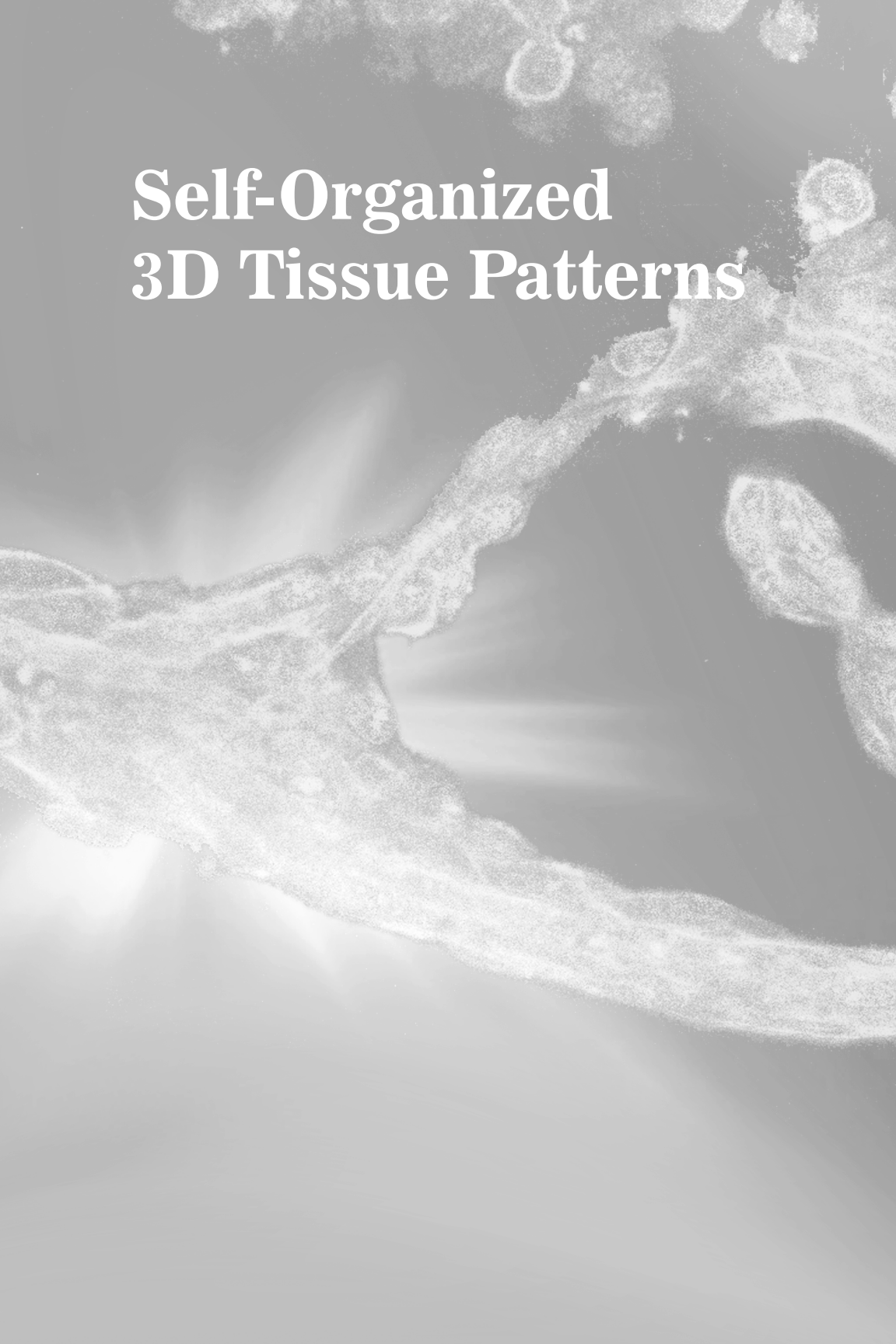
Self-Organized 3D Tissue Patterns

Fundamentals, Design, and Experiments

Xiaolu Zhu | Zheng Wang



Self-Organized 3D Tissue Patterns



Self-Organized 3D Tissue Patterns

Fundamentals, Design, and Experiments

Xiaolu Zhu
Zheng Wang



**JENNY STANFORD
PUBLISHING**

Published by

Jenny Stanford Publishing Pte. Ltd.
Level 34, Centennial Tower
3 Temasek Avenue
Singapore 039190

Email: editorial@jennystanford.com
Web: www.jennystanford.com

British Library Cataloguing-in-Publication Data

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

**Self-Organized 3D Tissue Patterns: Fundamentals,
Design, and Experiments**

Copyright © 2022 Jenny Stanford Publishing Pte. Ltd.

All rights reserved. This book, or parts thereof, may not be reproduced in any form or by any means, electronic or mechanical, including photocopying, recording or any information storage and retrieval system now known or to be invented, without written permission from the publisher.

For photocopying of material in this volume, please pay a copying fee through the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. In this case permission to photocopy is not required from the publisher.

ISBN 978-981-4877-77-0 (Hardcover)

ISBN 978-1-003-18039-5 (eBook)

Contents

<i>Preface</i>	ix
1. Introduction	1
1.1 Research in Tissue Engineering	1
1.2 Traditional Tissue Grafting and Typical Cell Implantation for Skin or Cartilage	4
1.3 Synthetic Structured Scaffold and Decellularized 3D Matrices	6
1.4 Tissue and Organoid Morphogenesis by Regulated Self-Organization Process	8
2. Fundamentals of Three-Dimensional Cell Culture in Hydrogels	17
2.1 Introduction	17
2.2 Experimental Methods for 3D Culture of Cells	19
2.2.1 Cell Culture	20
2.2.2 Hyaluronic Acid Modification	20
2.2.3 VMCs-Laden HA Hydrogel Formation	21
2.2.4 Fixation and Fluorescent Staining	22
2.2.5 3D Visualization Using Selective Plane Illumination Microscopy	22
2.2.6 Measuring Cell Proliferation in 3D HA Hydrogels	23
2.3 Results and Discussion for 3D Microtissue Patterns Emerged in HA Hydrogels	23
2.3.1 Generation of 3D Structures Composed of Aggregated Cells	23
2.3.2 Influence of Component Proportion on Self-Organization of VMCs in HA Hydrogel	27

2.3.3	Cytotoxicity of Modified Dextran Hydrogels and Cellular Proliferation measurement in Hydrogels	28
2.3.4	Discussion on Self-Organization of Cells in 3D Hydrogel with Quantitatively Tunable Components	32
2.4	Summary	33
3.	Three-Dimensional Patterns of Tissues Emerging in Hydrogels	39
3.1	Background	39
3.2	Experimental Methods for 3D Culture of Cells	41
3.2.1	Cell Culture	41
3.2.2	HA Modification	42
3.2.3	VMCs-Laden HA Hydrogel Formation	42
3.2.4	Fixation and Fluorescent Staining	43
3.2.5	Clustered Encapsulation of Cells in 3D HA Hydrogels	43
3.2.6	3D Visualization Using Selective Plane Illumination Microscopy	43
3.3	Results and Discussion for 3D Microtissue Patterns Emerging in Ha Hydrogels	44
3.3.1	3D Pattern Formation of VMCs in Modified HA Hydrogel	44
3.3.2	Generation of Varying Morphologies of 3D Structures Composed of Aggregated Cells	46
3.3.3	Mapping Combined Effects of Exogenous Factors	52
3.4	Summary	53
4.	Constructing 3D Tissue Structures via Cellular Self-Assembly at Patterned Interfaces inside Hydrogel	59
4.1	Background	59
4.2	Materials and Methods	61
4.2.1	Cell Culture	61
4.2.2	HA Modification	61

4.2.3	HA Hydrogel Synthesis	62
4.2.4	Rheology Measurement of Hydrogel	62
4.2.5	Fabrication of 2D Interface	63
4.2.6	3D Imaging	64
4.3	Experiment Results	64
4.3.1	Multicellular Network and Branching Structures inside HA Hydrogels	64
4.3.2	Multicellular Network inside HA Hydrogels with Low Stiffness and Higher Stiffness	67
4.3.3	Controllable Large-Dimensional Tube Formation at Interface of High-Stiffness and Low-Stiffness Gels	68
4.4	Summary	72
5.	Modeling Cellular Self-Assembly at Patterned Interfaces inside Hydrogel via Turing's Reaction-Diffusion Frame	77
5.1	Introduction	77
5.2	Theoretical Model	80
5.3	Simulation Results and Discussion	83
5.4	Summary	91
6.	Tuning Cellular Behaviors during Self-Organization of Cells in Hydrogel by Changing Inner Nano-Structure of Hydrogel	95
6.1	Introduction	95
6.2	Materials and Methods	98
6.2.1	3D Dextran Hydrogel	98
6.2.2	Cell Preparation	99
6.2.3	RGD-Homogenous Hydrogel Fabrication	100
6.2.4	RGD-Clustered Hydrogel Fabrication	100
6.2.5	SEM Imaging	101
6.2.6	Rheology Measurement	102
6.2.7	Live/Dead Test	102
6.2.8	Bright Field Imaging	103
6.2.9	F-actin Staining	103
6.2.10	DAPI Staining	103

6.2.11	LSCM Imaging	104
6.2.12	Nucleus Circularity Measuring Method	104
6.2.13	Gel Degradation	104
6.2.14	Data Statistics	105
6.3	Results	105
6.3.1	Microgeometry and Rheological Properties of Dextran Hydrogel	105
6.3.2	Cellular Morphology and Behaviors in RGD-Homogenous Dextran Hydrogel	106
6.3.3	Cell-Adhesive Efficacy of RGD Clustering Dextran Hydrogels	111
6.3.4	Cell Spreading, Elongation, and Connection in RGD-Clustering Dextran Hydrogels	113
6.4	Discussion	116
6.4.1	Fundamental Comparison on Cellular Morphology and Behaviors in 2D Petri Dishes and 3D Dextran Hydrogel	116
6.4.2	Cellular Morphology and Behaviors in RGD-Homogenous and RGD-Clustered Dextran Hydrogel	118
6.4.3	Local Stiffness Variation Influenced by RGD Distributions in Hydrogels	120
6.4.4	Significance of Cellular Behaviors Influenced by Averaged Concentration of RGD in Hydrogels	121
6.4.5	Effect of Stiffness-Heterogeneity with Large Fluctuation and RGD Clustering Induced Stiffness-Heterogeneity with Small Variation	123
6.5	Summary	124
	<i>Index</i>	131

Preface

Tissue engineering applies principles and methods from engineering and life sciences to create artificial constructs to direct tissue regeneration or enhance tissues and organs. Structured scaffolds are widely useful for providing structures supporting cells to form 3D tissue. However, it is non-trivial to develop a scheme, which can robustly guide cells to self-organize into a tissue with desired 3D spatial structures. The self-organization of cells is a natural process that occurs in various biological bodies. In the field of engineering, we may expect that the process of self-organization can be rationally predicted and controlled. Moreover, it will be better that the self-organization of a huge number of cells can be governed by a mathematical framework.

This book first introduces the advances in tissue and organ regenerating using diverse technologies from different disciplines (Chapter 1). Then, it focuses on the multicellular or tissue structure formation via self-assembly of cells in 3D hydrogels (Chapter 2 and 3). Based on the hydrogel fabrication technique, the tailored inner interfaces inside hydrogels have been proposed to stimulate the tubular microtissue formation via a self-assembly scheme (Chapter 4), and the corresponding mathematical framework and the simulation model are also presented and discussed (Chapter 5). Furthermore, in order to develop a more elaborate and sophisticated regulating method for tuning the collective cellular behaviors, we also propose a hydrogel system with 3D distribution of clustered compositions, which can tune the multicellular elongations and aggregations (Chapter 6). This book offers the fundamentals and innovative designs for the 3D hydrogel system and discusses the representatively experimental results on the self-organized 3D Tissue patterns.

The authors are grateful for the helpful discussion with Prof. Ting-Hsuan Chen at City University of Hong Kong, Prof. Chih-Ming Ho at the University of California, Los Angeles (UCLA), Prof. Tatiana Segura at Duke University and Prof. Alan Garfinkel and Prof. Yin Tintut at UCLA, while conducting the related projects. We hope this book will be useful for the readers in the interdisciplinary areas of engineering, biology, and life sciences.

Xiaolu Zhu
Zheng Wang
Hohai University, China

“This book offers multidisciplinary knowledge and diverse approaches for constructing 3D multicellular patterns. It also provides an easy access to the related multidisciplinary fundamentals, design strategies, and experimental procedures for professional researchers and students.”

Prof. Xiuli Cong
Zhejiang Hospital, China

“I am thrilled to see the authors putting their enthusiasm and professionalism in the research of 3D self-organized tissues using both experimental and simulation techniques. More importantly, the research is finally organized in such a smooth and logical way as a book. I recommend this book as a useful reference to researchers in both academia and industry, even students who are passionate about tissue engineering.”

Dr. Kesong Hu
Applied Materials, Inc., USA

“This book focuses on the fascinating self-organizing approaches that integrate multidisciplinary knowledge for constructing 3D cellular patterns. It provides interesting and rich information for the professional researchers in the related fields.”

Prof. Xianting Ding
Shanghai Jiao Tong University, China

Therapies for regenerating damaged tissue and organs have been attracting much attention. In order to efficiently regenerate the functions of living tissue and organs, diverse attempts have been made to utilize scaffolds to “mold” artificial tissue structures. However, the structural complexity of the reconstituted tissue is limited by the mechanical precision of scaffolds, which still causes problems arising from degradation, immunogenic reactions, and so forth. It is also being realized that ultimately the best approach might be to rely on the innate self-organizing properties of cells and the regenerative capability of the organism itself.

This book investigates the 3D-pattern formation and evolution mechanism in multipotent cells embedded in 3D semi-synthetic hydrogels and the control methodology for self-organized patterns. The authors theoretically and experimentally demonstrate several types of topological 3D-pattern formation by cells in a 3D matrix in vitro, which can be modeled and predicted by mathematical models based on the reaction-diffusion dynamics of various chemical, physical, and mechanical cues. The study, focused on the 3D pattern formation of cells, provides (i) a unique perspective for understanding the self-organized 3D tissue structures based on Turing instability, (ii) the scheme for rationally controlling the cellular self-organization via exogenous factors or tailored inner interfaces inside hydrogels, and (iii) the elaborate and sophisticated regulating method for tuning collective cellular behaviors in 3D matrices.



Xiaolu Zhu is associate professor at Hohai University, China. He graduated from Southeast University, China, in 2007 and obtained a PhD in 2014. He worked as a research scholar at the University of California, Los Angeles, from 2011 to 2013. His work is currently focused on understanding and controlling self-organized 3D patterns of cells and hydrogel-based biofabrication.



Zheng Wang is currently a master's student at the University of Hong Kong. He obtained his bachelor's degree from Hohai University in 2020. He is working on the development of the applications of hydrogels in the regulation of cellular behaviors and on the quantification of the relationships between cellular behaviors and the physical properties of the extracellular matrix.

 **JENNY STANFORD**
PUBLISHING

