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Liliang Ouyang

Study on Microextrusion-based 3D Bioprinting and Bioink Crosslinking Mechanisms

Doctoral Thesis accepted by
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Supervisor's Foreword

It is my great pleasure to introduce Dr. Ouyang's thesis work, which is recognized as an Outstanding Doctoral Thesis by Tsinghua University. Dr. Ouyang joined my laboratory and started his Ph.D. study in September 2012, working on extrusion-based 3D bioprinting, novel bioink development, and embryonic stem cell printing. In 2015, he received a scholarship from the China Scholarship Council and conducted one-year visiting research with Prof. Jason A. Burdick at the University of Pennsylvania. Dr. Ouyang completed his doctoral work in July 2017, ending up with an impressive list of high-impact publications in the field of biofabrication.

In this dissertation, Dr. Ouyang presents a comprehensive study on microextrusion 3D bioprinting as well as the mechanisms of bioink crosslinking. Three-dimensional bioprinting is normally referred to using living cells as building blocks for constructing cellular structures, where the cell-containing ink materials are termed as bioinks. In the last decade, 3D bioprinting has been witnessed with numerous progresses and applications, while the fundamentals of the printing technology, as well as the effect of printing process and bioink crosslinking on the structural printability and cellular function, have not been fully investigated. To bridge this gap, Dr. Ouyang has conducted a comprehensive study on the microextrusion-based cell-printing process, and the use of responsive bioinks and their crosslinking. A number of novel printing/crosslinking strategies were developed and presented in this thesis work, for example, a dual-crosslinking strategy for shear-thinning supermolecular bioink printing (Chap. 4), a synergetic optimization method for thermo-responsive bioink printing (Chap. 5), and an in-situ crosslinking strategy for printing non-viscous photo-crosslinkable hydrogels (Chap. 6). In addition, this thesis also reports some fundamental studies on a signal pathway activation, cell–matrix interaction, as well as a proliferation-induced embryoid body (EB) formation mechanism as results of bioprinting (Chap. 7). Findings, as well as the developed methodologies from those studies, have greatly enriched the field of bioprinting.

I am proud of Dr. Ouyang for what he has accomplished during his Ph.D. study and proud of presenting this thesis book to the bioprinting and biofabrication community.

Beijing, China/Philadelphia, USA Prof. Wei Sun, Ph.D.
May 2019

Parts of this Thesis have been Published in the Following Documents:

Journal Publications:

Ouyang L, Highley CB, Sun W, Burdick JA. A Generalizable Strategy for the 3D Bioprinting of Hydrogels from Nonviscous Photo-crosslinkable Inks. *Advanced Materials*. 2017; 29(8):1604983.

Ouyang L, Yao R, Zhao Y, Sun W. Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication*. 2016; 8(3):035020.

Ouyang L[#], Highley CB[#], Rodell CB, Sun W, Burdick JA. 3D Printing of Shear-Thinning Hyaluronic Acid Hydrogels with Secondary Cross-Linking. *ACS Biomaterials Science & Engineering*. 2016; 2(10):1743–1751. ([#] Equal contribution)

Ouyang L[#], Yao R[#], Mao S, Chen X, Na J, Sun W. Three-dimensional bioprinting of embryonic stem cells directs high-throughput and highly uniform embryoid body formation. *Biofabrication*. 2015; 7(4):044101. ([#] Equal contribution)

Ouyang L[#], Yao R[#], Chen X[#], Na J, Sun W. 3D printing of HEK 293FT cell-laden hydrogel into macroporous constructs with high cell viability and normal biological functions. *Biofabrication*. 2015; 7(1):015010. ([#] Equal contribution)

Zhang T, Yan KC, Ouyang L, Sun W. Mechanical characterization of bioprinted in vitro soft tissue models. *Biofabrication*. 2013; 5(4):045010.

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This thesis would not have been possible without the help and guidance from my mentors in both laboratories. I thank Dr. Ting Zhang for introducing me to Prof. Sun's group and mentoring me at the beginning of my project. I genuinely thank Dr. Rui Yao for instilling in me her passion for research during our close interaction in the first few years of my Ph.D. I owe a significant debt of gratitude to Dr. Christopher B. Highley as he selflessly provided me with tremendous help and encouragement when I was in Prof. Burdick's group.

Some of my Ph.D. work regarding embryonic stem cells is owed to the collaboration with Dr. Jie Na and Dr. Xi Chen from the School of Medicine at THU. I sincerely appreciate their invaluable inputs and contributions to the projects. I also would like to thank my friend and colleague, Dr. Yu Zhao, who has been just reliable and supportive to my Ph.D. work and life. This thesis would not come to fruition without the advice, comments, and tips from the colleagues (past and present): Prof. Feng Lin, Prof. Renji Zhang, Dr. Lei Zhang, Mr. Mingfeng Li, Mr. Laquan Zhang, Mr. Long Zhao, Mr. Yu Li, Mrs. Jia Wang, Mr. Yongyi Quan, Mrs. Shuangshuang Mao, and Dr. Karen Chang Yan at THU, as well as Dr. Yi-cheun Yeh, Dr. Christopher B. Rodell, and Dr. Kwang Hong Song at UPenn.

I am profoundly grateful to all the teachers and mentors who have helped me in the last twenty-one years since my primary education. I am also very grateful to my friends for accepting me with nothing but trust.

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Contents

Abbreviations

3DP	Three-dimensional Printing
3T3	NIH 3T3 Fibroblast
Ad	Adamantane
Ad-HA	Adamantane Hyaluronic Acid
Alg	Alginate
BSA	Bovine Serum Albumin
CAD	Computer-Aided Design
DAPI	4′,6-diamidino-2-phenylindole
DC	Dual Crosslinking
DMSO	Dimethyl Sulfoxide
DTT	DL-Dithiothreitol
EB	Embryoid Body
ECM	Extracellular Matrix
FBS	Fetal Bovine Serum
FDM	Fused Deposition Modeling
G′	Storage Modulus
G″	Loss Modulus
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
Gel	Gelatin
GelMA	Gelatin Methacryloyl
GH	Guest–Host
HA	Hyaluronic Acid
HEK 293FT	Human Embryonic Kidney 293FT cell
I2959	Irgacure 2959 (4-(2-hydroxyethoxy)phenyl-(2-propyl)ketone)
LAP	Lithium Phenyl-2,4,6-trimethylbenzoylphosphinate
LIF	Leukemia Inhibitory Factor
MeHA	Methacrylated Hyaluronic Acid
mESC	Mouse Embryonic Stem Cell
MMP	Matrix Metalloproteinase
MMP-deg	Matrix Metalloproteinase-degradable Crosslinker

Nanog	Homeodomain-bearing Transcriptional Factor
NorHA	Norbornene-functionalized Hyaluronic Acid
Oct-4	Octamer-Binding Transcription Factor 4
PBS	Phosphate-Buffered Saline
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PEGDA	Poly(ethylene Glycol) Diacrylate
RGD	Arginine–Glycine–Aspartic Acid
SSEA1	Stage-Specific Embryonic Antigen 1
βCD	β-Cyclodextrin
βCD-HA	β-Cyclodextrin–Hyaluronic Acid
η	Viscosity
τ	Shear Force
$\dot{\gamma}$	Shear Rate
C	Circularity
Pr	Printability
v_{ink}	Ink Velocity
v_{noz}	Nozzle Velocity