AWARD NUMBER: W81XWH-14-1-0522

TITLE: Layer-by-Layer Bioprinting of Stem Cells for Retinal Tissue Regeneration

PRINCIPAL INVESTIGATOR: Shaochen Chen

CONTRACTING ORGANIZATION: University of California, San Diego
La Jolla, CA 92093

REPORT DATE: October 2015

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	30 Sep 2014 - 29 Sep 2015

4. TITLE AND SUBTITLE

Layer-by-Layer Bioprinting of Stem Cells for Retinal Tissue Regeneration

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0522

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Shaochen Chen and Kang Zhang

E-Mail: chen168@eng.ucsd.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California
SME 245B, MC 0448
9500 Gilman Dr.
La Jolla, CA 92093

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We aim to investigate a Layer-by-Layer (LBL) bioprinting process using stem cells for retinal tissue regeneration. The LBL nature of the bioprinting process matches nicely with the native, multilaminar anatomy of the retina. This research will bioprint retinal stem cells (RSCs) and appropriate growth factors (GF) encapsulated in a biomaterial (e.g. hyaluronic acid, HA). We will investigate the mechanical, chemical and biological properties of the hydrogel materials. We will study the cell viability and analyze the biological functions of the 3D printed retina tissue.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

1. Introduction

We report on the project - "Layer-by-Layer Bioprinting of Stem Cells for Retinal Tissue Regeneration" for FY 2014-2015. We have developed the hydrogel biomaterials with comparable mechanical properties that are 3D printable using light-based polymerization process. We have demonstrated that these 3D-printed hydrogel materials are also biocompatible for retinal cell growth.

2. Keywords

retinal tissue regeneration, 3D bioprinting, Layer-by-Layer, hydrogel biomaterials.

3. Accomplishments

What were the major goals of the project?

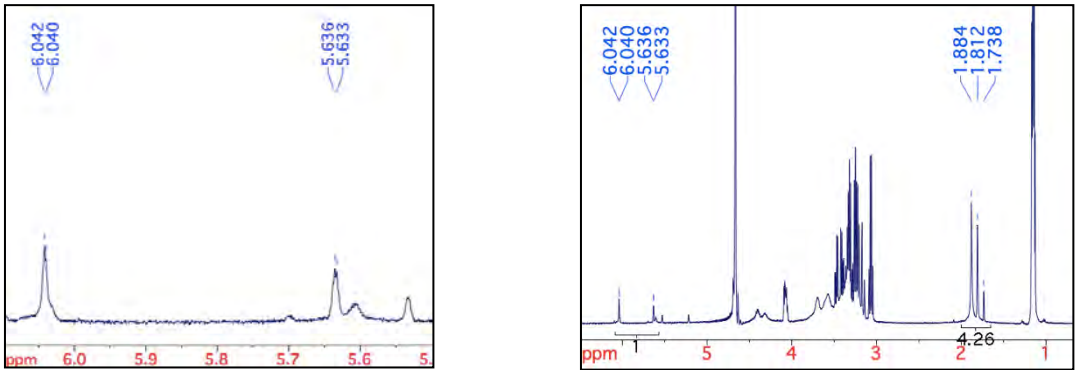
The major goal of this project is to investigate a Layer-by-Layer (LBL) bioprinting process using stem cells for retinal tissue regeneration. Our specific aims are as followings:

Specific Aim 1: Develop and optimize a 3D bioprinting method with encapsulated RSCs,

Specific Aim 2: Layer-by-layer bioprinting of *in vitro* retina PRs/RPE/Bruch's membrane tissues.

What was accomplished under these goals?

1) Major activities: There are two major tasks for Year 1: a) Biomaterial Development, b) Layer-by-layer Bioprinting
2) Specific objectives: a) Synthesis of hyaluronic acid - glycidyl methacrylate hydrogel; b) Mechanical Testing of the hydrogel; c) Bioprinter development and optimization; d) Layer-by-layer printing using hyaluronic acid - glycidyl methacrylate hydrogel; e) Layer-by-layer printing encapsulating retinal stem cells
3) Significant results or key outcomes:
   a) For the synthesis of HA-GM, 200 mg of hyaluronic acid (Lifecore Biomedical, MN) was added into 25ml of 50% acetone solution. The solution was mixed overnight before adding 1.8ml (20-fold molar excess) of triethylamine and glycidyl methacrylate. The reaction took 8 hours before dialysis and lyophilization. The methacrylation was confirmed by ^{1}H-NMR characterization (Joel 500) by the peaks at 5.6 and 6.0 ppm, as shown in **Fig. 1a.** The degree of methacrylation (DM) is determined by integration of the methacrylation group over that of the methyl groups in hyaluronic acid at 1.7, 1.8 and 1.9 ppm, as shown in **Fig. 1b**. For example the DM of the sample in Fig. 1a is 23%. The relation between the initial molar ratio of reagents and the degree of methacrylation of the product is shown in Table 1.



(a) (b)

Figure 1. *The ^{1}H-NMR results of HA-GM showing: a) the methacrylate group; b) the*

integration of methacrylate group over the methyl groups of hyaluronic acid to calculate the degree of methacrylation

***Table 1**. Degree of methacrylation of HA-GM synthesized by different initial stoichiometric ratio*

Initial molar ratio of glycidyl methacrylate to hyaluronic acid	Degree of methacrylation of final HA-GM
20:1	23%
10:1	11%
5:1	3%

b) For mechanical testing, the hydrogel sample was prepared by a 3D printing apparatus developed in the lab. A rectangular hydrogel sheet with a dimension of 5mm x 10mm x 1mm was made with the synthesized HA-GM. The elastic modulus of the 3D-printed sheets using HA-GM was determined by tensile tests using a thermomechanical analysis machine (TMA, Perkin Elmer). Briefly, the sample was clamped at two ends. The force applied on the sample started from 3mN with a constant increment of 5mN/min while the elongation of the sample was measured. A stress vs. strain curve is obtained and the elastic modulus is calculated as the slope of the curve. Samples made by HA-GM with methacrylation ratio lower than 23% could not be characterized by this method due to softness. Such softer hydrogels will be measured in the future using atomic force microscopy (AFM). The test result of 2% hydrogel made by HA-GM with 23% methacrylation is shown in **Fig. 2**. The elastic modulus of the hydrogel is about 30±5 kPa. Previous work suggested that the elastic modulus of native retina is about 20 kPa. Compare to other materials used for retina replacement, such as poly (glycerol-sebacate) which has an elastic modulus of 600kPa, HA-GM has closer mechanical property as native retina.

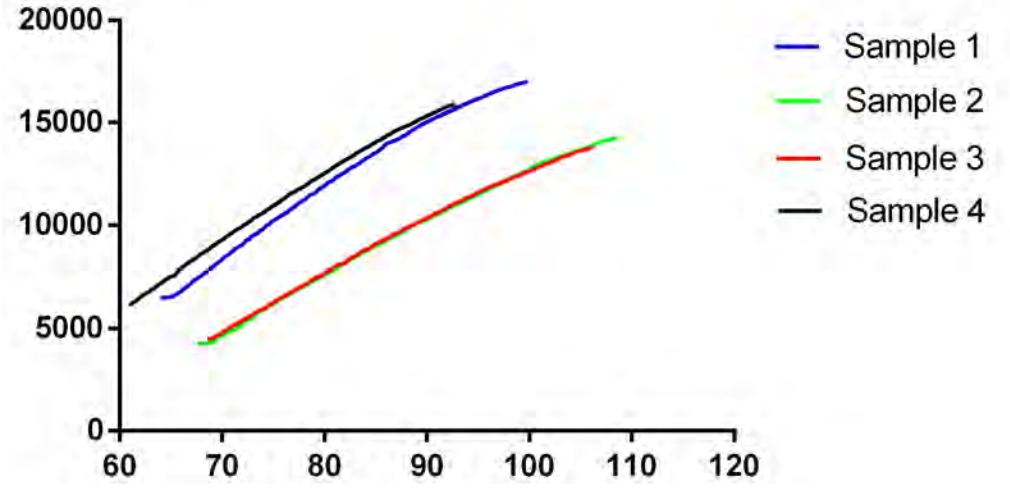


***Figure 2**. Stress vs. strain curve of HA-GM hydrogel with 23% methacrylation ratio*

c) We have established a strategy to print the HA-GM hydrogel into dome shapes to resemble the native environment for retina development. To improve the cell adhesion within the hydrogel, we added another printing material, methacrylated gelatin, GleMa, into the HA-GM hydrogel. As the hydrolysis product of collagen, gelatin could facilitate the adhesion of cells due to its chemical structure. We used lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) as the free radical generator. LAP has a local absorbance maximum at approximately 375 nm and significant absorbance at 365 nm. The pre-polymer resin included HA-GM, GelMa and LAP. Cell could be mixed into the resin if encapsulation is needed for year 2 tasks.

Figure 3 shows the 3D printing apparatus developed for this project. Briefly, a coherent light source (Omnicure S2000, 365nm) is used to provide the UV light for photo-polymerization. Patterns are generated by Adobe Photoshop and transferred to a digital-mirror array device (DMD) by in-house software. The DMD chip is used as an optical mask for projecting patterns onto the pre-polymer resin to allow the resin to polymerize. Once the light passes through the lenses, it is collimated to form a precise image on the resin. The resin is positioned on the stage. The stage could move in all three dimensions, dictated by the image from the computer. By changing the height of the stage, a 3D device can thus be fabricated.

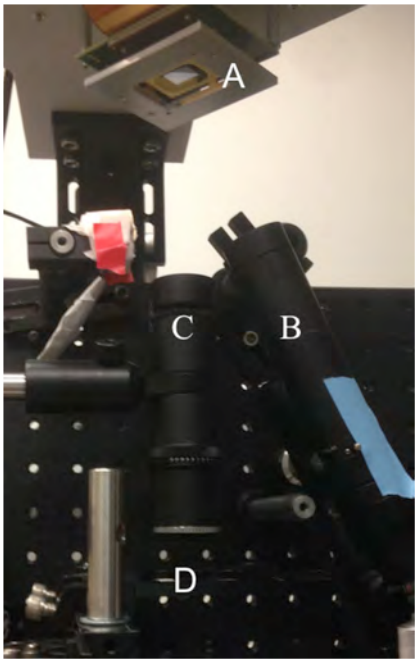


Figure 3*. Setup of the 3D printer. The labeled components are: A: DMD chip; B: lens system to project coherent light onto the DMD chip; C: lenses that collimate the light; D: stage that holds the pre-polymer resin.*

The printer enabled us to construct complex structures with the biomaterials. For example, we could print a dome structure with softer material as the core, followed by a harder material as a shell, as shown in **Figure 4**. Briefly, HA-GM was printed as the first layer in a cylindrical shape with thickness of 125 μm and diameter of 1,500μm. The shell was printed with HA-GM and GelMA mixture. It was printed in the same shape as the first layer with a thickness of 250 μm and diameter of 2,500 μm. With our printer and biomaterial, we could deliver a layer-by-layer printing strategy for retina regeneration *in vitro*.

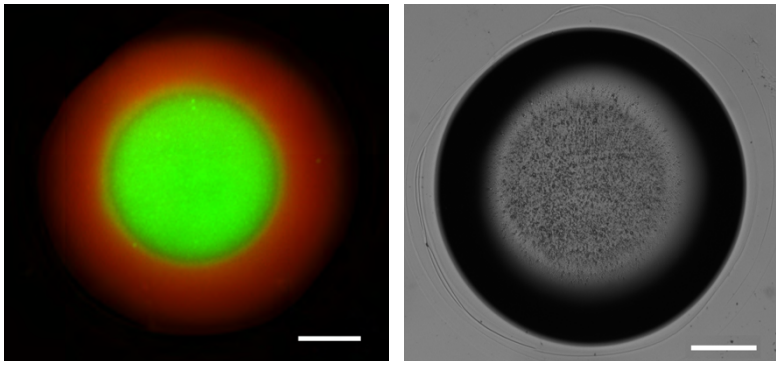


Figure 4*. A dome structure constructed by DMD based projection printing using biocompatible HA-GM and GelMA hydrogel. Green color comes from fluorescein isothiocyante-dextran in HA-GM as the first layer; red color comes from the tetramethylrhodamine isothiocyanate-dextran in HA-GM and GelMA as the second layer. Scale bar = 500 μm*

d) We developed the layer-by-layer (LBL) bioprinting system for the encapsulation of living cells, particularly retinal stem cells (RSCs). In the previous quarters, we were able to use the LBL system to print three-dimensional (3D) structures with hydrogel materials, such as hyaluronic acid-glycidyl methacrylate (HA-GM) and gelatin methacrylate (GelMa). During this quarter, we succeeded to incorporate living cells in the LBL bioprinting process, and the cell-loaded structures were cultured in vitro to evaluate the cell viability and investigate the interaction between cells and the microenvironment created by bioprinting.

Briefly, the RSCs were mixed with the monomer solution made of HA-GM and GelMa by gentle pipetting. The cell-loaded solution was then loaded to the stage for bioprinting. A core-shell structure was printed to mimic the curved layer-by-layer fashion of the native retina tissues (**Figure 5**). This design was also expected to introduce a gradient of nutrition and growth factor diffusion from the outside to the inside of the dome structure, which could guide the orientation of the cells to mimic the highly ordered retinal cells including photoreceptors, ganglion cells and bipolar cells. As shown in Figure 5, we were also able to vary the cell density in each layer to investigate the optimal condition for the differentiation of the RSCs into the cells of different layers in the retinal tissue. The cell density in the core part was 30 million per ml and the cell density in the shell was 15 million per ml.

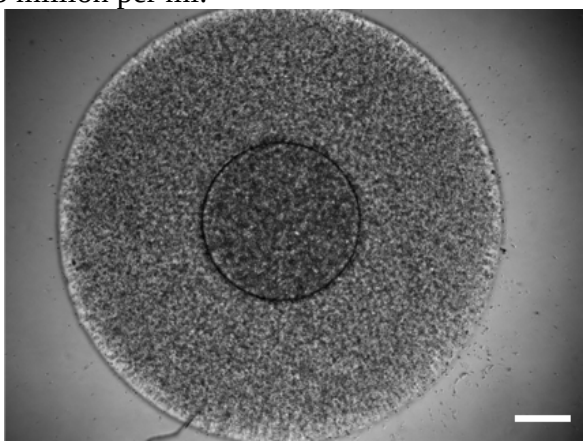


Figure 5. *Bioprinted core-shell structure encapsulated with RSCs. Scale bar: 500 μm.*

After 10 days of in vitro culture, we found that some RSCs were spreading in the hydrogel structure along the direction of the radius as well as the interface between the core and the shell (**Figure 6**). Immunofluorescence staining also showed the expression of photoreceptor-specific reporter IRBP-GFP (green) and recoverin (red), indicating photoreceptor induction with the help of differentiation medium (**Figure 7**).

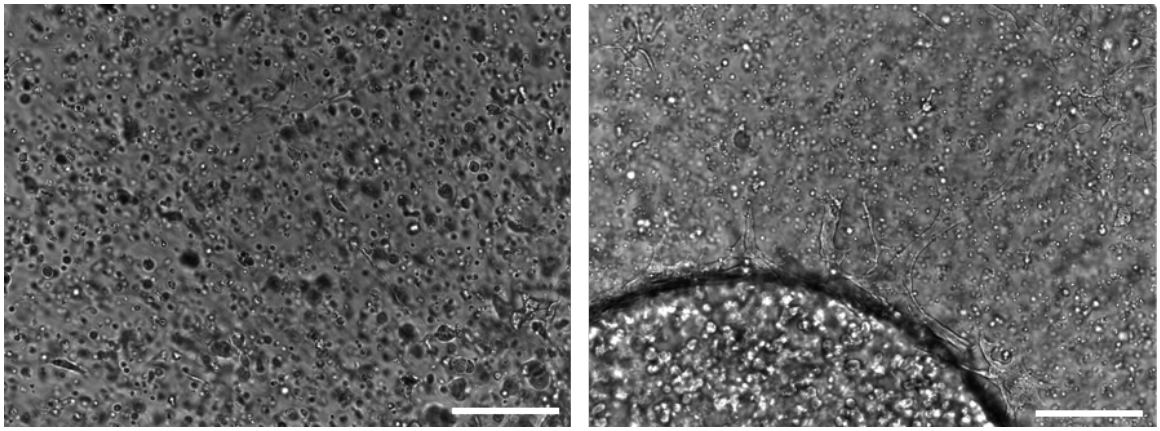


Figure 6. *Encapsulated cells spreading in the shell layer (left) and the interface between the core and the shell at day 10. Scale bar: 250 μm.*

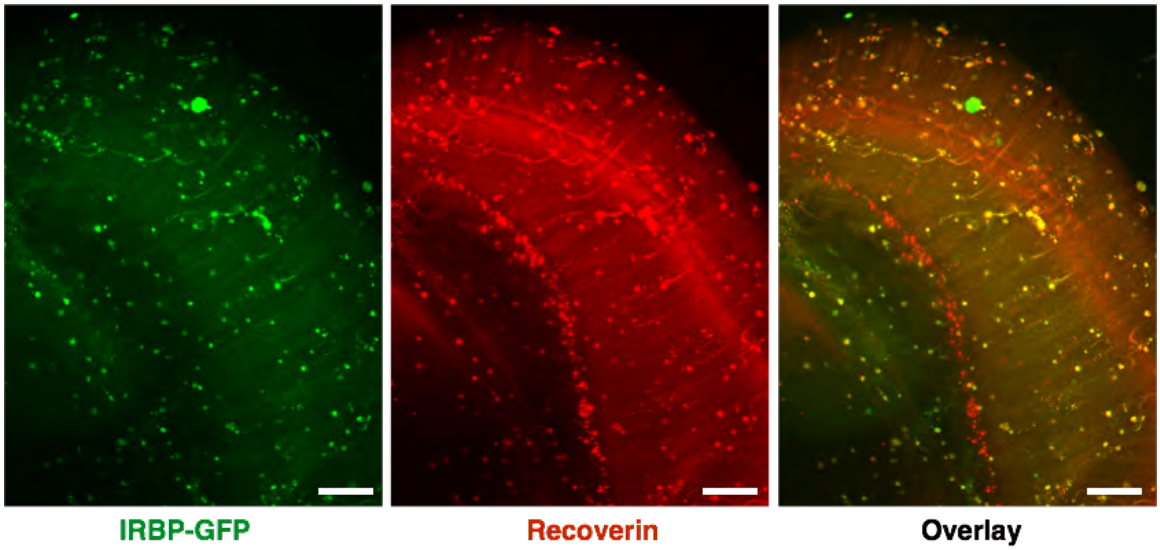


Figure 7. *Characterization of RSC differentiation to photoreceptors at day 10. Green: photoreceptor-specific reporter IRBP-GFP. Red: recoverin. Scale bar: 250 μm.*

In order to construct a multi-layered structure to facilitate the differentiation of RSCs, a single layered hRPE cells is desired. A uniform spreading of hRPE cells mimics the *in vitro* environment during the retinal formation, thus secreting the necessary growth factors and differentiation signals for the RSCs . We have prepared a single layered hRPE cells by encapsulating the cells into GelMa solution and printed a thin layer on the cover glass, as shown in **Fig. 8**. At day 2, the hRPE cells were still encapsulated in the gel without spreading. After 4 more days, the cells have detected the stiffer glass surface and migrated to form a single layer.

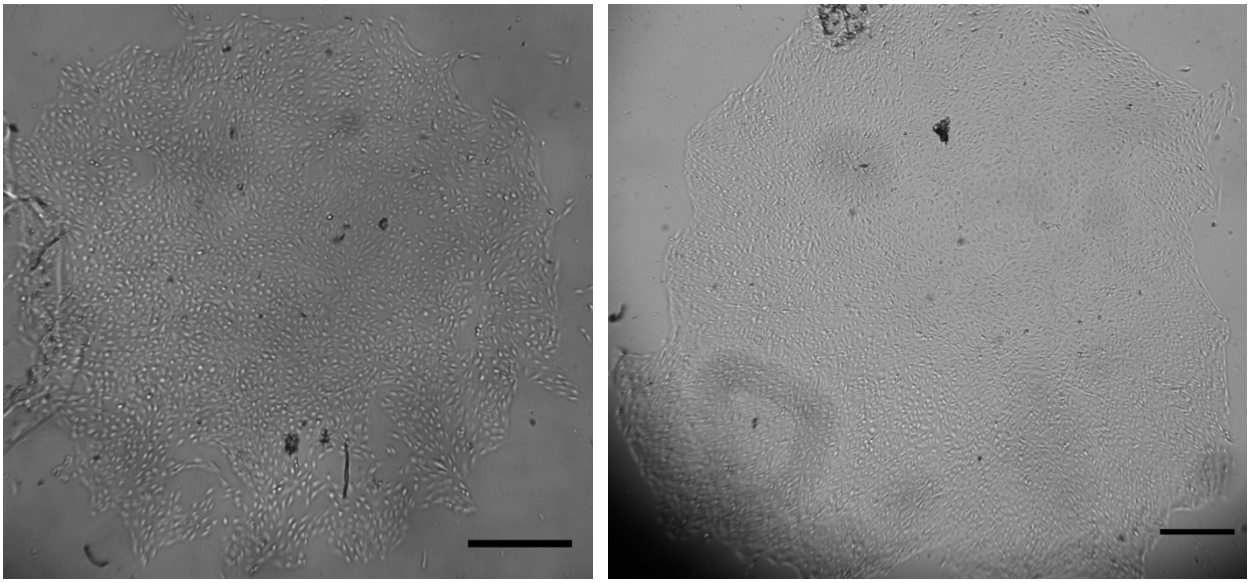


Figure 8. Formation of single layered hPRE cells: Left: 2 days after printing. Right: 6 days after printing.

What opportunities for training and professional development have the project provided?
Nothing to Report

How were the results disseminated to communities of interest?
We have presented our research results in several invited talks in university seminars and professional meetings such as the Pre Retina Society Annual Meeting, Philadelphia, PA.

What do you plan to do during the next reporting period to accomplish the goals?
We plan to carried out research for Specific Aim 2 - Layer-by-layer bioprinting of *in vitro* retina PRs /RPE/Bruch's membrane tissues. We will first print out three individual layers for PRs, RPE, and Brunch's membrane separately and study cell functions. Then we will print a multilayer constructs with all three cell types.

4. Impact

What was the impact on the development of the principal discipline(s) of the project?
The layer-by-layer 3D bioprinting method will be a viable tool for retinal research. It could also be translational for future clinical uses in retina tissue regeneration. The hydrogel biomaterials will also be useful for creating retinal tissue constructs that could be used for basic research.

What was the impact on other disciplines?
The 3D bioprinting process is versatile in that it can be applied to other cell and tissue types. The 3D-printed retinal tissues could be used as in vitro models for early drug screening.

What was the impact on technology transfer?
We will file a provisional patent when we have enough results.

What was the impact on society beyond science and technology?
Development of artificial retina tissues will change the clinical landscape by eliminating the current dependency on retina donor tissue and by providing a new strategy for restoring vision that would otherwise be lost in soldiers with severe retina blindness. The proposed 3D bioprinting and stem cell engineering represent an integration of emerging technologies that are truly novel for retina repair and regeneration.

5. Changes/Problems

Nothing to Report

6. Products

Publications, conference papers, and presentations:
Dr. Chen was invited to give the following seminars and presentation

- Corinne Bower Lecture, entitled "3D Bioprinting: Materials, Fabrication, and Tissue Engineering", in the Pre Retina Society Annual Meeting, Philadelphia, PA, 2014 (**Invited)**
- "Micro and Nanoscale 3D Bioprinting: Materials, Fabrication, and Tissue Engineering," (Distinguished Seminar) University of California at Davis, March 12, 2015. (**Invited)**
- "Applications and Breakthroughs in Tissue Engineering and Bioprinting – What's Next?" in *Rock Stars of Innovation Summit*, San Diego, June 2015. (**Invited)**
- W. Zhu, **S.C. Chen**, "Micro- and Nanoscale 3D Bioprinting for Functional Tissue Scaffolds" Functional Analysis and Screening technologies Congress, Boston, MA, Nov. 17-19, 2014. (**Invited)**
- **S.C. Chen**, "Nano and Microscale 3D Bioprinting: An Enabling Technology for Personalized Regenerative Medicine", National Academy of Engineering (NAE) China-American Frontiers of Engineering Symposium, Irvine, CA, June 1-3, 2015. (**Invited)**
- **S.C. Chen**, "Micro and Nanoscale 3D Bioprinting for Functional Tissue Scaffolds", TERMIS 2015, Boston. (**Invited)**

Dr. Zhang was invited to give a seminar - "Genetics, Epigenetics, and Stem Cell Based Therapies for Blinding Eye Diseases", Dept. of Bioengineering, UC San Diego, May 1, 2015.

Dr. Zhang and Nature Publishers are organizing a *Nature Conference on Tissue Engineering and Regenerative Medicine* in 2016.

Website(s) or other Internet site(s)
Dr. Chen's lab website updates news and publications: http://schen.ucsd.edu/lab/index.html
Dr. Chen's lab website updates news and publications: http://zhanglab.ucsd.edu

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
Nothing to report

Other Products
Nothing to report

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name	Shaochen Chen
Project Role	PI
Researcher Identifier	NA
Nearest person month worked	1
Contribution to Project	Supervised the project, designed the experiments, and advised the graduate students
Funding Support	Partially from this grant.

Name	Kang Zhang
Project Role	Co-PI
Researcher Identifier	NA
Nearest person month worked	1
Contribution to Project	Co-supervised the project, co-designed the experiments, and co-advised the graduate students
Funding Support	Partially from this grant.

Name	Wei Zhu
Project Role	Graduate Student
Researcher Identifier	NA
Nearest person month worked	6
Contribution to Project	Carried out the experiments, and analyzed the results
Funding Support	Partially from this grant.

Name	Pengrui Wang
Project Role	Graduate Student
Researcher Identifier	NA
Nearest person month worked	10
Contribution to Project	Carried out the experiments, and analyzed the results
Funding Support	Partially from this grant.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

New grants:

CMMI1547005 (Chen) 10/1//2015–9/30/2017 1%
NSF $39,734
EAGER/Cybermanufacturing: Cloud-based, Rapid, Microscale 3D Bioprinting
The major goal of this project is to develop a cyber-manufacturing system for 3D printing using cloud-based computational approach.
Our specific objectives are to: 1) Create and characterize the mCOP bioprinting platform for digital, projection 3D printing, 2) Demonstrate the use of mCOP for creating complex structures in hydrogel, and 3) Create live, functional 3D scaffolds with cell encapsulation.
POC: Dr. Bruce Kramer, (703) 292-5348, bkramer@nsf.gov

RT3-07899 (Chen and Xu) 6/01/15-7/31/18 16%
California Institute of Regenerative Medicine $300,000
Development of 3D Bioprinting Techniques using Human Embryonic Stem Cells Derived Cardiomyocytes for Cardiac Tissue Engineering
The goal of this project is to develop a 3D bioprinting platform using hESC for the direct-write of 3-dimensional functional cardiac tissue constructs.
Specific Aims are: 1) Develop and optimize a 3D printing technique to create biomimetic 3D micro-architectures using HA-based biomaterials and hESC-derived cardiomyocytes, 2) Create an advanced vascularization technique for 3D pre-vascularized cardiac tissues with precise control of spatial organization, 3) Test the *in vivo* functionality of the 3D bioprinted pre-vascularized cardiac tissue in Hu-mice and rat MI models.
POC: Dr. Ryan Wells, 415-396-9124, rwells@cirm.ca.gov

Completed projects / grants

R01EB012597 (Chen, Khademhosseini) 08/30/2010 – 08/31/2014 8%
NIH/NIBIB $240,000
A Microfabrication Platform for Direct Printing Vascularized Functional Tissue Constructs
The major goal of this project is to develop a microfabrication platform using projection printing for 3D scaffold fabrication and create vascularized functional tissue constructs.
Specific Aims are to: 1) develop and optimize the PSL system for the fabrication of 3D microstructures using HA with Arg-Gly-Asp (RGD) and matrix metalloproteinase (MMP), 2) direct-write 3D HA scaffolds encapsulating cardiomyocytes, 3) create vascularized structures in a 3D scaffold and analyze vasculature functions.
POC: Dr. Rosemarie Hunziker, 301-451-1609, hunzikerr@mail.nih.gov

CMMI 1130894 (Chen) 08/01/2011 – 07/31/2014 5%
National Science Foundation $53,841
Collaborative Research: Nano/Femtosecond Laser Processing of Gas Impregnated Polymer for Biomedical Applications
The major goals of this project are to develop a micro-manufacturing process by integrating a nanosecond laser with a femtosecond laser for polymer processing.
Our objectives are to: 1) study the heating and ablation mechanisms of the nano-/femtosecond lasers in gas impregnated polymers, 2) study the bubble nucleation and growth process under nanosecond laser heating, 3) evaluate the fabricated porous scaffolds for organotypic 3D cell culturing.
POC: Dr. Mary Toney, (703) 292-7008, mtoney@nsf.gov

What other organizations were involved as partners?

Nothing to report

8. Special Reporting Requirements

The Quad Chart is shown below.

Layer-by-Layer Bioprinting of Stem Cells for Retinal Tissue Regeneration

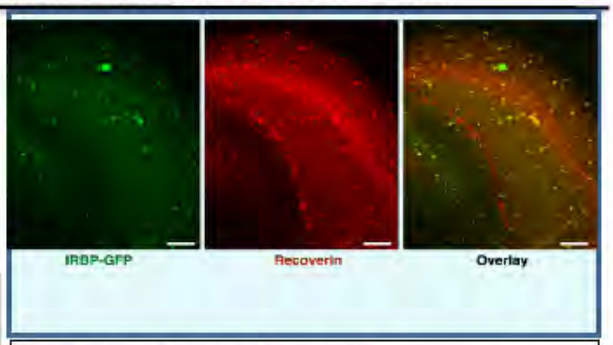
PI: Shaochen Chen and Kang Zhang **Org:** University of California, San Diego **Award Amount: $250,000**

Study/Product Aim(s)

- Aim 1: Develop and optimize a 3D bioprinting method with encapsulated RSCs
- Aim 2: Layer-by-layer bioprinting of *in vitro* retina PRs /RPE/Bruch's membrane tissues

Approach

We hypothesize that through layer-by-layer bioprinting with retinal stem cells (RSCs) and appropriate growth factors (GF) encapsulated in a biomaterial (e.g. hyaluronic acid, HA), we can regenerate the anatomically correct retina in a biomimetic fashion, thus creating a paradigm shift in retinal tissue engineering.



RSC differentiation to photoreceptors at day 10. Green: photoreceptor-specific reporter IRBP-GFP. Red: recoverin. Scale bar: 250 μm.

Timeline and Cost

Activities CY	14	15	16	17
Aim 1				
Aim 2				
Estimated Budget ($K)	**$125k**	**$125k**	**$000**	**$000**

Updated: 10/24/2015

Goals/Milestones

CY14 Goal –3D bioprinting development
- ☑ Synthesis of HA-GM
- ☑ Mechanical Testing of HA-GM
- ☑ 3D printing of HA-GM
- ☑ Create scaffolds with growth factors and RSCs

CY15 Goals – LBL bioprinting of *in vitro* retina tissues
- ☐ Print HA scaffolds with RSC and different growth factors
- ☐ Characterize biological function of the 3D retinal tissue constructs

9. Appendices

Abstracts for presentation in university seminars and conferences

1) *Corinne Bower Lecture, Pre Retina Society Annual Meeting, Philadelphia, PA, 2014*

3D Bioprinting: Materials, Fabrication, and Tissue Engineering
Shaochen Chen, Ph.D.
Professor of NanoEngineering and Bioengineering Departments
Co-Director, Biomaterials & Tissue Engineering Center, Institute of Engineering in Medicine,
University of California, San Diego

Abstract

The goal of our laboratory is to develop micro- and nano-scale bioprinting techniques for the direct-write of 3D designer scaffolds used for tissue engineering and regenerative medicine. In this talk, I will present my laboratory's recent research efforts in femtosecond laser nano-printing and projection 3D bioprinting to create 3D scaffolds using a variety of biomaterials. These 3D biomaterials are functionalized with precise control of micro-architecture, mechanical (e.g. stiffness and Poisson's ratio), chemical, and biological properties. Design, fabrication, and experimental results will be discussed. Such functional biomaterials allow us to investigate cell-microenvironment interactions at nano- and micro-scales in

response to integrated physical and chemical stimuli. From these fundamental studies we can create both *in vitro* and *in vivo* tissue models for precision tissue engineering and regenerative medicine.

2) *(Distinguished Seminar) University of California at Davis, March 12, 2015*

3D Bioprinting: An Enabling Technology for Tissue Engineering and Regenerative Medicine

Shaochen Chen, Ph.D.
Professor of NanoEngineering and Bioengineering Departments
Co-Director, Biomaterials & Tissue Engineering Center
Institute of Engineering in Medicine
University of California, San Diego

Abstract

My laboratory aims to develop micro- and nano-scale bioprinting techniques for the direct-write of 3D designer scaffolds used for tissue engineering and regenerative medicine. In this talk, I will present my laboratory's recent research efforts in 3D nano-printing and rapid 3D bioprinting to create 3D scaffolds using a variety of biomaterials. These 3D biomaterials are functionalized with precise control of micro-architecture, mechanical (e.g. stiffness and Poisson's ratio), chemical, and biological properties. Design, fabrication, and experimental results will be discussed. Such functional biomaterials allow us to investigate cell-microenvironment interactions at nano- and micro-scales in response to integrated physical and chemical stimuli. From these fundamental studies we can create both *in vitro* and *in vivo* tissue models for precision tissue engineering and regenerative medicine.

3) *National Academy of Engineering (NAE) China-American Frontiers of Engineering Symposium, Irvine, CA, June 1-3, 2015.*

Nano and Microscale 3D Bioprinting: An Enabling Technology for Personalized Regenerative Medicine

Shaochen Chen, Ph.D.
Professor of NanoEngineering and Bioengineering Departments
Co-Director, Biomaterials & Tissue Engineering Center, Institute of Engineering in Medicine,
University of California, San Diego

Abstract

In my laboratory, we develop micro- and nano-scale bioprinting techniques for the direct-write of 3D designer scaffolds used for tissue engineering and regenerative medicine. In this talk, I will present my laboratory's recent research efforts in femtosecond laser nano-printing and projection 3D bioprinting to create 3D scaffolds using a variety of biomaterials. These 3D biomaterials are functionalized with precise control of physical, mechanical, chemical, and biological properties, aiming for personalized medicine. Design, fabrication, and experimental results will be discussed. Such functional biomaterials allow us to investigate cell-microenvironment interactions at nano- and micro-scales in response to integrated physical and chemical stimuli. From these fundamental studies we can create both *in vitro* and *in vivo* tissue models for precision tissue engineering and regenerative medicine.