CA 15214
Inter-WG meeting proposal: **Probing the effects of 3D Bioprinting Processes on Stem Cells' Functional Properties and Integrity.**

Proposers:
Aylin Sendemir-Urkmez (sendemir@gmail.com)
Yannis Missirlis (yfmissirlis@gmail.com)
George Miloshev (gamilo73@yahoo.com)

Purpose: To discuss possibilities, and identify specific topics for collaborative proposals, spanning all WGs of our Action in the area of 3D Bioprinting. More specifically, to examine the fate and integrity of the embedded stem cells during and after the process. The outcome of this one-day brain-storming will be shared with all members of all WGs for further action.

Rationale: 3D bioprinting is a process that is fast gaining ground among other processes in Tissue Engineering. Although current bioprinting techniques allow versatile, layer by layer production of complex tissue constructs composed of multiple cells and ECM components with high resolution, the effects of mechanical and/or thermal stress the cells are exposed to during the process have not been thoroughly analyzed (see 2 relevant publications). Besides, the dynamics in their epigenetic programme during and after the bioprinting process has not yet been studied. It is essential to investigate and control short and long term effects of the processing parameters on stem cell survival, epigenetic make-up and functionality in order to utilize this emerging tool in clinical applications. Furthermore the proposers have experience in 3D bioprinting and tissue engineering, in assessing DNA damage and relevant markers of epigenetic and phenotypic changes, as well as in mechanobiology and fluid mechanics.

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Investigation of cell viability and morphology in 3D bio-printed alginate constructs with tunable stiffness

Pujiang Shi,[1] Augustinus Laude,[2] Wai Yee Yeong[1]

[1]Singapore Centre for 3D Printing, School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

[2]National Healthcare Group Eye Institute, Tan Tock Seng Hospital, Singapore 308433

Received 25 October 2016; revised 28 November 2016; accepted 30 November 2016

Published online 1 February 2017 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.35971

Abstract: In this article, mouse fibroblast cells (L929) were seeded on 2%, 5%, and 10% alginate hydrogels, and they were also bio-printed with 2%, 5%, and 10% alginate solutions individually to form constructs. The elastic and viscous moduli of alginate solutions, their interior structure and stiffness, interactions of cells and alginate, cell viability, migration and morphology were investigated by rheometer, MTT assay, scanning electron microscope (SEM), and fluorescent microscopy. The three types of bio-printed scaffolds of distinctive stiffness were prepared, and the seeded cells showed robust viability either on the alginate hydrogel surfaces or in the 3D bio-printed constructs. Majority of the proliferated cells in the 3D bio-printed constructs weakly attached to the surrounding alginate matrix. The concentration of alginate solution and hydrogel stiffness influenced cell migration and morphology, moreover the cells formed spheroids in the bio-printed 10% alginate hydrogel construct. © 2017 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 105A: 1009–1018, 2017.

Key Words: alginate, bio-printing, three-dimensional (3D) printing, additive manufacturing, rapid prototyping

IOP Publishing Biofabrication 8 (2016) 035020 doi:10.1088/1758-5090/8/3/035020

Biofabrication

CrossMark

RECEIVED
13 May 2016

REVISED
24 July 2016

ACCEPTED FOR PUBLICATION
31 August 2016

PUBLISHED
16 September 2016

PAPER

Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells

Liliang Ouyang[1,2], **Rui Yao**[1,2], **Yu Zhao**[1,2] **and Wei Sun**[1,2,3]

[1] Biomanufacturing Center, Department of Mechanical Engineering, Tsinghua University, Beijing 100084, People's Republic of China
[2] Biomanufacturing and Rapid Forming Technology Key Laboratory of Beijing, Beijing 100084, People's Republic of China
[3] Department of Mechanical Engineering, Drexel University, Philadelphia, PA 19104, USA

E-mail: yaorui@tsinghua.edu.cn

Keywords: 3D cell printing, embryonic stem cells, printability, viability, bioink properties, bioprinting

Supplementary material for this article is available online

Abstract

3D cell printing is an emerging technology for fabricating complex cell-laden constructs with precise and pre-designed geometry, structure and composition to overcome the limitations of 2D cell culture and conventional tissue engineering scaffold technology. This technology enables spatial manipulation of cells and biomaterials, also referred to as 'bioink', and thus allows study of cellular interactions in a 3D microenvironment and/or in the formation of functional tissues and organs. Recently, many efforts have been made to develop new bioinks and to apply more cell sources for better biocompatibility and biofunctionality. However, the influences of printing parameters on the shape

Journal of Mechanics in Medicine and Biology
Vol. 15, No. 5 (2015) 1550073 (15 pages)

DOI: 10.1142/S0219519415500736

MODELING MECHANICAL CELL DAMAGE IN THE BIOPRINTING PROCESS EMPLOYING A CONICAL NEEDLE

MINGGAN LI[*], XIAOYU TIAN[†], JANUSZ A KOZINSKI[‡,§], XIONGBIAO CHEN[†,¶,**] and DAE KUN HWANG[*,‖,**]

[*]*Department of Chemical Engineering, Ryerson University 350 Victoria Street, Toronto, Ontario, M5B 2K3, Canada*

[†]*Department of Mechanical Engineering, University of Saskatchewan 57 Campus Dr., Saskatoon, SK, S7N 5A9, Canada*

[‡]*Lassonde School of Engineering, York University 4700 Keele Street, Toronto, Ontario, M3J 1P3, Canada*

[§]*janusz.kozinski@lassonde.yorku.ca*
[¶]*xbc719@mail.usask.ca*
[‖]*dkhwang@ryerson.ca*

JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med 2015; 9: 1286–1297.
Published online 24 January 2013 in Wiley Online Library (wileyonlinelibrary.com) DOI: 10.1002/term.1682

RESEARCH ARTICLE

An additive manufacturing-based PCL–alginate–chondrocyte bioprinted scaffold for cartilage tissue engineering

Joydip Kundu[1†], Jin-Hyung Shim[1†], Jinah Jang[2], Sung-Won Kim[3] and Dong-Woo Cho[1,2*]
[1]*Department of Mechanical Engineering, Pohang University of Science and Technology (POSTECH), Kyungbuk, South Korea*
[2]*Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology (POSTECH), Kyungbuk, South Korea*
[3]*Department of Otolaryngology-Head and Neck Surgery, The Catholic University of Korea, College of Medicine, Seoul, Korea*

Abstract

Regenerative medicine is targeted to improve, restore or replace damaged tissues or organs using a combination of cells, materials and growth factors. Both tissue engineering and developmental biology currently deal with the process of tissue self-assembly and extracellular matrix (ECM) deposition. In this investigation, additive manufacturing (AM) with a multihead deposition system (MHDS) was used to fabricate three-dimensional (3D) cell-printed scaffolds using layer-by-layer (LBL) deposition of polycaprolactone (PCL) and chondrocyte cell-encapsulated alginate hydrogel. Appropriate cell dispensing conditions and optimum alginate concentrations for maintaining cell viability were determined. *In vitro* cell-based biochemical assays were performed to determine glycosaminoglycans (GAGs), DNA and total collagen contents from different PCL–alginate gel constructs. PCL–alginate gels containing transforming growth factor-β (TGFβ) showed higher ECM formation. The 3D cell-printed scaffolds of PCL–alginate gel were implanted in the dorsal subcutaneous spaces of female nude mice. Histochemical [Alcian blue and haematoxylin and eosin (H&E) staining] and immunohistochemical (type II collagen) analyses of the retrieved implants after 4 weeks revealed enhanced cartilage tissue and type II collagen fibril formation in the PCL–alginate gel (+TGFβ) hybrid scaffold. In conclusion, we present an innovative cell-printed scaffold for cartilage regeneration fabricated by an advanced bioprinting technology.

SCIENCE ADVANCES | RESEARCH ARTICLE

MATERIALS SCIENCE

3D printing of bacteria into functional complex materials

Manuel Schaffner,[1]* Patrick A. Rühs,[1]*† Fergal Coulter,[1,2] Samuel Kilcher,[3] André R. Studart[1]†

Despite recent advances to control the spatial composition and dynamic functionalities of bacteria embedded in materials, bacterial localization into complex three-dimensional (3D) geometries remains a major challenge. We demonstrate a 3D printing approach to create bacteria-derived functional materials by combining the natural diverse metabolism of bacteria with the shape design freedom of additive manufacturing. To achieve this, we embedded bacteria in a biocompatible and functionalized 3D printing ink and printed two types of "living materials" capable of degrading pollutants and of producing medically relevant bacterial cellulose. With this versatile bacteria-printing platform, complex materials displaying spatially specific compositions, geometry, and properties not accessed by standard technologies can be assembled from bottom up for new biotechnological and biomedical applications.

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Controlling Shear Stress in 3D Bioprinting is a Key Factor to Balance Printing Resolution and Stem Cell Integrity

*Andreas Blaeser, Daniela Filipa Duarte Campos, Uta Puster, Walter Richtering, Molly M. Stevens, and Horst Fischer**

Bioprinting is an emerging tissue engineering (TE)[1–4] discipline that pursues the goal of generating viable tissue constructs using additive manufacturing technologies. Cell-laden hydrogels are printed layer-by-layer according to a predefined, in cell biology,[29,30] e.g., in cell signaling and protein expression.[31–33] For instance, it is reported that shear stress promotes maturation of megakaryocytes.[34] Moderate shear stress was found to have an influence on stem cell differentiation.[35]

Biotechnology Journal
DOI 10.1002/biot.200900004
Biotechnol. J. 2009, 4, 1168–1177

Research Article

Characterization of cell viability during bioprinting processes

Kalyani Nair[1], *Milind Gandhi*[1], *Saif Khalil*[1], *Karen Chang Yan*[2], *Michele Marcolongo*[3], *Kenneth Barbee*[4] *and Wei Sun*[1]

[1]Department of Mechanical Engineering and Mechanics, Drexel University, Philadelphia, PA, USA
[2]Department of Mechanical Engineering, The College of New Jersey, Ewing, NJ, USA
[3]Department of Materials Science and Engineering, Drexel University, Philadelphia, PA, USA
[4]School of Biomedical Engineering, Science & Health Systems, Drexel University, Philadelphia, PA, USA

Available online at www.sciencedirect.com

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Current Opinion in
Biomedical Engineering

Three-dimensional bioprinting for bone tissue regeneration

Shivakalyani Adepu[a], Nandini Dhiman[b], Anindita Laha[c], Chandra S. Sharma[d], Seeram Ramakrishna[e] and Mudrika Khandelwal[a]

Abstract
Three-dimensional bioprinting can prove to be a promising technology for bone tissue regeneration as it facilitates good spatio-temporal distribution of cells in scaffold. The feed for bio-printing is bioink, which comprises of cells incorporated in the scaffold material. Progress has been made on the incorporation of growth factors in the bioink, which not only enables efficient regeneration but at the same time proves the feasibility of large constructs. Important parameters which determine the suitability of bioink have been discussed here. Lack of vascularization limits the success of this technology in its present form. Advances in inducing vascularization and growth factors have also been discussed. Towards the end, challenges and opinions in the area of bioprinting of bone tissue regeneration have been presented.

or organs. The conventional 3D printing approach involves the predefined layered printing of scaffolds followed by cell seeding and perfusing the construct before implantation. However, this method suffers from a lack of uniform spatial and temporal distribution of cells and growth factors in the construct. A new class of 3D printing called bioprinting – printing along with the cells – promises to overcome all these limitations.

In this mini review, we report the most recent advances in the field of 3D bioprinting of bone with respect to methods, bioink properties and growth factors/drug aided vascularization in the constructs. Later, we present our opinion and the challenges needed to overcome, to advance the field of bone bioprinting.