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BIOADDITIVE MANUFACTURING OF HYBRID TISSUE SCAFFOLDS FOR CONTROLLED RELEASE KINETICS

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Abstract
Development of engineered tissue scaffolds with superior control over cell-protein interactions is still very much infancy. Advancing through heterogeneous multifold scaffolds with controlled release fashion enables synchronization of regenerating tissue with the release kinetics of loaded biomolecules. This might be an engineering challenge and promising approach for improved and efficient tissue regeneration. The most critical limitations: the selection of proper protein(s) incorporation, and precise control over concentration gradient and timing should be overcome. Hence, tissue scaffolds need to be fabricated in a way that proteins or growth factors should be incorporated and released in a specific spatial and temporal orientation to mimic the natural tissue regeneration process. Spatial and temporal control over heterogeneous porous tissue scaffolds can be achieved by controlling two important parameters: (i) internal architecture with enhanced fluid transport, and (ii) distribution of scaffold base material and loaded modifiers.

In this research, heterogeneous tissue scaffolds are designed considering both the parameters. Firstly, the three-dimensional porous structures of the scaffold are geometrically partition into functionally uniform porosity regions and controlled spatial micro-architecture has been achieved using a functionally gradient porosity function. The bio-fabrication of the designed internal porous architecture has been performed using a single nozzle bioadditive manufacturing system. The internal architecture scheme is developed to enhance fluid transport with continuous base material deposition

Next, the hybrid tissue scaffolds are modeled with varying material characteristics to mediate the release of base material and enclosed biological modifiers are proposed based on tissue engineering requirements. The hybrid scaffolds are fabricated for spatial control of biomolecules and base material to synchronize the release kinetics with tissue regeneration. A pressure-assisted multi-chamber single nozzle bioadditive manufacturing system is used to fabricate hybrid scaffolds.

1. Introduction

Current medical procedures aim to restore tissue function to patients with diseased or damaged tissues through tissue transplantation and implants. Tissue Engineering, an interdisciplinary field of biology, biomaterials and engineering, is seeking to restore tissue functions by developing engineered scaffolds providing optimum environment for cell attachment and growth, tissue regeneration, fluid movement and structural integrity [1]. Engineered scaffold attempts to mimic the complexity of both external and internal architecture of replaced tissue in a way that optimal microenvironment is designed for the cells to culture and develop into tissues. Optimal microenvironment can be achieved by signaling cellular activity through delivering vital biological modifiers such as proteins, growth factors and drugs. Release of these modifiers with spatial and temporal gradient concentration mediates tissue regeneration process. It develops a mental biology, in which cells are guided by a mechanism with respect to obtained spatial and directional cues [2]. Moreover, mediating the degradation by controlling the scaffold geometry in micro-scale could have a big impact on cell growth and proliferation. MacKay and Miller [3] studied effects of nutritional and botanical support on wound healing process and concluded that adequate protein intake is necessary for proper wound healing.

Therefore, it is essential to release encapsulated biological modifier in a controlled fashion since the incorporated modifiers need to maintain their integrity to stimulate desirable cells to achieve biological response and thereafter the degradation of the release system [4]. As a result, scaffolds in tissue engineering should be fabricated in way that they enable controlled release scheme of biological modifiers with distinct spatial gradient to guide specific cues to the cellular microenvironment. Weiss *et al* [5] have presented a Bayesian methodology for computer-aided experimental design of heterogeneous fibrin-based scaffolds having spatial distribution of growth factors designed to induce and direct the growth of new tissue as the scaffold degrades, however bioactive molecules are discrete entities and need to be spatially distributed in a controlled fashion within the porous structure.

In this paper, proteins, growth factors and cells etc. are named with a general term "biological modifiers". Development of engineered tissue scaffolds with superior control over cell-biological modifier interactions is still very much infancy. Advancing through heterogeneous multifold scaffolds with controlled release fashion enables synchronization of regenerating tissue with the release kinetics of loaded biological modifiers. This might be an engineering challenge and promising approach for improved and efficient tissue regeneration. The most critical limitations: the selection of proper biological modifier(s) incorporation, precise control over concentration gradient and timing should be overcome. Hence, tissue scaffolds need to be fabricated in a way that biological modifiers should be incorporated and released in a specific spatial and temporal orientation to mimic the natural tissue regeneration process. In this paper, spatial and temporal control over heterogeneous tissue scaffolds is achieved by controlling two important parameters: (i) internal architecture with controlled fluid transport, and (ii) distribution of scaffold base material and loaded modifiers.

2. Materials and Methods

2.1 Materials

Sodium alginate (SA) from brown algae and calcium chloride were purchased from Sigma-Aldrich, USA. Nozzle tips for dispensing systems were purchased from Nordson EFD, USA. 3-5% (w/v) alginate solutions were prepared by suspending alginate into deionized (DI) water and stirred at room temperature for a day. Calcium chloride was suspended in DI water to obtain 0.6% (w/v) calcium chloride solution for crosslinking purposes. Micro-glass beads in powder form were purchased from Corpuscular Inc. to represent biomolecules.

2.2 Experimental Setup

2.2.1 Single Nozzle Bioadditive Manufacturing Platform

Biofabrication of the porous hybrid scaffolds with controlled internal architecture is performed using a 3D micro-nozzle bioadditive manufacturing system (see Fig.1). The fabrication process is biologically compatible. Sodium alginate, a type of hydrogel widely used in cell immobilization, cell transplantation, and tissue engineering, is preferred as biomaterial due to its biocompatibility and formability. Sodium alginate solution is filled in a reservoir and a pneumatic system is deployed to flow the solution via the micro-nozzles (150 μm). The system runs at room temperature under low pressure (0–8 psi). The reservoir is mounted on the dispensing system that is driven by a 3D motion control. The system is driven by a FlashCut CNC 3D motion controller. A PC is connected to the system to control the motion in 3D. Toolpath for the motion is realized through importing CAD models in stereolithography (STL) format followed by G-code generation using visual basic-based in-house developed script. The calcium chloride solution is then dispensed onto the printed alginate structure through another nozzle to provide crosslinking between alginate anions and the calcium cations to form the hydrogel. A pink color pigment is used only for proper visualization purposes.

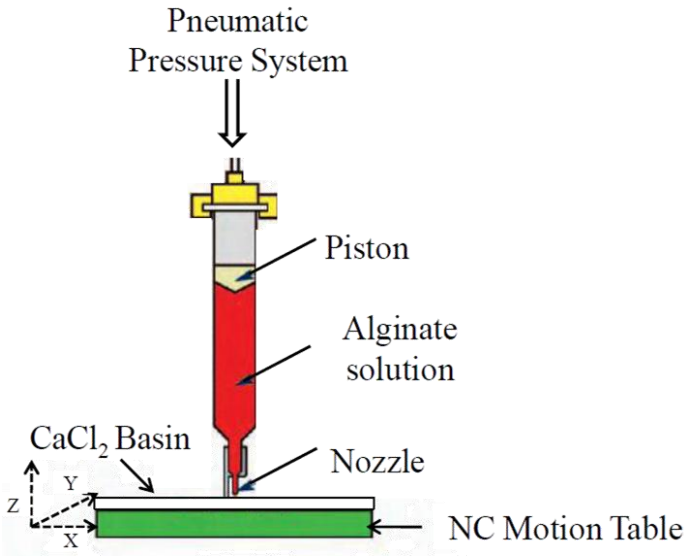

Figure 1: Single Nozzle Bioadditive Manufacturing Platform

2.2.2 Multi-Chamber Single Nozzle Bioadditive Manufacturing Platform

Biofabrication of multi-material hybrid tissue scaffolds is performed by using a multi-chamber single nozzle bioadditive manufacturing system. Similar to single nozzle bioadditive manufacturing platform, fabrication process is biologically compatible and the system runs in room temperature under low pressure (0-8 psi) to reduce fluidic shear forces that can damage incorporated modifiers or diminish their active properties. The multi-chamber single nozzle assembly is mounted on the 3D motion control unit. The multi-chamber single nozzle assembly (see Fig. 2) consists of multiple biomaterial chambers and a single converging nozzle unit, where two different biomaterials can be mixed statically and extruded with unique properties. The volume of the static mixture nozzle is optimized while nozzle volume determines the quality and the evenness of physical properties of the dispensed biomaterial. Biomaterial

chambers are connected to a pressure control unit, where the air pressure assists in extruding the biomaterial through the dispensing unit. Material flow and concentration through the nozzle is controlled by regulating positive chamber pressures connected to the air pressure control unit. Mixing two different concentrations from each chamber enables dispensing varying concentration of alginate and loaded microspheres by time. Final solutions were deposited through 250 µm nozzle tip. The system allows extrusion of continuous customized filaments with variational biomaterial and biological modifier properties enabling controlled release kinetics. A similar method is used to crosslink alginate solution with calcium chloride solution as described in section 2.2.1. Dark field images of fabricated samples are taken by 5.0x1.0x1.0 optic lenses.

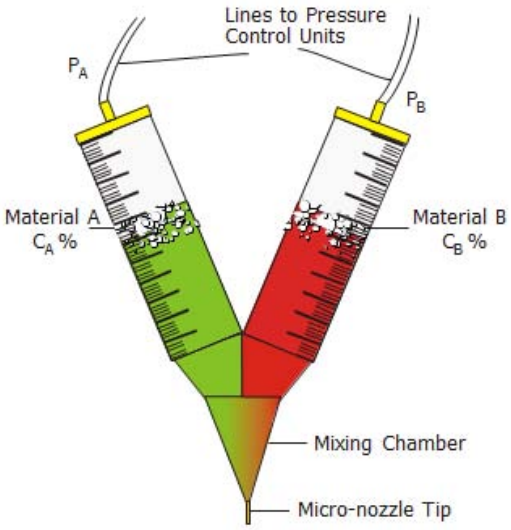

Figure 2: Schematic of the multi-chamber single nozzle bioadditive manufacturing platform

2.3 Modeling of Geometric Variation in Tissue Scaffolds

Natural tissues and organs are not homogenous rather they have layers of tissue with one or more specific function that generate natural functional gradients over their structure [6]. Thus, tissue scaffolds with controlled variation in porosity over their architectures could have significant impact on functional, mechanical and biological behavior of scaffolds [7]. Thus, porosity level in tissue scaffolds should vary spatially to mimic the formation of natural tissues based on location dependent mechanical, biological and functional requirements over their structures. Moreover, permeability needs to be controlled spatially to provide enhanced nutrient, water and oxygen transport throughout the tissue scaffold. This can be mimicked by partitioning the entire scaffold into different functionally uniform regions each having different porosity levels. This enables improved flow of the media through the internal regions and mediates the release of loaded modifier in internal regions. To mimic the functional gradient in porosity of the replaced tissue, the scaffold area can be discretized into a number of uniform regions. To achieve these uniform porosity regions, a non-uniform-offsetting operation [8] is used to partition the structure. Similar discretization approach is used to partition tissue scaffolds with variational biomaterial and biomolecule composition to control release kinetics spatiotemporally.

3. RESULTS

For implementation purpose, a lumbar vertebra solid model is obtained by extracting the 3D geometry through reserve engineering. Roland Picza LPX-60, USA laser scanner is used to generate scan data and converted into CAD model through Pixform software. Then, the model is inputted into NURBS modeling software Rhinoceros 4.0. The desired number of regions is generated and sliced. Next, toolpath optimization method presented in our earlier work [9] is used to develop toolpath plan for the lumbar vertebrae slices. The generated toolpath plan is then verified using Flashcut software and exported into solid freeform fabrication system to build the designed scaffold. Then, the single nozzle bioadditive manufacturing platform is used to fabricate the model with nozzle tip diameter of 150 µm with controlled variational porosity along the scaffold architecture. Figure 3 illustrates the fabricated lumbar vertebrae scaffold. Two consecutive vertebra slices are printed with five different porosity regions of 84.6%, 83.6%, 79.3%, 78.1% and 76.2%, where the prosoity decreases through the innnermost region to meet tissue engineering requirements (see Fig. 3(a)). A sample scaffold with 18 layers is also demonstrated to show the effectivesses of the system in 3D.

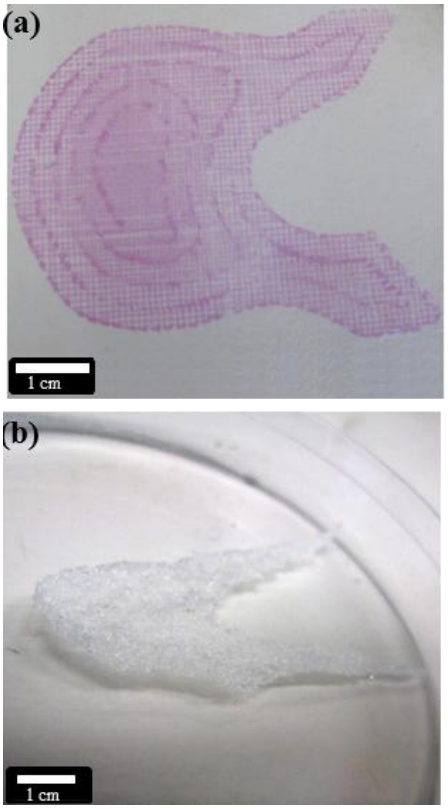

Figure 3: (a) Two consecutive fabricated vertebra slices with five different porosity region of 84.6%, 83.6%, 79.3%, 78.1% and 76.2% (b) and 3D fabricated vertebra with 18 slices.

Fabricated vertebra sample is analzed through imaging study using Image J software. As presented in Table 1, the porosity level of fabricated sample is slightly different than that of designed scaffold. The permeability of the fabricated scaffold is also characterized by the imaging study to understand the effectiveness of the proposed optimization model. By appliying the toolpath optimization strategy developed in our earlier work [9], permeability of the fabricated vertebrae is improved by 21.1% compare to that without toolpath optimization.

Table 1 Characterization of the fabricated vertebra scaffold with variational porosity using imaging.

		Vertebra Region 1	2	3	4	5
Fabricated Model	Average Filament Diameter (μm)	144				
	Average Unit cell length, L (mm)	0.75	0.73	0.58	0.55	0.52
	Calculated Porosity,%	83.4	82.9	78.5	77.5	76
Designed Model Porosity, %		84.6	83.6	79.3	78.1	76.2
% Error		1	0.8	1	0.8	0.3
Avg Error, %		0.78				

The multi-chamber single nozzle bioadditive manufacturing platform is used to manufacture hybrid tissue scaffolds with variational biomaterial and biomolecule concentrations. Figure 4 shows concentration level of sodium alginate and biomolecules in different regions on a tissue scaffold illustrated in Fig. 5(a). Requirements presented in Fig. 4 are assumed to be tissue engineering needs. In general, alginate concentration over 5% is not suitable for cell viability and concentration under 1% does not provide good mechanical integrity [10]. Thus, alginate concentration is preferred to be in the range of 3% to 5%. Moreover, modifier concentration under 1% is in biologically relevant range [11]. Then, the concentration information is inputted into the fabrication system. A double layer of CAD model is printed through the deposition system with blending of two different alginate and modifier concentrations. Yellow and red inks in RGB are used for each alginate solutions to represent regional differentiation for visualization purposes. Only top layer is printed due to diffusive characteristics of color inks.

Based on requirements presented in Fig. 4, a sample structure is printed in which the alginate concentration increases from the outermost region (Region 1) through the innermost region (Region 4). Fig. 5(a) shows a sample fabricated wound dressing with 4 regions, in which regions are occupied by different alginate and modifier concentration based on the profile presented in Fig. 4. Fig. 5(b) demonstrates dark field image of biomolecules of a randomly picked region on the tissue scaffold. As can be seen in Fig. 5(b), biomolecules are randomly distributed over the filaments.

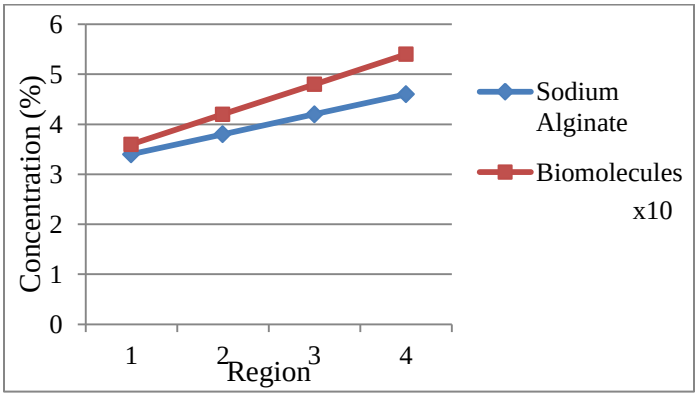

Figure 4: Region-based concentrations of sodium alginate and biomolecules in a hybrid tissue scaffold.

Controlling material composition in different regions provides control release of loaded biomolecules temporally. Changing the incorporated bio-molecules distribution and material concentrations provides a way to control release kinetics spatially. Higher bio-molecule concentrations in specific sites result in higher amount of cumulative release. Material concentration however mediates release kinetic rate rather than changing cumulative release such as higher alginate concentration slows down release kinetics and vice versa [12].

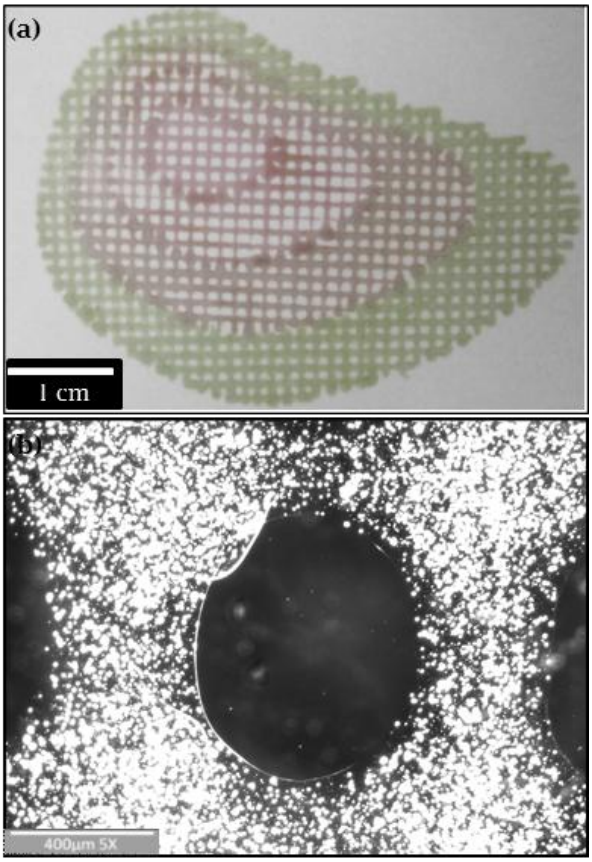

Figure 5: (a) Fabricated porous scaffold with highlighted differentiation in microsphere concentration between 0.3-0.6% (w/v) (b) and dark field image highlighting loaded microsphere.

4. CONCLUSION

In this paper, release kinetics through engineered tissue scaffolds is controlled spatiotemporally to enhance tissue regeneration process. Spatial and temporal control enables synchronization of tissue regeneration with the release kinetics to advance cell proliferation and tissue healing. Spatiotemporal control of release kinetics in this paper is achieved by fabricating hybrid tissue scaffolds with (i) controlled structural geometry and internal architecture with variational porosity and (ii) controlled distribution of base material and loaded biological modifiers spatially.

Transition from incorporation of a single modifier to multiple modifiers for scaffolding applications might be an engineering challenge and promising approach for improved and efficient tissue regeneration. As a future direction, tissue scaffolds needs to be designed and fabricated in a way that multiple proteins or growth factors should be impregnated and released in a specific spatial and temporal orientation to mimic the natural tissue regeneration process.

5. ACKNOWLEDGEMENT

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