3D Printing with Peptide-Polymer Conjugates to Create Spatially Organized Scaffolds for Tissue Engineering

Hafiz Busari[1], Peter Schwarzenberg[2], Katherine D. Hudson[1,2], Hannah L. Dailey[2], Lesley W. Chow[1,3]
[1]Department of Materials Science and Engineering, Lehigh University, Bethlehem, PA, USA
[2]Department of Mechanical Engineering and Mechanics, Lehigh University, Bethlehem, PA, USA
[3]Department of Bioengineering, Lehigh University, Bethlehem, PA, USA

Statement of Purpose: Functional regeneration of biological tissues remains a challenge because native tissues contain gradients in structure, mechanical properties, and biochemical composition critical for tissue function. Biomaterials for tissue engineering must be designed to provide spatially organized mechanical, structural, and biochemical cues to guide tissue formation.[1] Here, we introduce a novel platform that combines 3D printing technology with peptide-functionalized polymer-based materials to produce scaffolds with spatially organized peptides. This strategy allows us to independently and simultaneously control the spatial concentration of multiple peptides and scaffold architecture within a single construct.

Methods: Poly(caprolactone) (PCL) was modified with peptides to produce peptide-PCL conjugates using methods previously described.[2] Briefly, peptides were synthesized using standard Fmoc solid phase peptide synthesis techniques before covalently coupling to PCL.[2] The peptide-PCL conjugates included the following sequences: biotin-GGGYKKKGGGC (biotin-PCL), CGGGAAAEEE (E3-PCL), and CGGGRYPISRPRKR (HAbind-PCL). Peptide-functionalized scaffolds were 3D printed using a customized solvent-cast 3D printer with inks containing peptide-PCL conjugates dissolved at desired concentrations with unmodified PCL in a volatile solvent hexafluoro-2-propanol (HFIP). Multiple printer heads containing inks with different peptide-PCL at varying concentrations were used to independently control peptide organization and scaffold structure. The presence and location of the biotin-PCL, E3-PCL, and HAbind-PCL peptides were detected by labeling with streptavidin-gold nanoparticles or fluorescein isothiocyanate (FITC), FITC-hyaluronic acid (HA), or amino-Cyanine-5, respectively. The 3D-printed scaffolds were characterized by scanning electron microscopy (SEM) and confocal fluorescence microscopy to observe scaffold structure and peptide spatial concentration.

Results: Solvent-based 3D printing with peptide-PCL conjugates allowed for single-step peptide functionalization of scaffolds without the need for post-processing. SEM of scaffolds 3D printed with inks containing PCL only or PCL with 2 mg/ml, 10 mg/ml, or 30 mg/ml biotin-PCL and labeled with streptavidin-gold nanoparticles confirmed the presence of peptides on the scaffold surface and demonstrated a direct correlation between the number of gold nanoparticles and peptide-PCL concentration. Biotin-PCL scaffolds labeled with streptavidin-FITC showed how these local changes in peptide density translated to a global increase in fluorescence in the entire scaffold. Scaffolds were also printed with two different inks to demonstrate how the surface functionalization can be controlled within a single construct. Figure 1A illustrates alternating biotin-PCL and PCL only inks produced scaffolds with user-defined peptide organization. Similarly, printing with E3-PCL and HAbind-PCL verified that two distinct peptides can be organized in the same scaffold (Figure 1B). In parallel, scaffolds were 3D printed with variable spacings to create scaffolds with different porosities (Figure 1C). Combined with peptide-PCL inks, this showed how scaffold functionalization and porosity can be modified independently and simultaneously.

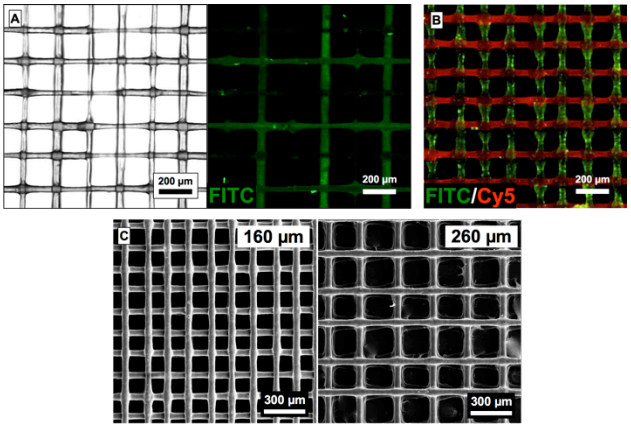

Figure 1. (A) Bright-field and corresponding fluorescence images of alternating inks containing PCL with 0 mg/mL or 2mg/mL biotin-PCL labeled with streptavidin-FITC illustrate spatial organization of peptides in user-defined locations within a continuous 3D-printed scaffold. (B) Fluorescence image of a scaffold 3D printed with HAbind-PCL and E3-PCL and labeled with FITC-HA and amino-Cy5, respectively, show a dual peptide-functionalized scaffold. (C) SEM of scaffolds printed with 160 µm or 260 µm spacing.

Conclusions: This work introduces a versatile platform to control peptide concentration and organization and scaffold structure within a continuous, 3D-printed scaffold to guide tissue formation. The organization of biochemical and physical cues can be optimized to fine-tune local and global cell behavior. *In vitro* studies are currently underway to investigate how human mesenchymal stem cells (hMSCs) respond to variations in porosity and single or dual-peptide functionalization.

References: [1] Chow & Fischer, *Exp Biol Med* 241(10): 1025-1032, 2016.; [2] Chow et al., *Adv Healthc Mater* 3(9): 1381-1386, 2014.