Simultaneous and Independent Control of Biochemical and Physical Properties in 3D-Printed Biomaterials

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Introduction

- Fabricating hierarchically organized biomaterials to mimic native tissue environment is challenging.
- Solvent-cast direct-write (SC-DW) 3D printing uses computer-controlled deposition of polymer-based ink solutions to create complex 3D structures.
- During extrusion, inks undergo a liquid-to-solid phase change as solvent evaporates, allowing the printed fiber to maintain shape.[1,2]
- We developed a platform using poly(caprolactone) (PCL) and peptide-modified PCL (peptide-PCL) conjugates[4] to fabricate peptide-functionalized scaffolds in a single step (**Fig. 1**).
- Conjugates with hyaluronic acid binding (HAbind-PCL) and mineralizing (E3-PCL) peptides, which have been shown to promote chondrogenesis[5] or osteogenesis[6], respectively, were used to demonstrate multi-peptide organization within a single scaffold (**Fig. 2**).
- Print pattern was modified to create a variety of scaffold architectures
- This platform enables us to simultaneously and independently control both the biochemical and physical organization within a continuous construct.

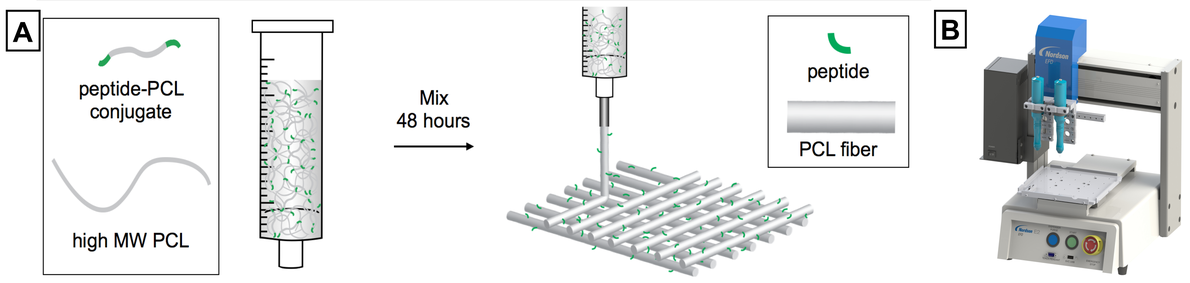


Figure 1. Schematic of (A) ink prepared with unmodified (80kDa) PCL and peptide-PCL conjugate co-dissolved in HFIP then printed into a peptide-functionalized scaffold. (B) Multiple printer heads used to print different ink compositions for dual-peptide scaffolds.

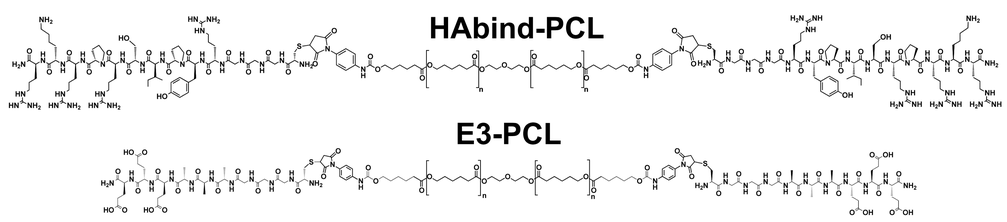


Figure 2. Chemical structures of the HAbind-PCL and E3-PCL conjugates.

Materials and Methods

Inks: All inks were prepared with 37% w/v unmodified PCL (MW 80 kDa) in hexafluoroisopropanol (HFIP); peptide-PCL conjugates were co-dissolved with PCL at varying concentrations prior to 3D printing

3D Printing: Inks were 3D printed using a 32-gauge needle (100 µm ID) at a print speed of 0.2 to 0.4 mm/sec and deposited layer-by-layer into 14-layer scaffolds

Characterization: To confirm peptide distribution, scaffolds were labeled using fluorescein-HA and/or amino-Cy3 to specifically label HAbind and E3 peptides, respectively, and imaged using a confocal fluorescence microscope (Nikon C2). Architecture and morphology was visualized using a scanning electron microscope (SEM; FEI XL30 ESEM) after sputtering coating samples with conducive iridium.

Controlling Physical Properties

Scaffold architecture was varied using different fiber spacing and print patterns in individual scaffolds to modify fiber spacing and pore shape (**Fig. 3**).

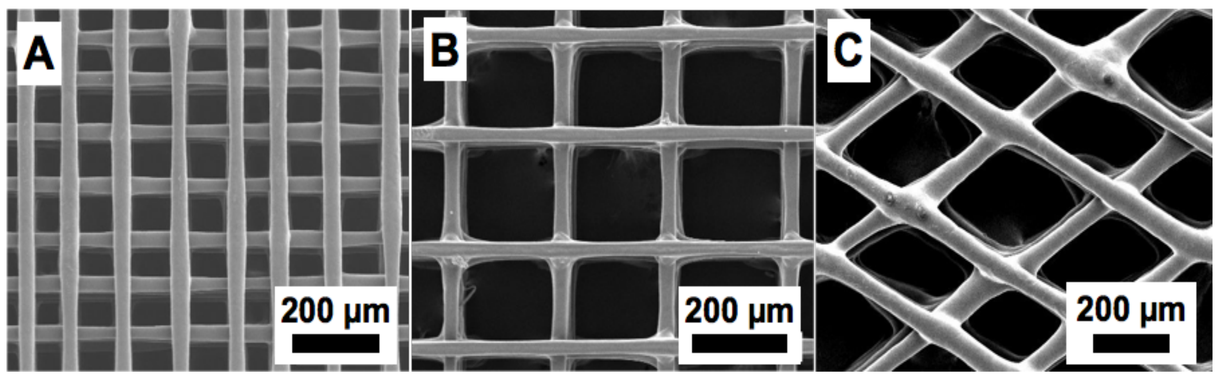
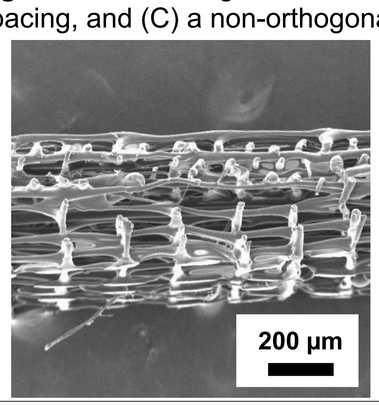


Figure 3. SEM images of PCL scaffolds 3D-printed with (A) 120 µm fiber spacing, (B) 260 µm fiber spacing, and (C) a non-orthogonal print pattern with 260 µm fiber spacing at 60º intersections



Print patterns can also be modified during a single fabrication step to generate variable architecture within a single construct (**Fig. 4**). This enables us to mimic gradients in tissue structure.

Figure 4. SEM image of the cross-section of a 14-layer dual fiber spacing PCL scaffold. The first seven layers were printed with 120 µm fiber spacing while the remaining layers were printed with 260 µm fiber spacing.

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References: [1] MA Woodruff, WH Dietmar, Prog Polym Sci 35:1217-1256, 2010; [2] LW Chow +, Adv Healthc Mater 3:1381-1386, 2014. [3] SZ Guo +, Langmuir 30:1142-1150, 2014; [4] SZ Guo +, Small 9:4118-4122, 2013. [5] PA Parmar +, Adv Healthc Mater 5:1656-1666, 2016; [6] OJ Karaman +, Tissue Eng Regen Med 10:E132-E146, 2016

Controlling Biochemical Properties

- Scaffolds successfully 3D printed with different peptide-PCL concentrations
- Surface peptide concentration controlled by changing peptide-PCL concentration in ink before 3D printing without requiring post-fabrication steps (**Fig. 5**).
- Both peptides were printed in discrete regions within the same construct to mimic the spatial composition of the native osteochondral interface (**Fig. 6**).

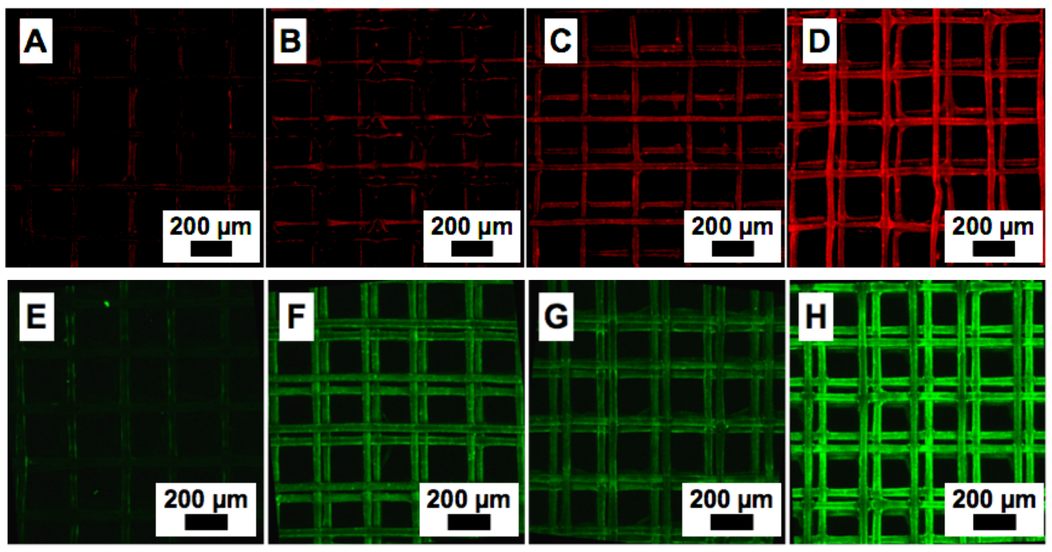

Figure 5. Confocal fluorescence microscopy images of scaffolds with concentrations of E3-PCL at A) 0 mg/ml, (B) 6 mg/ml, (C) 12 mg/ml, and (D)18 mg/ml labeled with amino-Cy3 (**red**) or HAbind-PCL at (E) 0 mg/ml, (F) 1 mg/ml, (G) 3 mg/ml, and (H) 6 mg/ml labeled with fluorescein-HA (**green**).

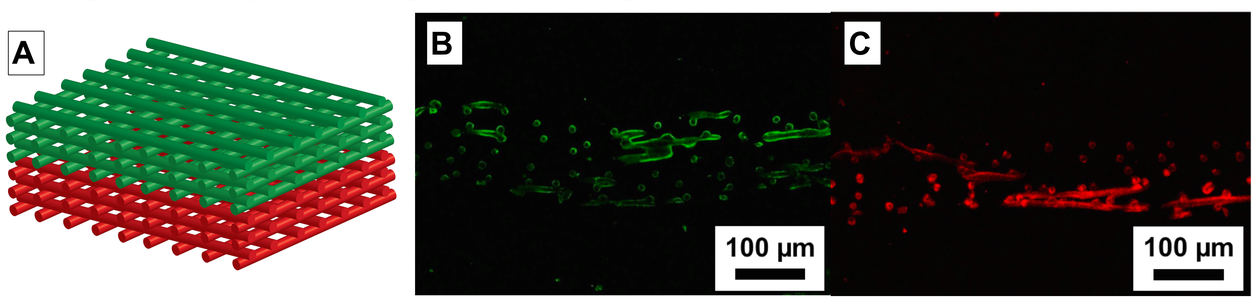

Figure 6. (A) Schematic of dual peptide-functionalized scaffold with corresponding confocal fluorescence microscopy images of cross-sections of a single scaffold showing (B) HAbind labeled with fluorescein-HA (green) and (C) E3 labeled with amino-Cy3 (**red**) in discrete spatial regions.

Conclusion and Future Work

- This works demonstrates a tunable platform to control scaffold architecture and biochemical organization independently and simultaneously.
- Multiple peptides and varying structures were spatially organized within a single 3D-printed construct.
- This platform introduces a versatile strategy to fabricate scaffolds for a wide range of tissue-specific applications.
- Ongoing and future work focuses on characterizing and tuning the mechanical properties of the constructs