3D Printing Peptide-Functionalized Scaffolds for Osteochondral Regeneration

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Peptide-Functionalized 3D-Printed Scaffolds

- Biodegradable polymers like poly(caprolactone) (PCL) can be functionalized with bioactive cues to drive desired cell response[1,2]
- Our lab has developed strategies to 3D print with multiple peptide-PCL conjugates to fabricate peptide-functionalized scaffolds[2] **(Fig. 1)**
- However, long-term culture may influence the surface chemistry over time[3]
- **Goal**: Investigate how peptide presentation on the surface of solvent-cast 3D-printed peptide-functionalized scaffolds changes during *in vitro* culture.
- Here, we synthesized peptide-PCL conjugates with sequences CGGGRYPISRPRPKR (HAbind-PCL) or CGGGAAAEEE (E3-PCL)
- Inks containing 37% (w/v) PCL in hexafluoroisopropanol (HFIP) with or without each conjugate were 3D-printed into 14-layer scaffolds.

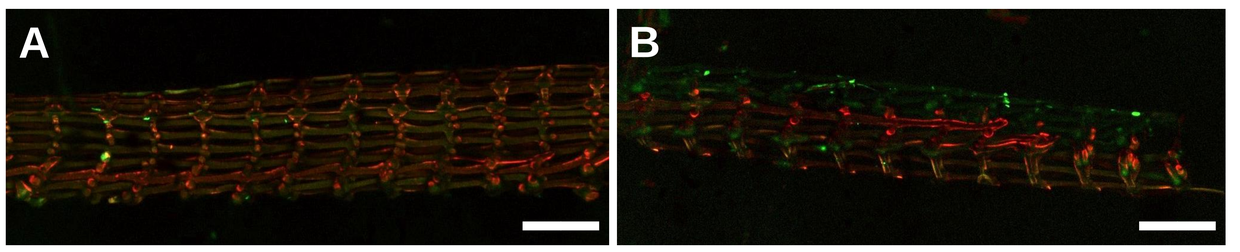

Figure 1. Fluorescence microscopy images of scaffold cross-sections with E3 and HAbind peptides presented (A) homogeneously or (B) spatially in discrete regions, demonstrating our ability to organize multiple peptides within a single construct. E3 and HAbind peptides were labeled with amino-Cy3 and fluorescein-HA, respectively (scale bar = 400 µm).

Morphology after 3 Months *In Vitro*

- All scaffold groups were sterilized in 70% ethanol for 30 minutes and incubated in 0.1% bovine serum albumin (BSA) overnight
- Scaffolds were incubated in cell culture media (DMEM, 10% fetal bovine serum (FBS), 5% antibiotic/antimycotic) at 37˚C and 5% CO_2 for up to three months before imaging with scanning electron microscopy (SEM)

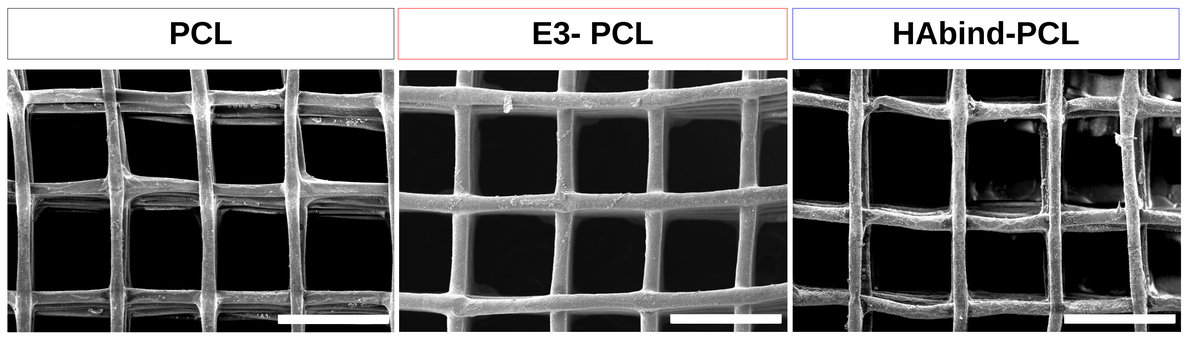

Figure 2. Representative SEM images of PCL, E3-PCL, and HAbind-PCL scaffolds showed minimal differences in scaffold morphology between groups. (scale bar = 300 µm).

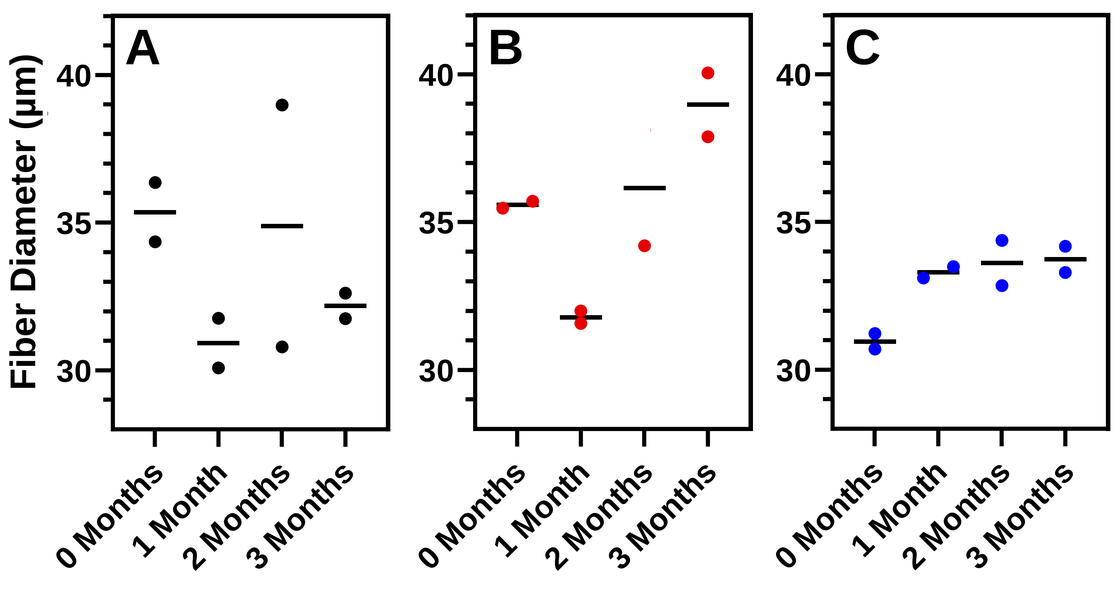

Figure 3. Average fiber diameters of (A) PCL, (B) E3-PCL, and (C) HAbind-PCL scaffolds for two separate scaffolds per group measured from SEM images at five randomly selected locations using ImageJ software. No significant changes were observed over the culture period for each scaffold or across groups.

Fluorescence Intensity after 3 Months *In Vitro*

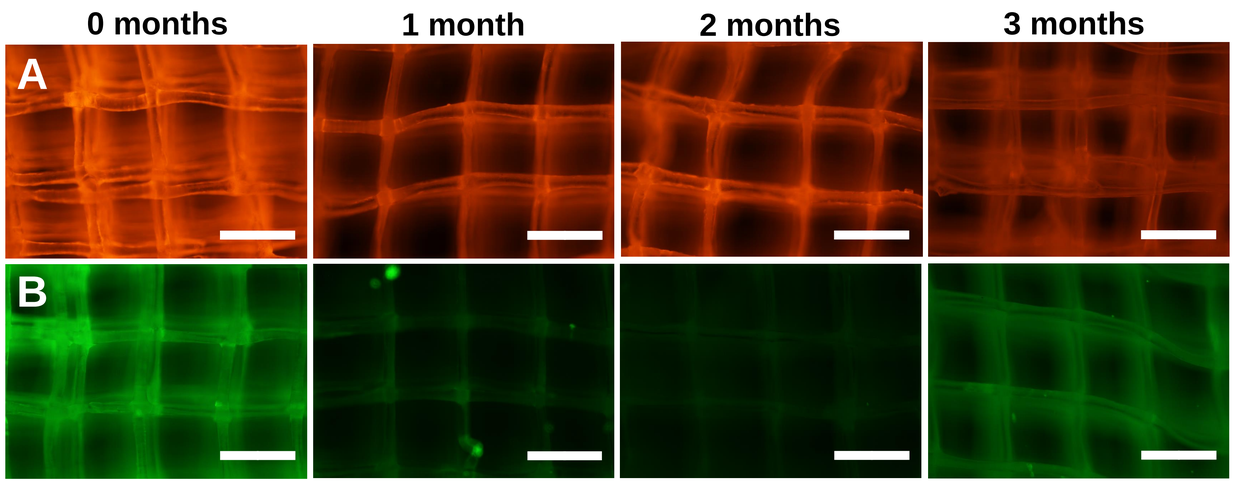

Figure 4. Representative fluorescence microscopy images of (A) E3-PCL scaffolds labeled with amino-Cy3 and (B) HAbind-PCL scaffolds labeled with fluorescein-HA at 0, 1, 2, and 3 month time points (scale bar = 200 µm).

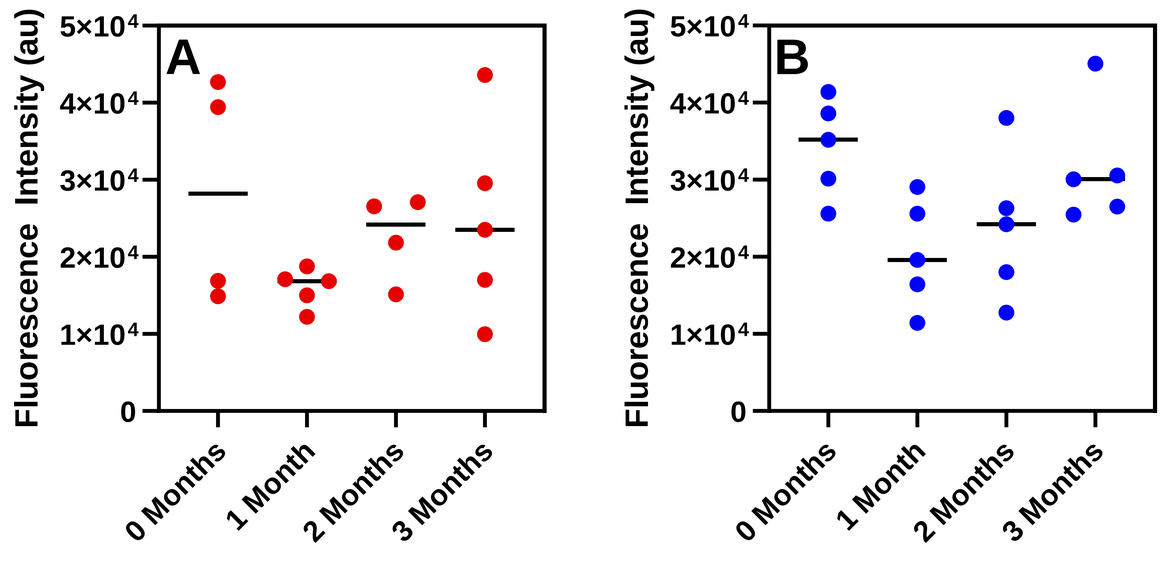

Figure 5. Average fluorescence intensity of (A) E3-PCL scaffolds labeled with amino-Cy3 and (B) HAbind-PCL scaffolds labeled with fluorescein-HA after 0, 1, 2, or 3 months in culture. Fluorescence was measured for whole scaffolds using a plate reader and normalized to the respective PCL control for each time point. No significant differences between groups were observed using a Kruskal-Wallis test for non-parametric data (**Mean ± SE,** N = 4-5).

Conclusions and Ongoing Work

- Fiber morphology did not change after 3 months in culture media for PCL, HAbind-PCL, or E3-PCL scaffolds
- Variability in fluorescence data may result from non-specific labeling of biomolecules adsorbed from cell culture media

Ongoing Work:

- HAbind-PCL and E3-PCL will be modified with biotin and azide chemistries to enable highly specific labeling of peptides after exposure to media
- PCL, biotinylated HAbind-PCL, and azide-modified E3-PCL scaffolds will be characterized by scaffold weight, polymer molecular weight, and peptide surface presentation over time for up to one year

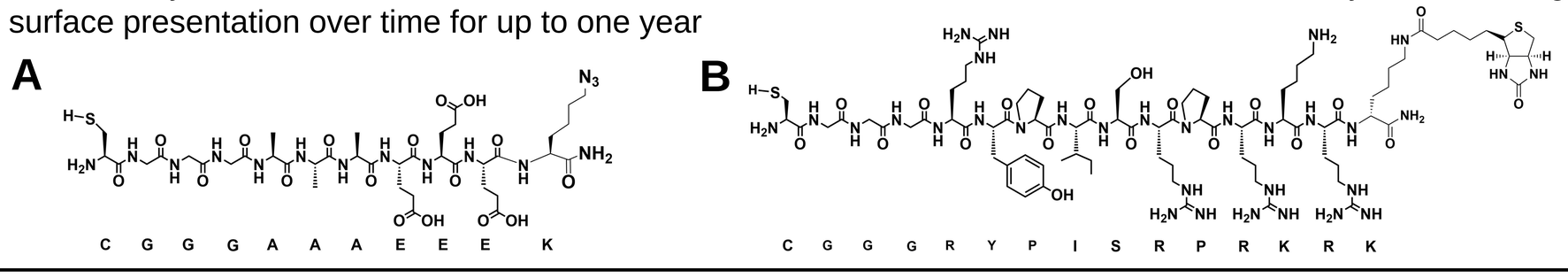

Figure 6. Chemical structures of the (A) azide-modified E3 peptide and the (B) biotinylated HAbind peptide.

References: [1] Chow, L. W., *Adv. Healthc. Mater.* 2014; 3; 1381-1386. [2] Camacho, P., *Biomater Sci.* 2019; 7(10); 4237-4247. [3] Li, B. *J. Biomed. Mater. Res*. 2006; A.

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