Spatiotemporal Presentation of Bioactive Peptides on 3D-Printed Scaffolds

Fenet G. Demissie[1], F. Gerardo Ortega Oviedo[2], Kelly B. Seims[3], Lesley W. Chow[2,3]

[1]Integrated Degree in Engineering Arts and Sciences Program, [2]Department of Bioengineering, [3]Department of Materials Science and Engineering, Lehigh University, Bethlehem, PA, USA

GF-mimetic Peptides to Treat Osteoarthritis

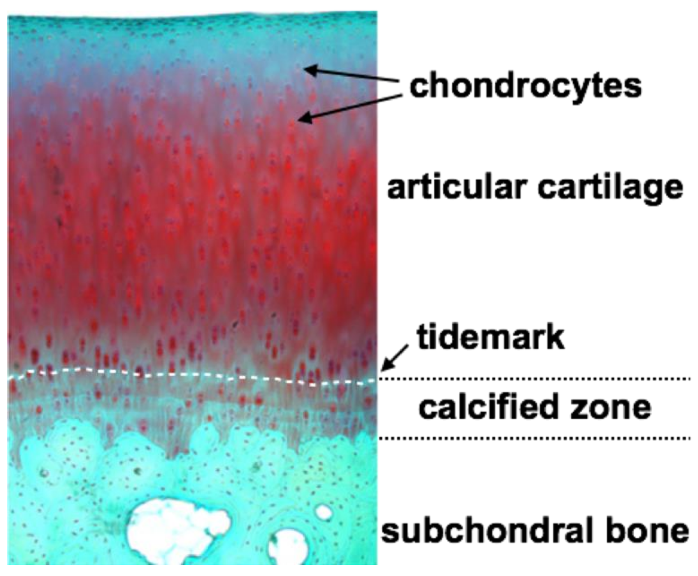

Figure 1: Osteochondral tissue features gradients in biochemical and physical properties from articular cartilage to subchondral bone (adapted from [1]).

- Osteoarthritis is a debilitating disease caused by progressive degeneration of the osteochondral (OC) tissue interface in articulating joints.
- Total joint replacement is the current gold-standard treatment and can be prevented with early-stage interventions. [1]
- Spatiotemporal growth factor (GF) patterns guide OC organization across the bone-cartilage interface (**Figure 1**).[1]
- Exogenous GFs are commonly used to enhance osteochondral tissue repair and regeneration but have short half-lives and are costly. [2]

TGF-β1 Mimetic Peptide Design

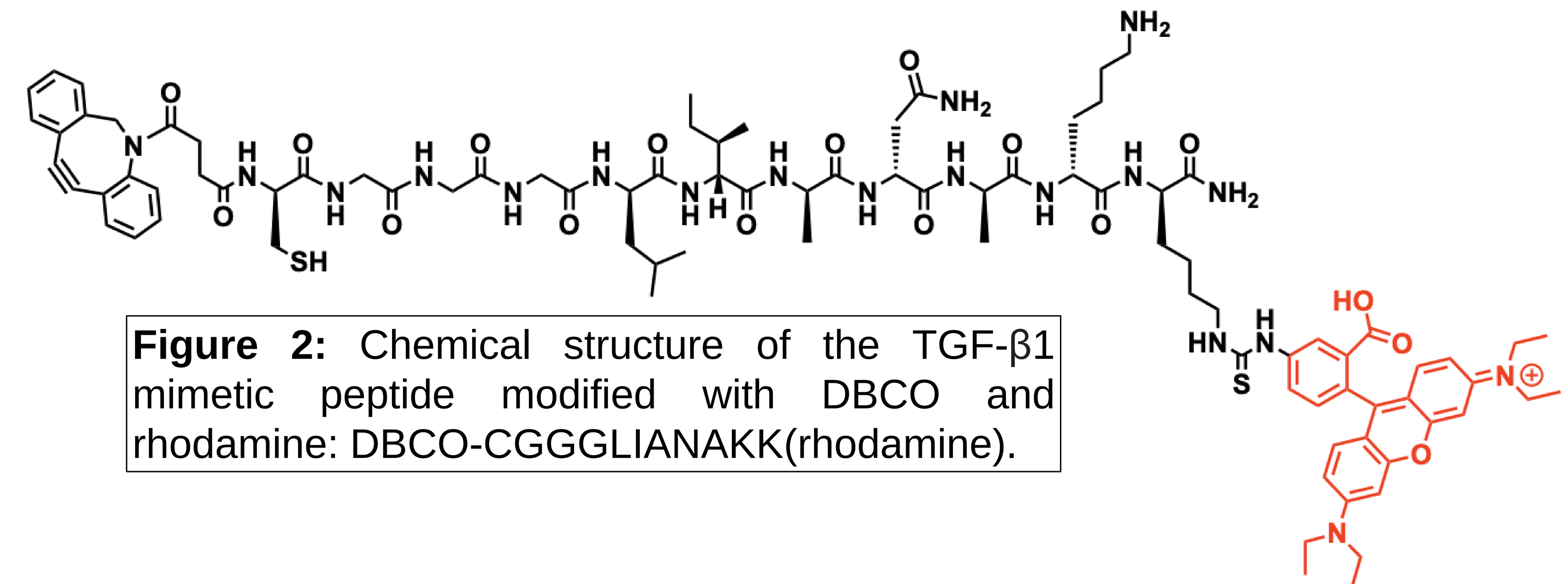

Figure 2: Chemical structure of the TGF-β1 mimetic peptide modified with DBCO and rhodamine: DBCO-CGGGLIANAKK(rhodamine).

- GF-mimetic peptides are stable alternatives to whole GFs.
- Transforming growth factor-β1 (TGF-β1) is a key GF in chondrogenesis. [3]
- LIANAK has been shown to mimic TGF-β1 activity.[4]
- We modified LIANAK with dibenzocyclooctyne (DBCO) to react with azide and rhodamine to visualize peptide location (**Figure 2**).
- We discovered that the peptide had a mass of 1600 g/mol indicating an additional alanine

Synthesis of PCL-azide

Poly(caprolactone) (PCL) was modified with azide to create PCL-azide conjugates for 3D printing (**Figure 3**)

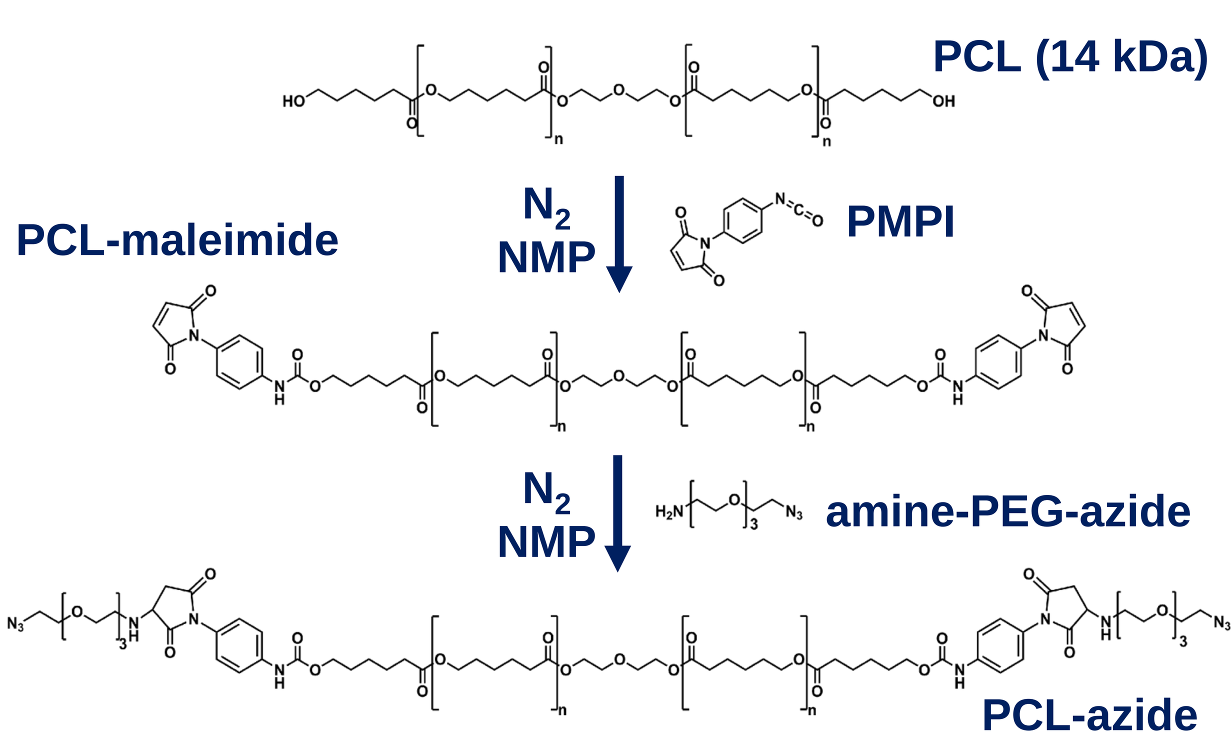

Figure 3: PCL-azide conjugate synthesis

3D Printing Azide-Functionalized PCL Scaffolds

20 mg/mL PCL-azide conjugate is dissolved with 370 mg/mL unmodified high MW PCL (80 kDa) in hexafluoroisopropanol (HFIP), and 3D printed to produce PCL-azide scaffolds. (**Figure 4**).

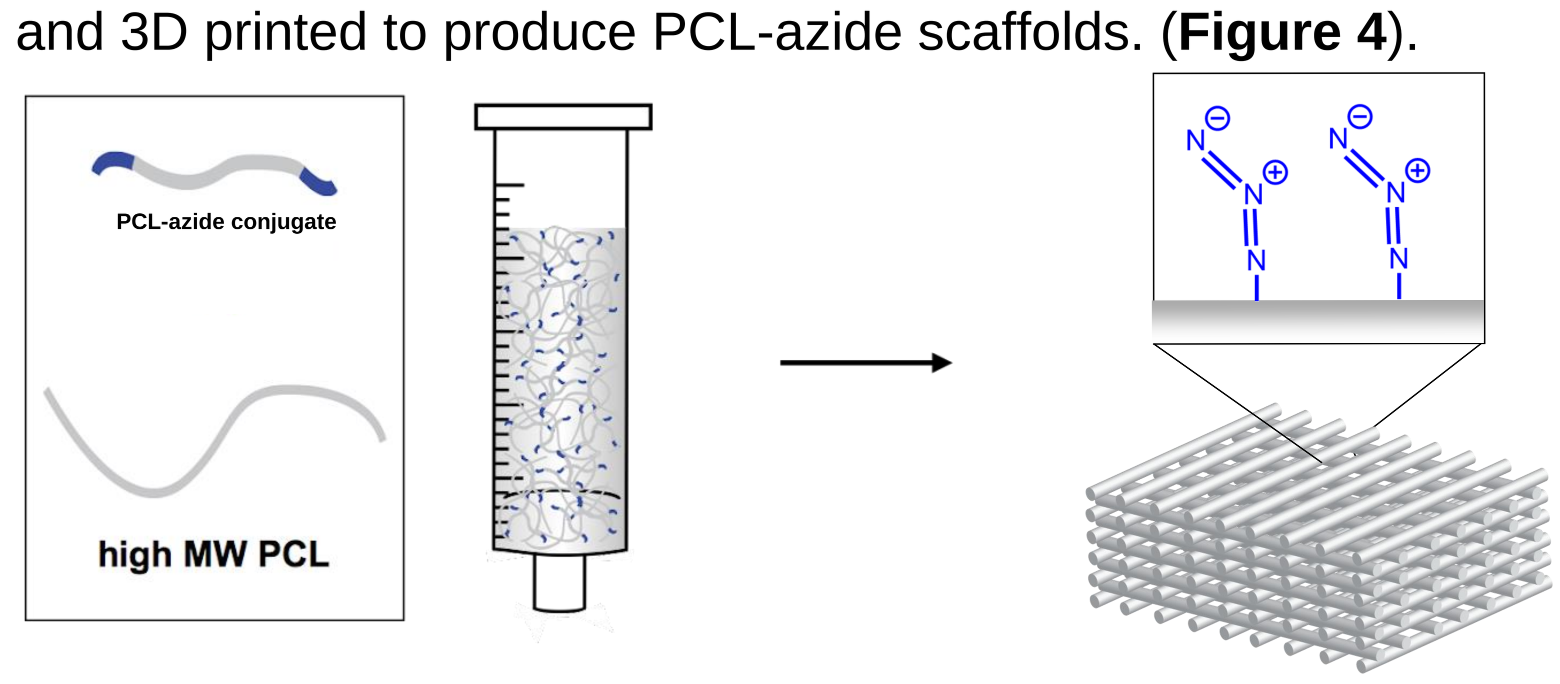

Figure 4: PCL-azide was dissolved with unmodified PCL to print PCL-azide scaffolds (adapted from [5]).

Click Chemistry to Modify Scaffold Surface

- Bio-orthogonal click chemistry refers to fast and highly selective reactions that do not interfere with cell activity.[4]
- We used DBCO to selectively react the peptide to azide on the surface of PCL scaffolds (**Figure 5**).

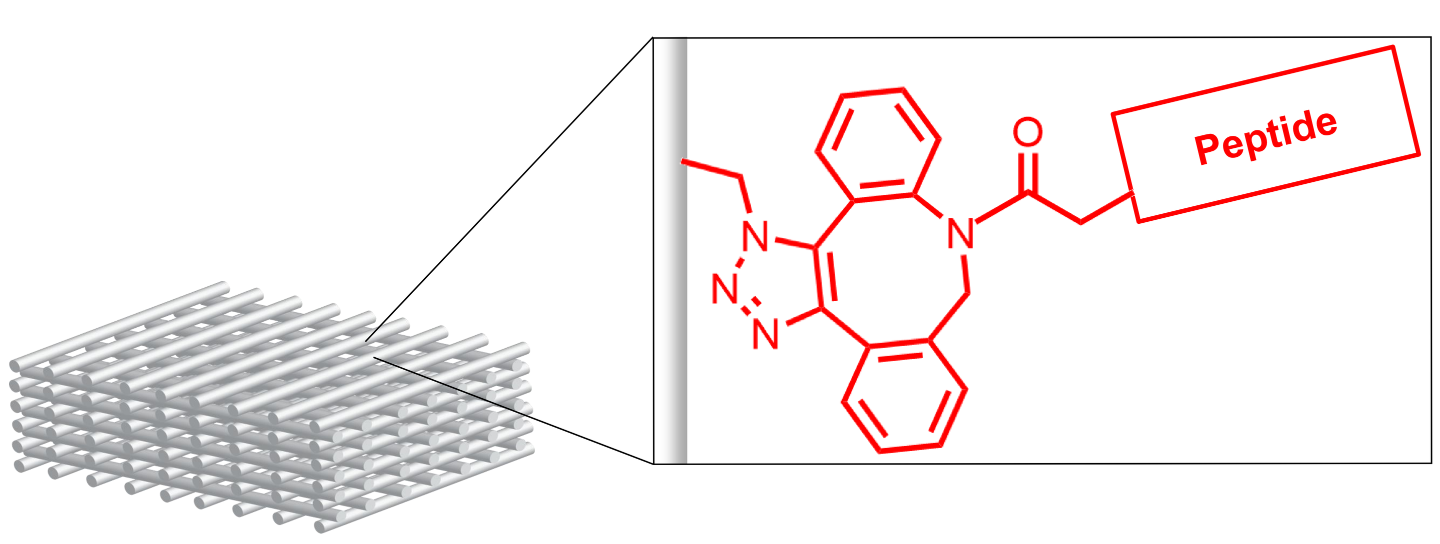

Figure 5: The DBCO group on the peptide reacts with the azide (N_3) on the azide-functionalized PCL scaffold.

Peptide-Functionalized Scaffolds

- PCL-only and PCL-azide scaffolds were labeled with DBCO- TGF-β1.
- The PCL-azide scaffold fluoresced (red) indicating peptide attachment (**Figure 6**).

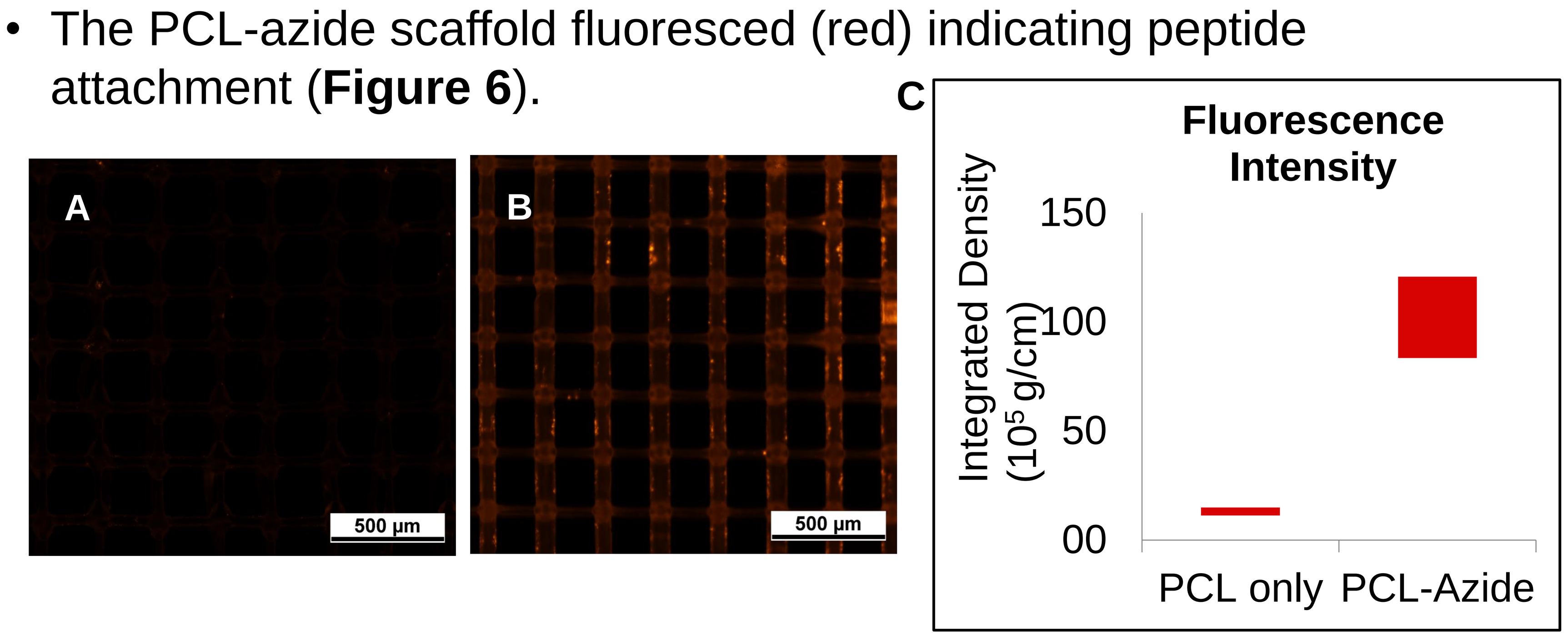

Figure 6: Representative fluorescence microscopy images of scaffolds 3D printed with **(A)** PCL only or **(B)** PCL with 20 mg/mL azide-PCL and labeled with DBCO-pep (red). **(C)** Quantification of mean fluorescence intensity using integrated density values (product of mean gray value by area).

Conclusions and Future Work

- TGF-β1 mimetic peptide modified with rhodamine (rhod) was synthesized and reacted with DBCO-acid to make DBCO-TGFpep(rhod)
- PCL-azide scaffolds were successfully 3D printed and fluorescently labeled with DBCO-TGFpep(rhod)

Future Work

- We will demonstrate peptide attachment in the presence of cells over time
- We are also synthesizing a BMP-2 mimetic peptide to promote bone regeneration
- Scaffolds presenting two distinct bioorthogonal chemistries will be fabricated to spatially and temporally deliver TGF-β1 and BMP-2 mimetic peptides during culture

DAVID AND LORRAINE FREED UGRS UNDERGRADUATE RESEARCH SYMPOSIUM

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