



Spatiotemporal Presentation of Bioactive Peptides on 3D-Printed Scaffolds



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GF-mimetic Peptides to Treat Osteoarthritis

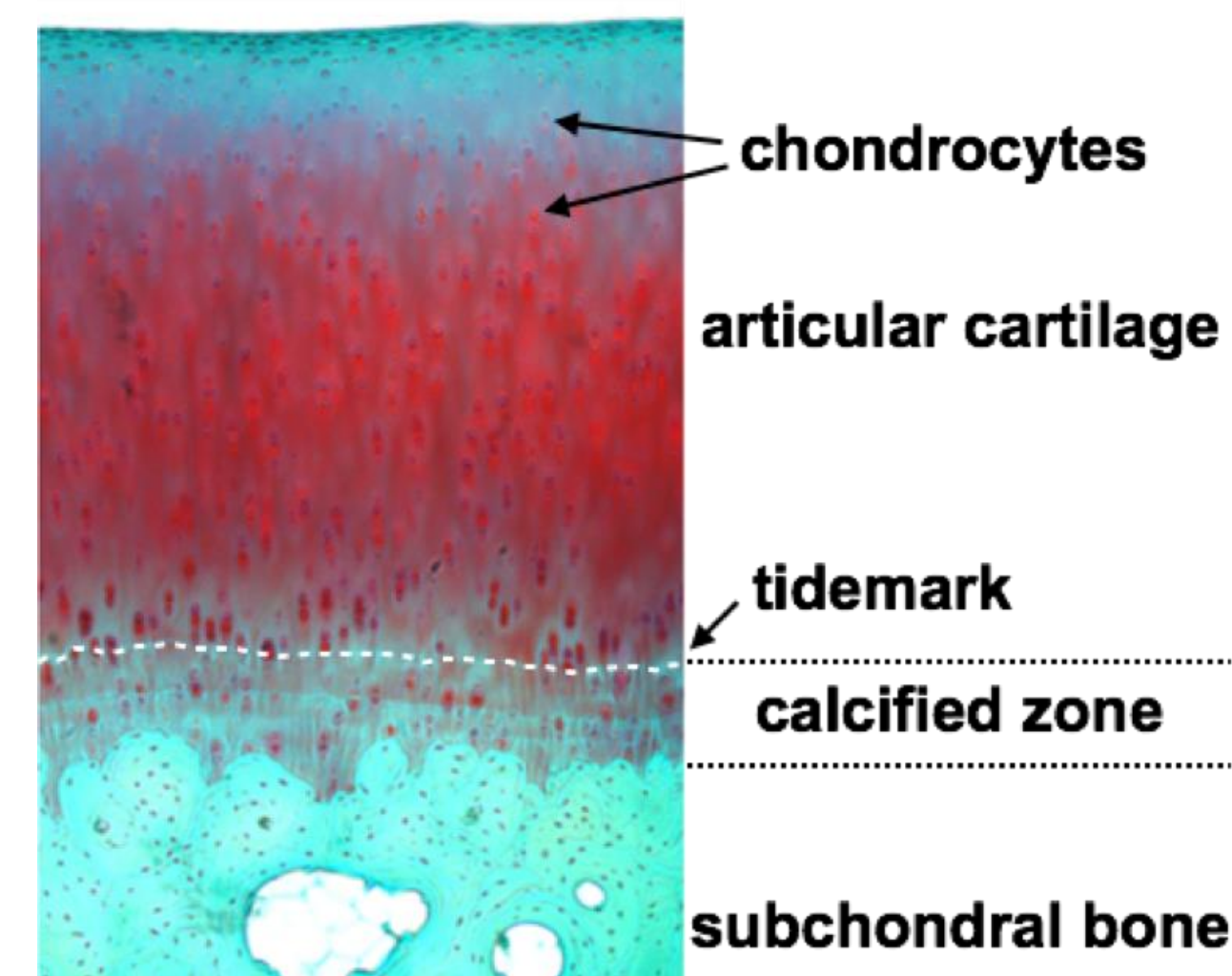


Figure 1: Osteochondral tissue features gradients in biochemical and physical properties from articular cartilage to subchondral bone (adapted from [1]).

- 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]

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).

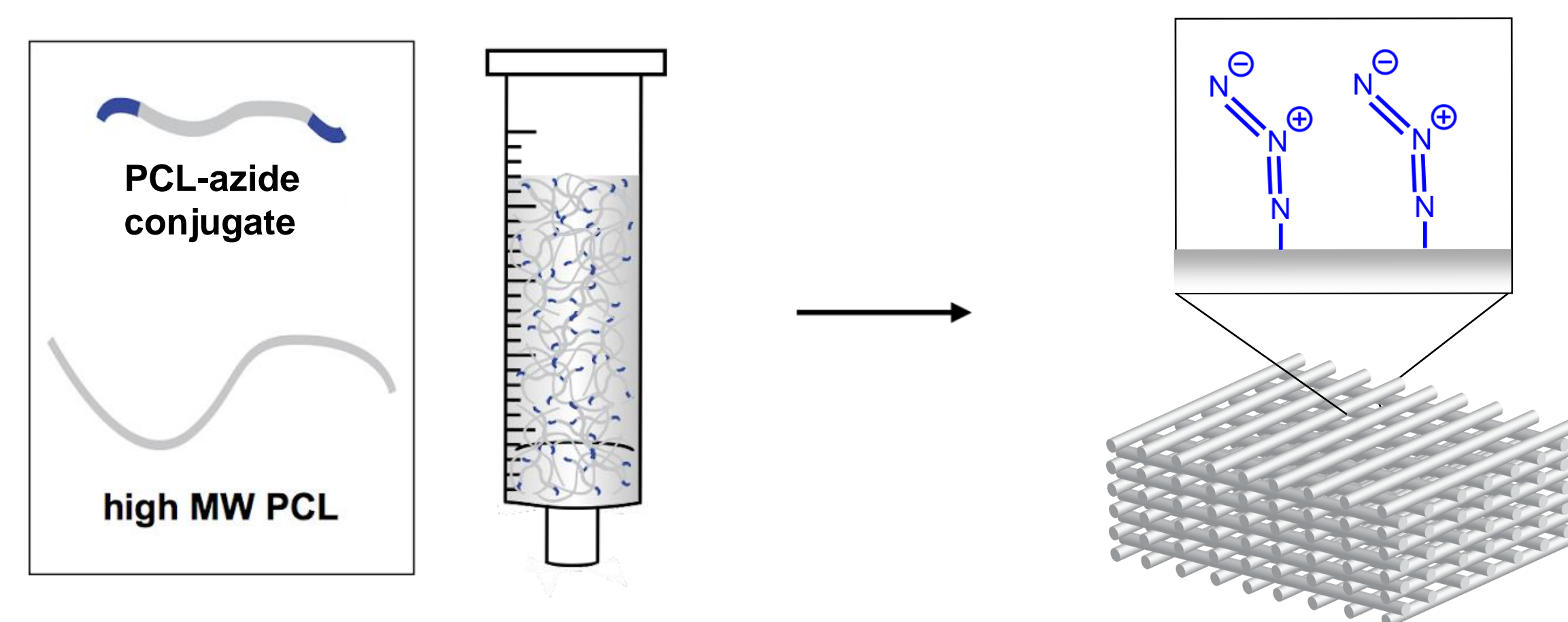


Figure 4: PCL-azide was dissolved with unmodified PCL to print PCL-azide scaffolds (adapted from [5]).

TGF-β1 Mimetic Peptide Design

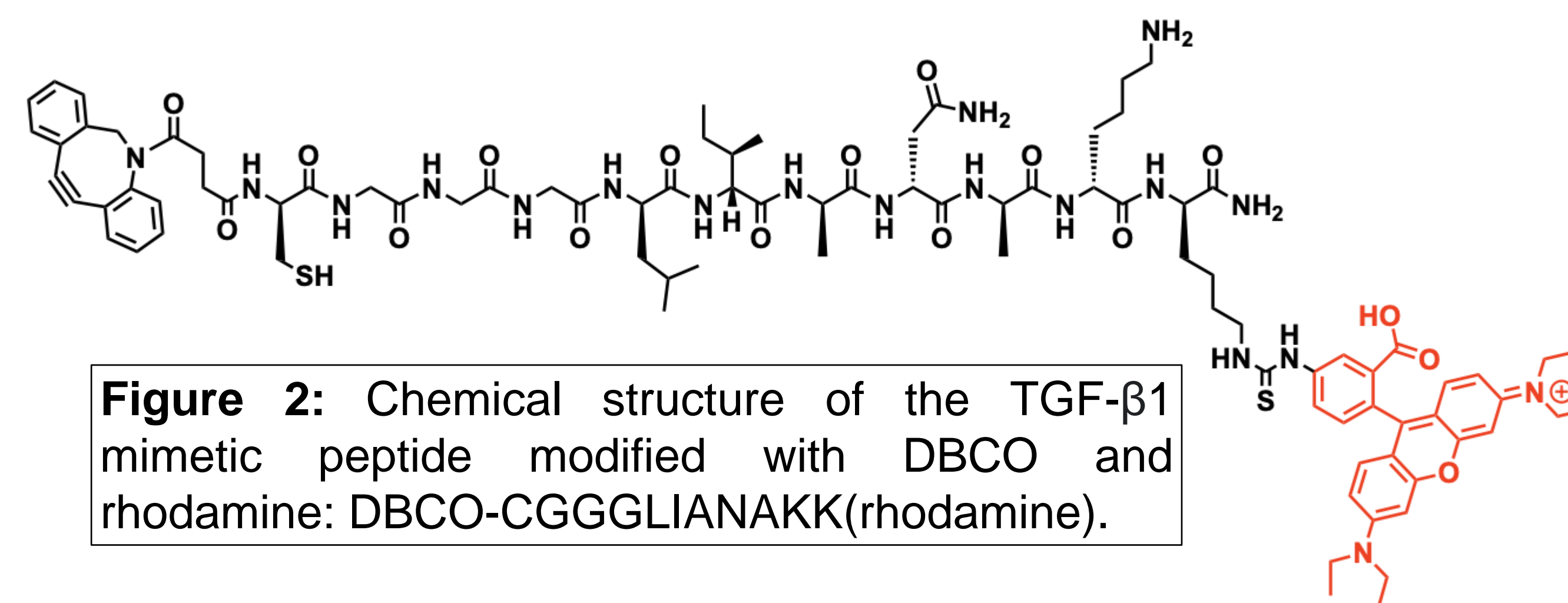


Figure 2: Chemical structure of the TGF-β1 mimetic peptide modified with DBCO and rhodamine: DBCO-CGGGLIANAKK(rhodamine).

- 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

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).

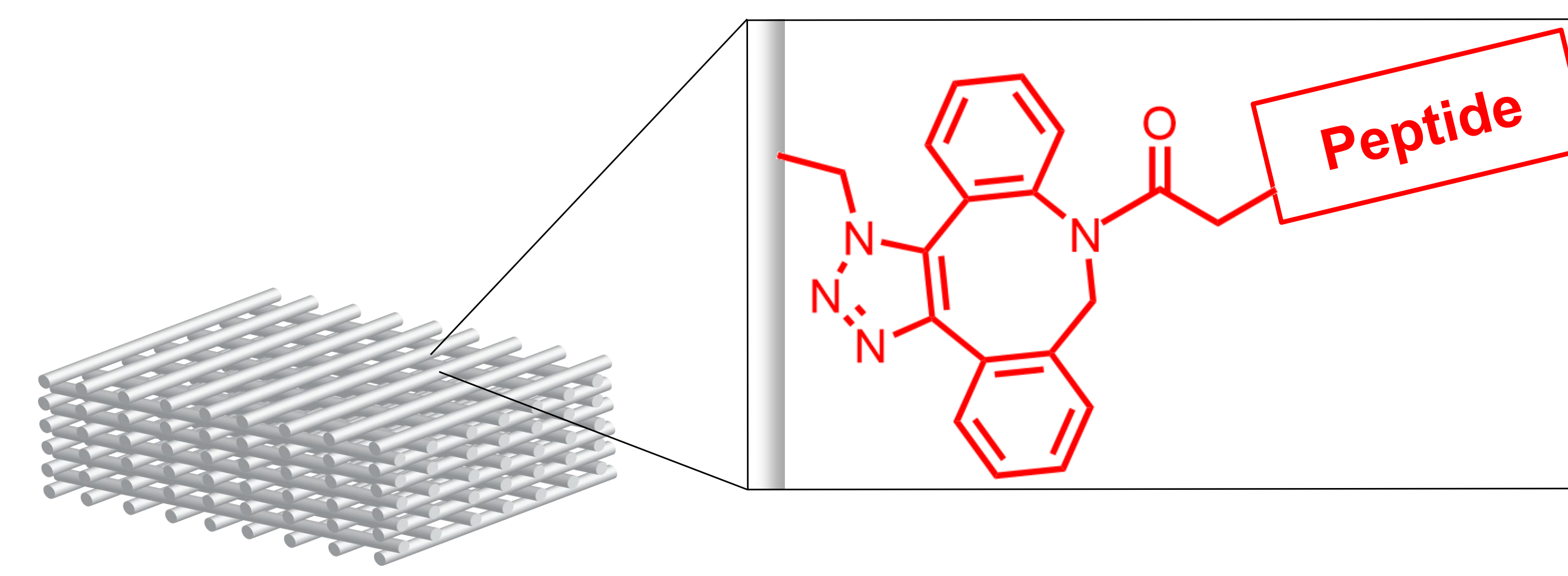


Figure 5: The DBCO group on the peptide reacts with the azide (N₃) on the azide-functionalized PCL scaffold.

Synthesis of PCL-azide

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

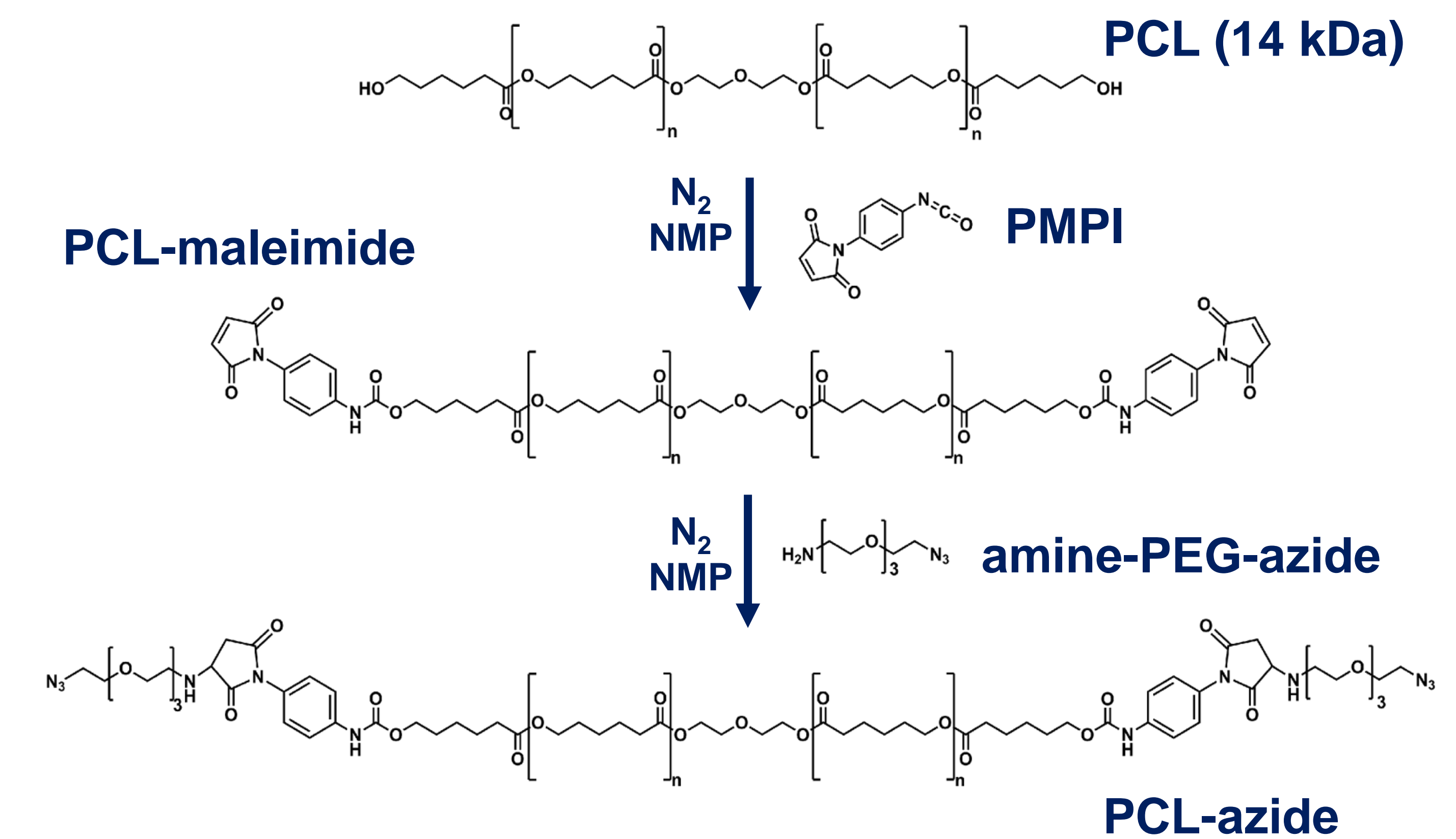


Figure 3: PCL-azide conjugate synthesis

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).

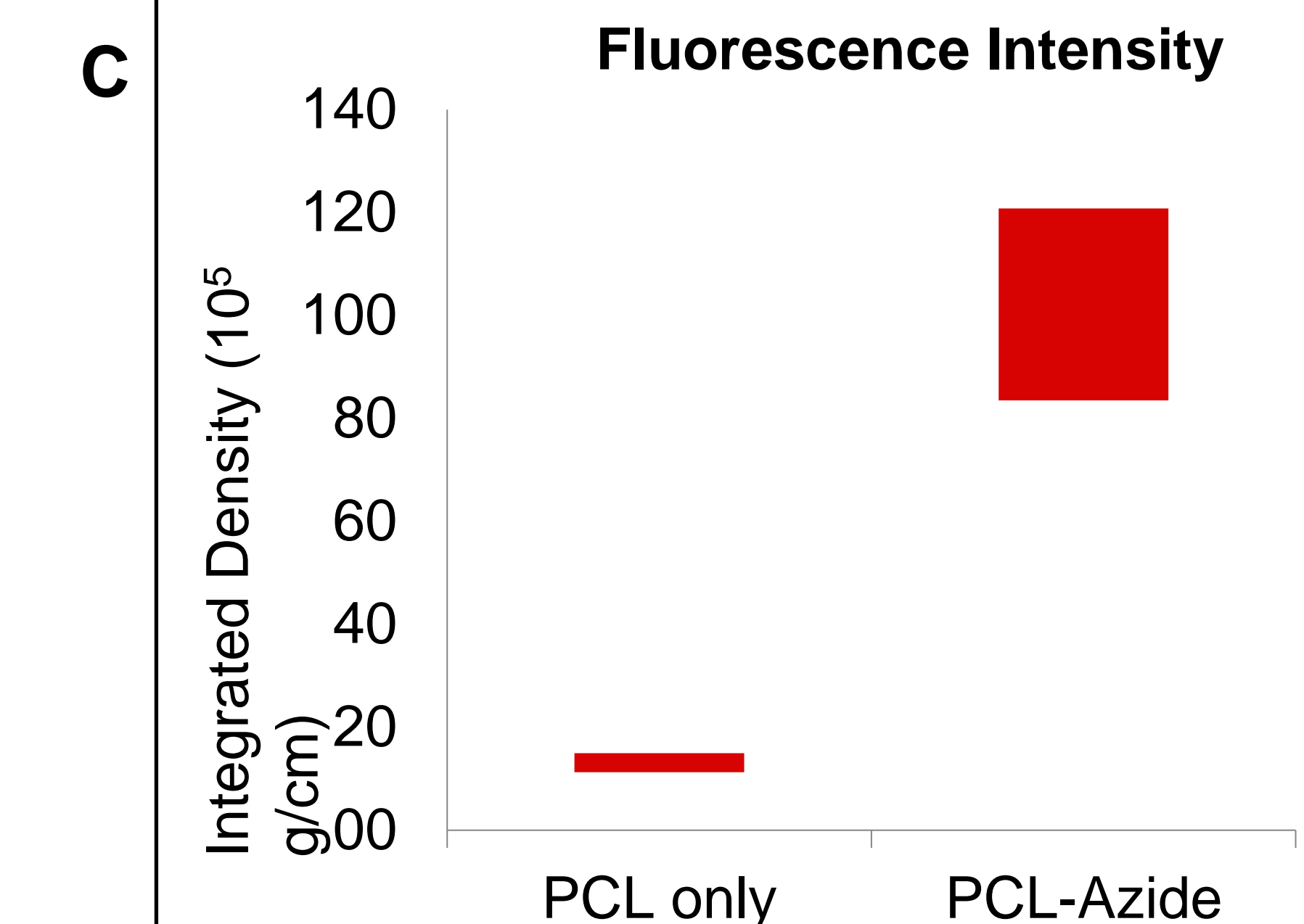
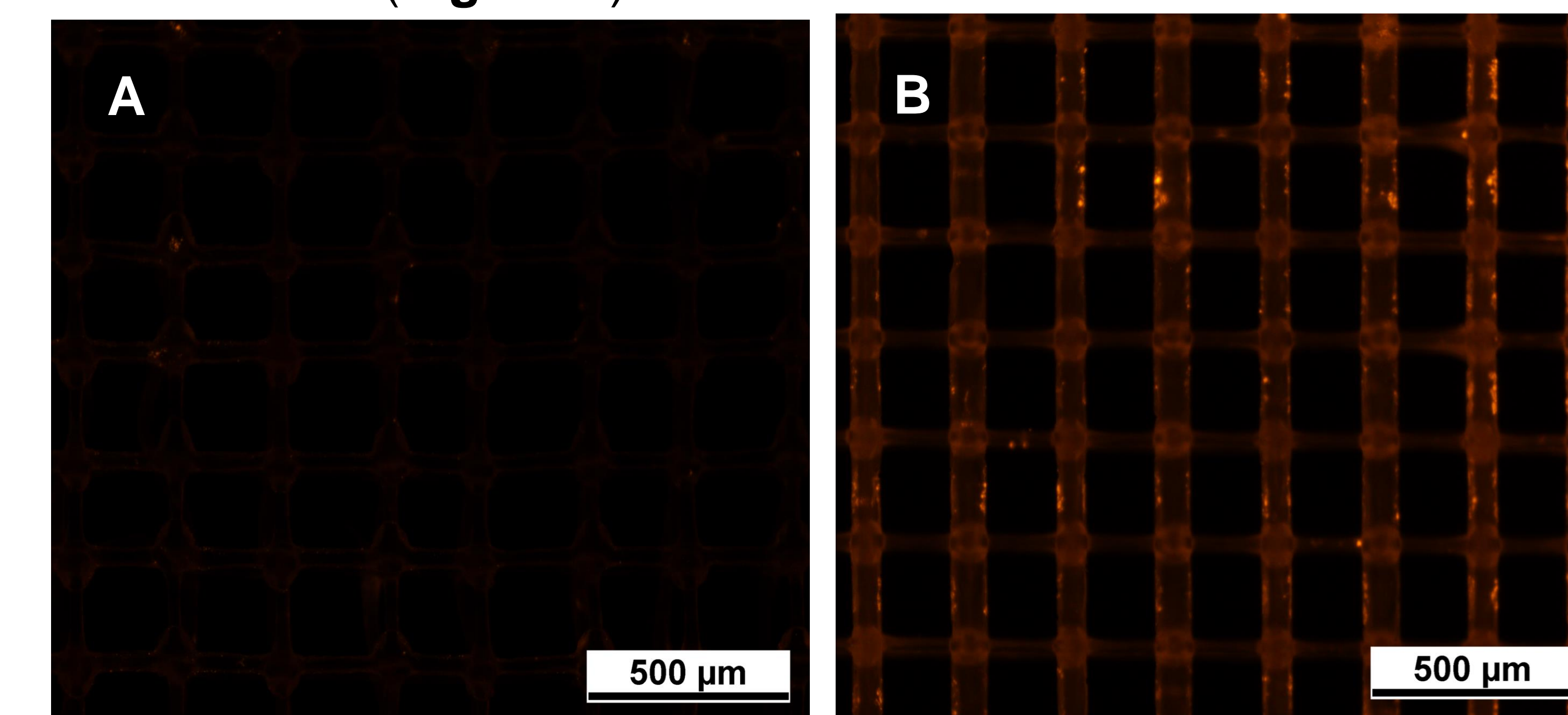


Figure 6: Representative fluorescence microscopy images of scaffolds 3D printed with (A) PCL only or (B) PCL with 20 mg/mL azide-PCL and labeled with DBCO-pep (red). (C) Quantification of mean fluorescence intensity using integrated density values (product of mean gray value by area).

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



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References: [1] S Glyn-Jones+ Osteoarthritis 386(9991): 376-387,2015. [2] K. B. Seims+ Bioconjugate Chemistry 32(5): 861-878, 2021. [3] Di Luca, A+ Birth Defects Res. Part C 105 (1), 34–52, 2015. [4] M. A. Azagarsamy+ ACS Macro Letters 2(1): 5-9,2013. [5] P. Camacho+ Biomater. Sci., 2019, 7, 4237, 2019. [6] Orth et al. Prog Histochem Cytochem 2015, 50, 19–36. [7] Occhetta, P.; et.al, I. PNAS 115 (18), 4625–4630, 2018. [8] Zhang, et al. Adv. Funct. Mater. 25(3): 350–360, 2015

