Developing Functionalized Bioresorbable Membranes Using Natural and Synthetic Polymer Blends

Nicole Malofsky[1], Paula Camacho[1], Hafiz Busari[2], Amelia Zellander[3], Lesley W. Chow[1,2]

[1]Department of Bioengineering, [2]Department of Materials Science and Engineering, Lehigh University, Bethlehem, PA, USA, [3]Scigofer, LLC Philadelphia, PA, USA

Introduction

- Biodegradable polymers, such as poly(caprolactone) (PCL), are used in 3D scaffolds to support cells and degrade as new tissue forms[1]
- Physical, mechanical, and biochemical properties of materials must be tailored to mimic native tissue to promote functional regeneration
- Bioresorbable membranes for soft tissue applications, such as skin, cornea, muscle, and blood vessels, may alleviate the need for native donor tissue
- Here, tunable membrane scaffolds are produced by blending synthetic PCL and a natural polymer chitosan (CHI)[2]
- Membrane properties can be modified by changing PCL:CHI ratio, specifically influencing optical, mechanical, and degradation behavior
- Using a strategy developed in the Chow Lab[3], membranes can be functionalized with bioactive peptides (i.e., canonical adhesion motif RGDS[4]) by adding peptide-PCL conjugates during membrane formation (Fig. 1)
- This work provides a platform for tuning multi-component tissue engineering constructs that mimic native tissue properties

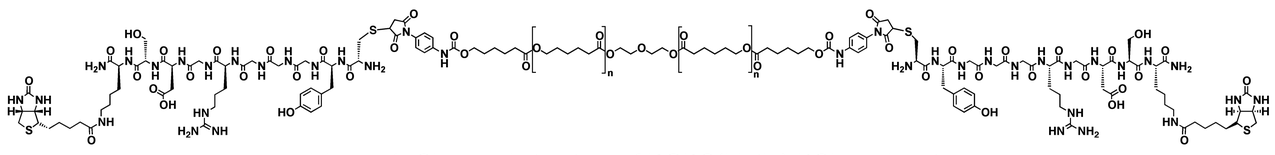


Figure 1. Chemical structure of RGDS(biotin)-PCL conjugate.

Peptide-Functionalized Membranes

- PCL/CHI membranes were prepared with range of PCL:CHI ratios (25:75, 50:50, 75:25) by dissolving PCL and CHI separately in a common solvent formic acid (Fig. 2)
- RGDS(biotin)-PCL conjugate (1 mg/mL or 5 mg/mL) was added to the PCL solution prior to membrane formation to create peptide-functionalized membranes
- After 24 hours, membranes were washed in phosphate buffered saline (PBS) and 0.5M sodium hydroxide to neutralize residual formic acid
- Membranes were labeled with streptavidin-fluorescein isothiocyanate (FITC) to confirm presence of biotin-tagged RGDS peptide
- All membranes were imaged using DSLR camera and optical microscopy
- Fluorescence was quantified by analyzing FITC-labeled membranes using a Tecan microplate reader
- NIH 3T3 fibroblast cells were seeded at 100,000 cells/membrane to investigate effect of peptide modification on cell adhesion
- Cell viability was evaluated using a Live/Dead Cell Viability Assay after 24 hours of culture on the membrane

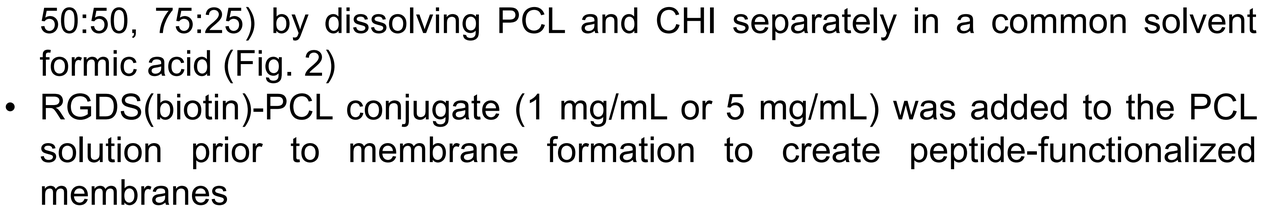


Figure 2. Schematic of (A) PCL (+/- RGDS(biotin)-PCL) and CHI dissolved separately in formic acid then (B) combined at varying PCL:CHI ratios. The mixed polymer solution was cast into glass petri dish then (C) heated for 24 hours at 50ºC until solvent evaporates to form a (D) PCL/CHI membrane.

PCL/CHI Transparency and Handleability

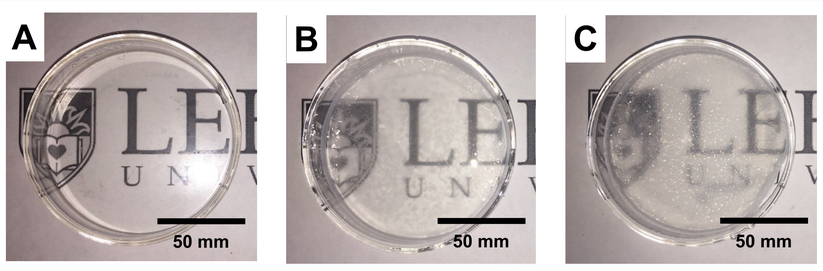


Figure 3. Macroscopic images of PCL/CHI membranes composed of (A) 25PCL/75CHI, (B) 50PCL/50CHI, and (C) 75PCL/25CHI. The 25PCL/75CHI membranes were selected for fluorescence and cell adhesion studies based on their transparency and mechanical stability.

- Membranes with varying PCL:CHI ratios were successfully fabricated
- Increasing CHI content increased optical transparency and homogeneity as well as mechanical stability (Fig. 3)

Controlling Peptide Concentration

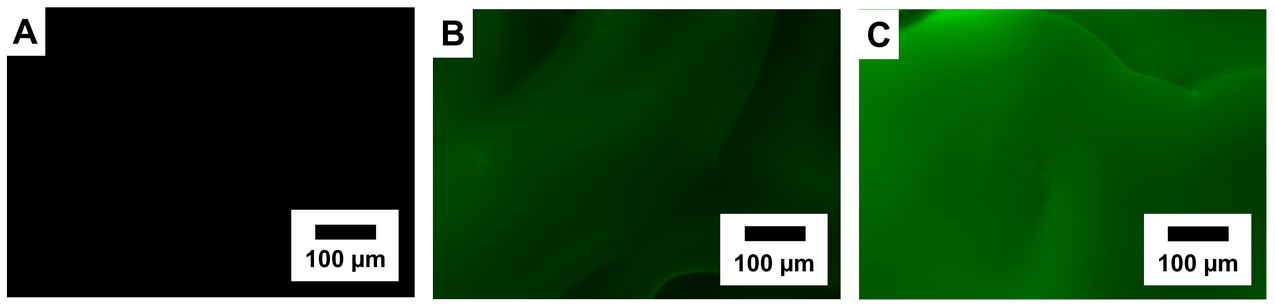
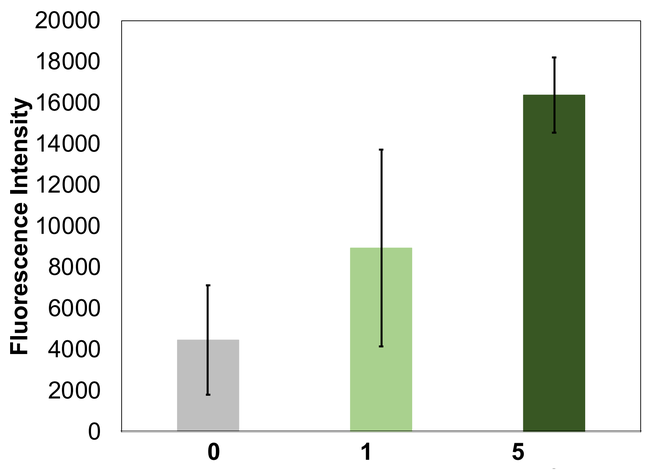


Figure 4. Fluorescence microscopy images of 25PCL/75CHI membranes made with RGDS(biotin)-PCL conjugate at (A) 0 mg/mL, (B) 1 mg/mL, and (C) 5 mg/mL and labeled with streptavidin-FITC (green).

- Membrane scaffolds were functionalized with peptides during formation without the need for post-modification steps
- Fluorescence was only detected in membranes containing RGDS(biotin)-PCL conjugate, indicating presence of peptide on membrane surface (Fig. 4)



- Peptide-functionalized scaffolds maintained similar optical properties as those without the conjugate
- Increasing RGDS(biotin)-PCL concentration correlated with an increase in fluorescence intensity, indicating higher RGDS surface concentration (Fig. 5)

Figure 5. Fluorescence intensity measurements of membranes formed with RGDS(biotin)-PCL conjugate showing fluorescence intensity correlates with conjugate concentrations (N=12 per group, error bars representing standard error).

Cell Adhesion and Viability on Functionalized Membranes

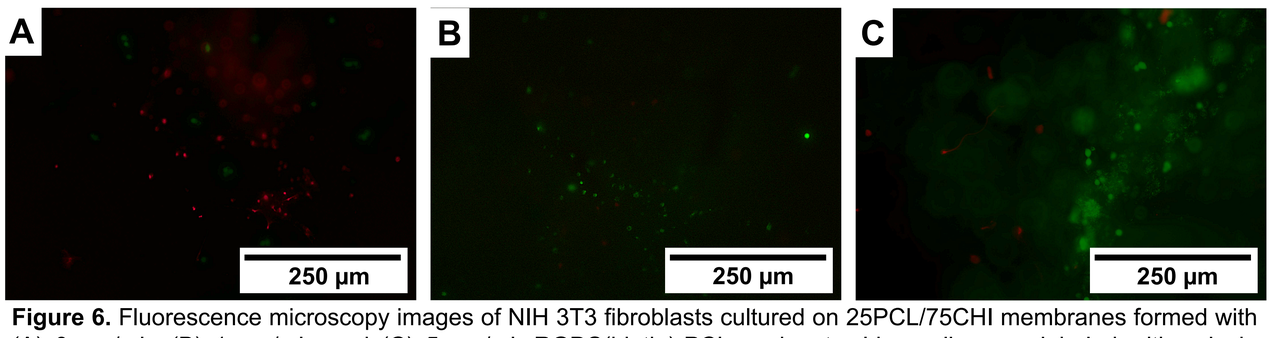


Figure 6. Fluorescence microscopy images of NIH 3T3 fibroblasts cultured on 25PCL/75CHI membranes formed with (A) 0 mg/mL, (B) 1 mg/mL, and (C) 5 mg/mL RGDS(biotin)-PCL conjugate. Live cells were labeled with calcein (green) and dead cells were labeled with ethidium homodimer 1 (red).

- Increased RGDS(biotin)-PCL concentration positively correlated with increased cell adhesion and viability on the membranes (Fig. 6)

Conclusions and Future Work

- This study showed membranes can be reproducibly formed with desired optical transparency, structural integrity, and cell adhesion properties using a single-step functionalization strategy with peptide-PCL conjugates
- Future work involves long-term cell culture studies and mechanical testing and membrane suturing to verify membranes satisfy clinical use requirements for tissue regeneration applications
- This platform introduces tunable multi-component tissue engineering constructs that more closely resemble the diverse mechanical and chemical properties of native tissues

Acknowledgements

The authors acknowledge Lehigh's Electron Microscopy and Nanofabrication Facility, Health Research Hub, Department of Chemistry Shared Instrumentation, and the Center for Advanced Materials and Nanotechnology. This work was partially supported by a collaborative grant with Polysciences, Inc. through the Pennsylvania Department of Community and Economic Development and start-up funds awarded to LWC from Lehigh University.

References: [1] Lopes MS, Jardini AL, Filho RM. *Procedia Engineering* 42(1): 1402-13, 2012; [2] Young TH, et al. *Colloids and Surfaces B: Biointerfaces* 116: 403-10, 2014; [3] Chow LW, et al. *Advanced Healthcare Materials* 3(9): 1381-1386, 2014; [4] Bellis SL, *Biomaterials* 32(18):4205-10, 2011.

LEHIGH UNIVERSITY

thechowlab.com

Center for Advanced Materials and Nanotechnology