Four Dimensional Printing of Gradient Scaffolds for Cartilage Tissue Engineering

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Introduction

- Osteoarthritis (OA) is a degenerative joint disease with symptomatic pain and discomfort [1].
- By 2030, 67 million Americans are expected to have an OA diagnosis; 33% of this population will be workforce contributors aged between 45 and 64 years [2].
- Existing minimally-invasive methods of treatment or mitigation of disease progression exist, but in most severe cases, all treatments eventually lead to total joint arthroplasty.
- A significant challenge with tissue engineering scaffolds at joint surfaces is the lack of natural repair and recovery of cartilaginous tissue.
- This research hypothesizes that a porous 3D scaffold, supplemented with polydopamine (PD) and transforming growth factor beta-1 (TGF-β1), will have the ability to direct chondrogenic differentiation of human mesenchymal stem cells (hMSCs).

Experimental Methods

- Fused Deposition Modeling (FDM) of Poly (vinyl alcohol) (PVA) created the scaffold molds.
- A novel, smart, PVA-based resin solution filled each mold and thermally cured for 24 hours.
- Heated sonication in ultrapure water assisted in leeching the PVA out of the cured resin leaving a porous, channeled, PCL-based smart scaffold.

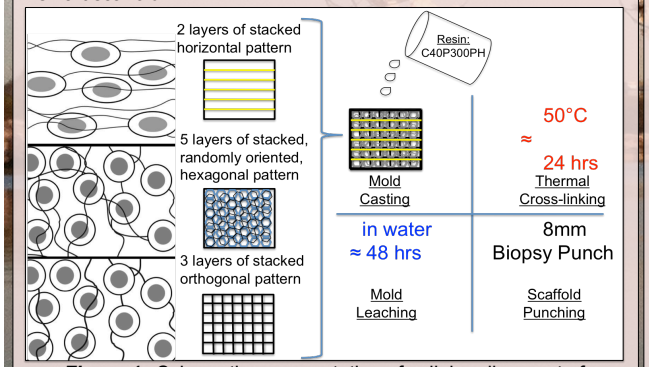

Figure 1. Schematic representation of cellular alignment of cartilage with corresponding schematics of scaffold molds and flow chart representing the investment casting process.

- Cell adhesion, proliferation, and differentiation were executed by covering each scaffold with a solution of hMSCs in media.
- Scaffolds were removed after 4 hours (adhesion) and 1, 3, and 5 days (proliferation) and cells lifted with Trypsin; these cells were then counted using a spectrometer to determine cell adhesion values for each scaffold.
- For differentiation, scaffolds were removed after 1, 2, and 3 weeks, transferred to a fresh well-plate, lysed, and evaluated for various chondrogenic markers.

Results

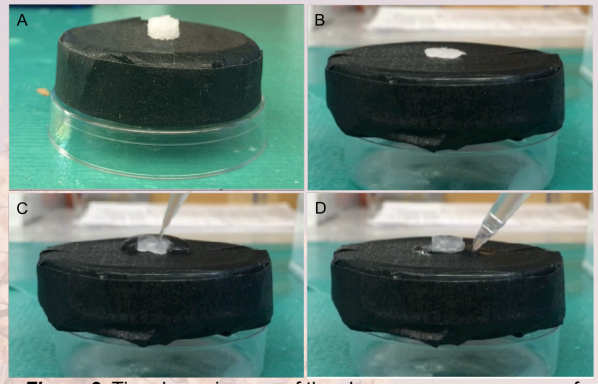

Figure 2. Time-lapse images of the shape memory response of the scaffolds. (A) Undeformed scaffold; (B) Deformed scaffold; (C) Scaffold mid-recovery; (D) Fully recovered scaffold.

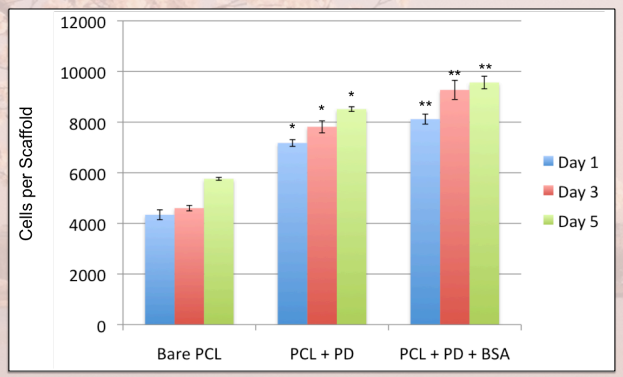

Figure 3. Enhanced hMSC proliferation on PCL-based scaffolds augmented with both PD and PD+BSA; data are mean ± standard error of the mean, n=9; *p<0.05 when compared to the bare PCL-based control, **p<0.05 when compared to all other scaffolds.

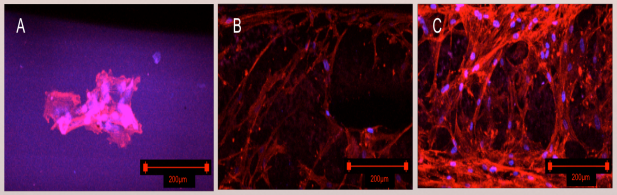

Figure 3. Confocal micrographs of PCL-based polymer scaffolds after 5 days of culture. (A) Bare PCL-based scaffold; (B) PCL-based scaffolds coated with PD; (C) PCL-based scaffolds coated with PD+BSA. Scaffolds in B and C show good cell attachment, growth, and viability.

Results

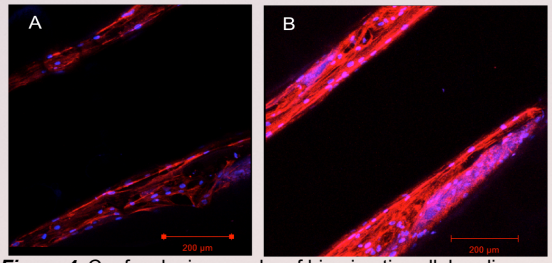

Figure 4. Confocal micrographs of biomimetic cellular alignment on top surface of PCL-based polymer scaffolds after 5 days of culture. (A) PCL-based scaffolds coated with PDA; (B) PCL-based scaffolds coated with PD and BSA.

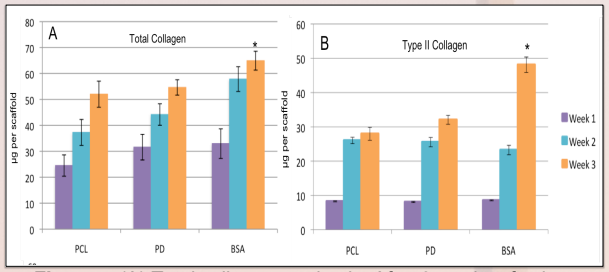

Figure 5. (A) Total collagen synthesis. After 3 weeks of culture, scaffolds coated with PD and BSA/TGF-β1 had higher total collagen content (*p<0.05) when compared to other scaffolds. (B) Type II collagen synthesis. After 3 weeks of culture, scaffolds coated with PD and BSA/TGF-β1 had significantly higher levels of Type II collagen (*p<0.05) when compared to all other scaffolds with. Data are mean ë standard error of the mean, n=9.

Conclusions

- The shape memory response of the PCL-based scaffolds shows promise for minimally invasive cartilage replacement surgeries as well as a medium for in situ mechanical stimulation of implants targeting load bearing applications.
- The qualitative hMSC proliferation results verified in the confocal micrographs show promise for favorable biocompatibility as well as biomimetic cellular alignment on the scaffold surface similar to that on the surface of the articulating joints.
- Differentiation analysis indicates both PD and TGF-β1 improve the chondrogenic response on 3D printed, PCL-based, smart scaffolds.

References

[1] Buckwalter, et.al., **Osteoarthritis.** Adv Drug Deliv Rev, 2006. ***58***(2).
[2] Hootman, et.al., **Projections of US prevalence of arthritis and associated activity limitations.** Arthritis and Rheumatism, 2006. ***54***(1).

Acknowledgement

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