Mineralized Collagen-Chitosan Scaffolds Promote Osteogenic Potential of Rat Mesenchymal Stem Cells

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Introduction: Three-dimensional (3D) *in vitro* bone microdevices use a bone-like substrate to provide environmental cues and support cell growth. A limitation of current bone substrates is they do not provide a model of the cancellous (porous) bone environment, prioritizing either porosity [1] or protein mineralization for dense scaffolds [2] despite the importance of both properties. We previously developed porous collagen-chitosan scaffolds of differing weight % ratios and confirmed their composition and architecture were comparable to cancellous bone [3]. We have yet to determine the biocompatibility of these scaffolds or their ability to influence osteogenic differentiation. The goal of this study was to determine which scaffold formulation demonstrates mesenchymal stem cell viability over 90% and promotes alkaline phosphatase activity.

Methods: Scaffold Fabrication. Commercial type I collagen (3 mg/mL) and chitosan were homogenized in three ratios (10:0, 8:2, 6:4 wt% collagen:chitosan) and mixed with 20 vol% phosphate buffered saline (10x). Protein solutions were freeze-dried in molds, creating porous cylindrical protein scaffolds. Scaffolds were crosslinked in a 2:1 molar ratio of EDC:NHS, mineralized for 14 days in modified simulated body fluid (mSBF) or polymer-induced liquid precursor (PILP) [2,4], and then lyophilized. Biocompatibility. Scaffolds (n=3 per formulation) were trimmed to 1 mm thick, biopsy-punched to fit in a 96-well plate, and sterilized. Bone-derived rat mesenchymal stem cells (rMSCs) were seeded in growth medium directly on scaffolds, or on tissue culture plastic as a control, and cultured for 7 days. Cell-conditioned media was collected on days 1, 3, 5, and 7 for fluorometric measures of alkaline phosphatase (ALP) activity to assess rMSC function, normalized by cell density. Live/dead staining and imaging on day 7 were used to quantify cell viability. Statistics. Viability was compared across formulations using ANOVA, and ALP activity was compared using repeated measures two-way ANOVA, with Tukey post hoc tests (α=0.05).

Results: Biocompatibility. No scaffold formulation was detrimental to overall cell health, with similar viability between scaffold and control groups. All groups had average viability over 90%. ALP activity, measured by 4-MU generation normalized by cell density, remained constant in the control group but increased over days 1-7 for all scaffold groups ($p<0.001$, **Figure 1**) and was more pronounced for both 10:0 formulations and 6:4 PILP. Day 7 4-MU production was higher for 10:0 mSBF compared to 8:2 mSBF, 6:4 mSBF, and control ($p<0.05$ for all).

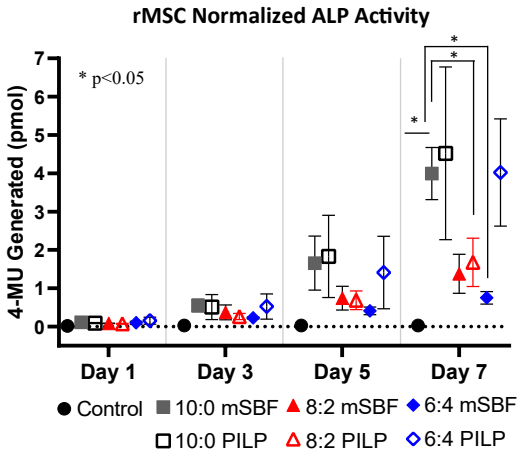

Figure 1. 4-MU generated by rMSC alkaline phosphatase (ALP) over 7 days of culture on mineralized scaffolds.

Conclusions: In our previous work, we confirmed through spectral analysis that our porous scaffold composition reflected collagen, chitosan, and bone-like mineral content. We found that the scaffolds were porous, 85% on average, with pores ranging 10-200 µm in diameter, comparable to cancellous bone. In this study, we demonstrated the scaffolds were biocompatible, with no significant differences in viability compared to controls. Of note, plating rMSCs on scaffolds increased their ALP activity compared to control and over time for various formulations. Early osteoblast differentiation is characterized by increased ALP production and activity, suggesting plating MSCs on mineralized scaffolds is osteogenic without supplementing the media. *In situ* osteogenic differentiation of MSCs in a 3D platform is desirable, allowing gradual differentiation based on environmental cues instead of through addition of supplements, reflecting a more natural process and saving resources. Overall, we have created cancellous bone-like mineralized collagen-chitosan scaffolds that promote *in vitro* osteogenic potential of mesenchymal stem cells.

References: 1. Lee SJ. Biomaterials. 2006; 27:3466-3472. **2.** Li Y. Biomacromolecules. 2012; 13:49-59. **3.** Stangeland-Molo S. SB3C Meeting. 2022; *abstract 267, podium*. **4.** Oyane A. J Biomed Mater Res A. 2003; 65:188-195.