PCL Reinforcement of a Mineralized Collagen-GAG Scaffolds for In Vivo Implantation

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Statement of Purpose: This work describes the reinforcement of a previously developed mineralized collagen-glycosaminoglycan (CGCaP) scaffold using a polycaprolactone (PCL) support structure for implantation in a porcine mandibular defect. We hypothesize that the resulting material composite (CGCaP-PCL) retains the inherent osteogenic bioactivity, nutrient retention, and increased cell attachment of the CGCaP scaffold and mechanical robustness associated with the PCL support.

Methods: The mineralized collagen-glycosaminoglycan (CGCaP) scaffold was fabricated via lyophilization from a precursor suspension composed of collagen, chondroitin sulfate, and calcium salts in phosphoric acid. The polycaprolactone (PCL) support structure was fabricated by the selective laser sintering of a polycaprolactone-hydroxyapatite powder. The composite (CGCaP-PCL) was fabricated by lyophilization of the CGCaP precursor suspension interpenetrated within the PCL support.

Preliminary investigations of the CGCaP-PCL composite has assessed changes in mechanical properties, permeability as a measure of fluid retention, and initial cell attachment compared to its individual constituents, CGCaP and PCL. Changes in mechanical properties were assessed via unconfined compression, while changes in permeability were determined via a constant head permeability test. Cell adhesion was investigated using porcine adipose derived stem cells (pASCs) 24 h after seeding via changes in metabolic activity (AlamarBlue).

Results: Preliminary data has indicated a 50 fold increase in elastic modulus between the CGCaP-PCL composite and the mechanically soft CGCaP scaffold, 932 kPa and 18 kPa respectively (**Figure 1**). Similarly, a 460 fold decrease in permeability or increase in fluid retention was observed between the CGCaP-PCL composite and the PCL support structure alone, 2.4E-14 m^2 and 1.6E-9 m^2 respectively. Finally, an increase in initial cell activity was observed in the CGCaP-PCL composite compared to the PCL support structure alone (**Figure 2**).

Current work is investigating differences between CGCaP, PCL, and CGCaP-PCL healing in vivo. Non-critical porcine mandibular defects are being assessed over a period of 56 days with bone ingrowth, density, and quality being assessed by micro-computed tomography (µCT), dual energy x-ray absorptiometry (DEXA), and standard histological analysis of tissue slices.

Ongoing efforts are also evaluating the effects of glycosaminoglycan content and biomolecule signaling on the innate osteogenic potential of the CGCaP matrix for in vivo implantation. Currently, heparin is being evaluated as an alternative to chondroitin sulfate due to its inherent use in regenerative applications. Efficacy is being investigated by differences in metabolic activity, gene expression via RT-PCR, and matrix remodeling assessed by µCT, histology staining of matrix components, and changes in mechanical properties. Additional work is also assessing the passive incorporation of VEGF for enhanced angiogenic incorporation during in vivo implantation.

Conclusions: We have indicated improved mechanical robustness and bioactivity of the CGCaP-PCL composite compared to its constituents. Future work will assess these qualities in vivo with treatment of a porcine mandibular defect. Additionally, alterations in scaffold composition and biomolecule incorporation for a more tunable osteogenic response are being investigated.

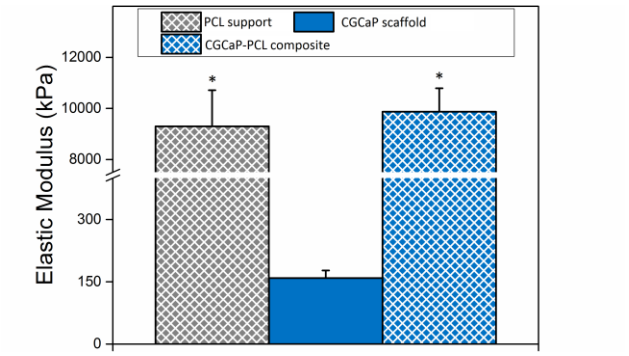

Figure 1. Changes in mechanical behavior of the CGCaP-composite compared to its constituents as measured in unconfined compression. *: significant ($p<0.05$) difference from the CGCaP scaffold group.

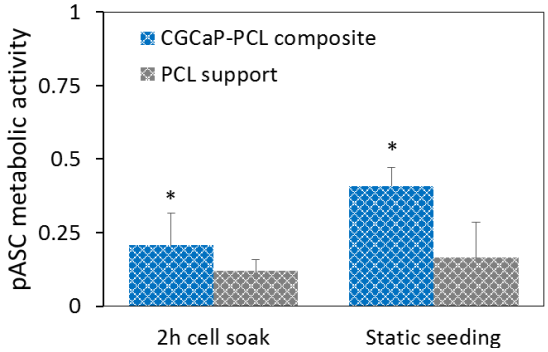

Figure 2. Metabolic activity as an indication of cellular attachment after 24h. Cells were either pipetted onto the scaffold with media added after 2 h (static seeding) or scaffolds were submerged in an equivalent cell solution for 2 h before transfer to fresh media (2h cell soak). *: significantly ($p<0.05$) different from group PCL support.