A Preliminary Evaluation of Electrospun Amelogenin for Various Tissue Engineering Applications

Isaac A. Rodriguez[1], Jennifer M. McCool[2], Yang Han[2], Scott A. Sell[3], and Gary L. Bowlin[1]

[1]Dept of Biomedical Engineering, The University of Memphis and Joint University of Memphis-UTHSC-Memphis Biomedical Engineering Program, Memphis, TN; [2]Dept of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA; [3]Dept of Biomedical Engineering, Parks College of Engineering, Aviation, and Technology, Saint Louis University, St. Louis, MO

Statement of Purpose: Amelogenin accounts for about 90% of the developing enamel matrix proteins. In addition to facilitating enamel mineralization, amelogenins have been identified to function as cell adhesion molecules and shown to promote cell adhesion and osteoblast differentiation for bone tissue engineering applications. More recently, amelogenin has been studied for applications outside of biomineralization considering that it is expressed by many different types of cells within the brain, cartilage, nerve, spinal cord, blood vessels, periodontal ligament, and more. Due to the wide variety of tissues in which amelogenin is found, it is thought that this protein may play a multifunctional role in tissue development.[1] This study introduces the first attempts of incorporating amelogenin within an electrospun scaffold. Amelogenin-loaded electrospun poly(glycolic acid) (PGA) and poly(ε-caprolactone) (PCL) scaffolds were characterized for their degradation and mechanical properties, mineralization, and fibroblast cell attachment.

Methods: Amelogenin was extracted from unerupted tooth buds of 6 month old pigs.[1] Scaffolds were fabricated by electrospinning PGA and PCL with 0, 1 and 5 mg/mL amelogenin. PGA and PCL were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol at 100 and 150 mg/mL, respectively. Prior to electrospinning, lyophilized amelogenin was dissolved in 150 µL acetic acid then added to the polymer solutions. Electrospinning parameters were as follows: 4 mL/hr, +26 kV, 20 cm airgap, and grounded mandrel and target. Scanning electron microscopy (SEM) was performed in order to evaluate the scaffold and fiber surface characteristics. Scaffolds were incubated for 1, 4, 7, 10, and 14 days in 1x phosphate buffered saline (PBS). Uniaxial tensile testing was performed on hydrated dogbone cut samples to obtain mechanical properties while protein release from scaffolds was characterized using a generic protein assay (Pierce BCA). 3mm discs of each scaffold type were soaked in 1x and 5x conventional simulated body fluid (c-SBF) overnight in static culture conditions to evaluate the mineralization potential of the electrospun incorporated amelogenin. Scaffold discs were also seeded with 20,000 human dermal fibroblasts (HDFs) and imaged after 4',6-diamidino-2-phenylindole (DAPI) staining to evaluate initial cell attachment.[1]

Results: After 4 days incubation in PBS, electrospun PGA scaffolds degraded to the point where mechanical tensile testing could not be performed. The initial modulus for PGA and PGA+1mg/mL amelogenin were 102 and 175 MPa, respectively. This was significantly higher than PGA+5mg/mL amelogenin and all PCL scaffolds which ranged between 4-9 MPa. Even after 4 days incubation, this significant trend was still observed. Over the course of the 14 day study, a significant increase of released protein was observed from the PGA+5 mg/mL amelogenin samples. The majority of the protein was released by day 10. The PGA+1 mg/mL amelogenin samples released some protein, but the trend was not as robust as for the 5 mg/mL samples. While the PGA samples exhibited a continuous increase in released protein, the PCL samples had small bursts of protein over the 14 day incubation period. After immersion in SBF, the PGA control scaffolds induced little to no mineralization. The incorporation of 1 mg/mL amelogenin into the PGA scaffold did not greatly increase scaffold mineralization. However, PGA+5 mg/mL amelogenin scaffolds nucleated small clusters of crystals with 1x c-SBF, while those incubated in 5x c-SBF had widespread large mineralization clusters (Figure 1). On the other hand, PCL and PCL+ amelogenin scaffolds did not mineralize overnight. After 24 h in static culture, PCL scaffolds with +1 mg/mL and +5 mg/mL amelogenin appeared to have increased numbers of attached HDFs compared to the controls. The PGA samples appeared to have greater cell attachment with +5 mg/mL amelogenin than those with +1 mg/mL amelogenin or the controls.

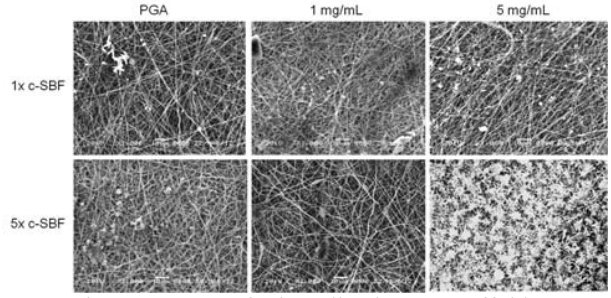

Figure 1. SEM of mineralized PGA scaffolds

Conclusions: This preliminary study demonstrates the successful incorporation of amelogenin into an electrospun scaffold and its subsequent release. Amelogenin withstands the electrospinning process and exposure to harsh organic solvents, while remaining detectable and bioactive. The controlled release of amelogenin from an electrospun scaffold can be tailored by the choice of polymer and its subsequent degradation rate. The preliminary nature of this study was intended to serve as a proof of concept of the first attempts of electrospinning amelogenin. The results from this study suggest that various types of tissue repair and regeneration may benefit from electrospun amelogenin scaffolds, such as bone and wound healing.

References: [1]McCool JM et al. J Bioactive Compatible Polymers. Jan. 2014.