Bone morphogenetic protein release from polyelectrolyte multilayers for orthopedic applications: Effect of multilayer deposition conditions

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Statement of Purpose: As joint replacements occur more often thanks to longer lifespans in the developed world, it is essential to improve the understanding of biointerfaces and to tailor the surface of implanted devices for specific applications. Joint replacement failure occurs because of implant loosening that can be caused by non-ideal surface properties, stress shielding and/or biofilms. Polymer coatings capable of release of relevant growth factors are one option to improve fixation of joint implants.

We have previously developed polyelectrolyte multilayer (PEM) coatings capable of µg cm^{-2}-scale release of a model polypeptide.[1] In this work, we compare pre-osteoblast outcomes and tissue formation on PEM-coated anodized titanium surfaces in which the PEMs were prepared under different conditions. Specifically, we compare coatings that were prepared from solutions at their natural pH, at their natural pH with 0.1M NaCl, and at pH=6.

Methods: Titanium foil (99.5%, Alfa Aesar) was anodized in 165 g L^{-1} sulfuric acid at a potential of 30 V for 5 min. Anodization under these conditions results in a porous oxide structure with pores ranging in size from 40-200 nm in diameter. Coating of titanium foil with the PEMs was achieved by first immersing the anodized titanium specimen in a 0.1 mg mL^{-1} solution of recombinant human bone morphogenetic protein 2 (BMP-2, Peprotech) for 15 minutes. The PEM was then formed on this adsorbed layer by dipping the plate in 1 mg mL^{-1} PMAA for 15 minutes, then in 1 mg mL^{-1} PH for 15 minutes. Alternating layers of PMAA and PH were deposited until 10 layers were achieved. PEMs without BMP-2 were also prepared as a control. Coatings were prepared under three conditions: 1. Solutions not modified 2. Solutions pH adjusted to 6; 3. 0.1M NaCl added to the solutions.

The amount of BMP-2 released from specimens immersed in PBS was quantified using enzyme linked immune sorbent assay (ELISA). ELISA was performed in accordance with the instructions provided with the development kit.

MC3T3-E1 preosteoblast cells were used to evaluate the biocompatibility of coatings. MC3T3-E1 cells were cultured in α-MEM with 4.5 g L^{-1} glucose, 10 vol.% fetal calf sera, 10 µg mL^{-1} Gentamicin and 50 µg mL^{-1} ascorbic acid. Approximately 6x10³cells cm^{-2} were suspended in culture medium, dispersed over the titanium plates and cultured in an incubator (Binder) at 37°C in a humidified atmosphere containing 5% CO_2. Fresh medium was given every 2nd or 3rd day of culture.

Preosteoblast differentiation was characterized with alkaline phosphatase (ALP) enzyme activity. Results are expressed in units of U L^{-1} and are normalized to a surface area of 1 cm^2.

Results: After 25 days, the unmodified coating (PE w/ BMP-2) was capable of releasing 21±5 ng cm^{-2} BMP-2. While promising, the unmodified coating perform poorly as compared to the coatings prepared at pH = 6 and with 0.1M NaCl, which released 86±5 ng cm^{-2} and 114±6 ng cm^{-2}, respectively.

ALP activity results for 21 days after culture are presented in Figure 1.

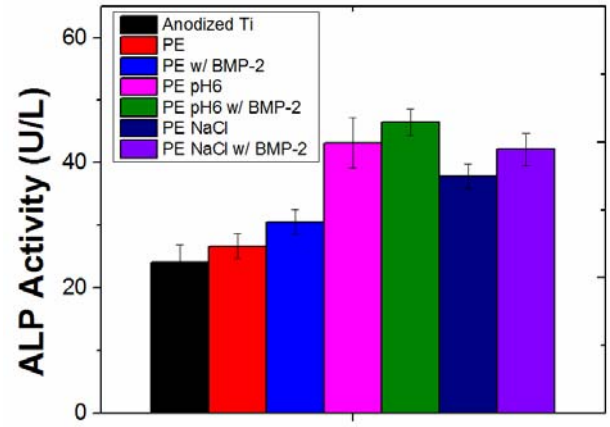

Figure 1. ALP activity on BMP-2-eluting and control surfaces after 3 weeks.

Conclusions: For all coatings, 50% of BMP-2 release occurred within the first three days, suggesting that similar release mechanisms are at play in all systems. Different amounts of release may primarily result from the different amounts of adsorption that can occur under different conditions. This possibility will be investigated in future work.

ALP enzyme activity results are most promising for the modified coatings. Interestingly, the addition of growth factor does not have a statistically significant effect on ALP enzyme activity for the pH=6 deposition condition. Additionally, the coatings made under different processing conditions are significantly more effective than the BMP-2-eluting coating prepared from unmodified solutions. Since the chemistries of these three systems are identical, there must be differences in surface roughness that play a significant role in preosteoblast differentiation and tissue formation. Ongoing work is focused on measuring and controlling surface roughness. These results suggest that coating roughness can play a greater role than growth factor release on tissue formation. Additionally, roughness optimization combined with controlled release of growth factors may result in systems capable of better fixation than systems with just one of those properties.

References: 1. Peterson AM. Biomacromolecules 2012;13:3120-3126.