Antibacterial Polyhydroxybutyrate (PHB) Membranes for Guided Bone Regeneration

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Statement of Purpose:
Guided bone regeneration (GBR) for titanium-based dental implants may involve a membrane placed in post-extraction sockets. The technique utilizes a bioresorbable membrane as a mechanical barrier to create a secluded space to permit bone regeneration while preventing connective tissue migration.[1] Although growing new bone is one of the most important issues in dental-tissue engineering, inhibition of infection is equally as crucial due to the microbial nature of the mouth flora. Polyhydroxybutyrate (PHB) is a natural polyester synthesized by a wide variety of microorganisms. It is biocompatible, biodegradable, invokes a minimal immune response, and has been proposed for various biomedical aplications.[2] In addition, over the last decade, implants have been modified to possess nanoscale surface features to enhance cellular functions and decrease bacterial growth. In fact, studies have demonstrated that cellular functions are highly affected by nanophase surface topographies.[3] Therefore, the purpose of this research was to combine the above two approaches using GBR and nanoscale surface features to develop an antibacterial membrane for improving dental applications.

Methods: PHB (Sigma) was dissolved in chloroform at 60ºC for 1 hour to create a 5wt% PHB solution. The solution was cast onto a glass-petri dish to create a PHB membrane, followed by overnight evaporation. The PHB membranes were treated with NaOH concentrations (0.1, 0.5 and 1 N) for various time points (5 and 10 min) to alter nanoscale surface features. For cell growth studies, human osteoblasts (C-12720, Promocell) were seeded onto each PHB membrane at 5000 cells/cm^2 and cultured for up to 3 days. An MTT assay (CellTiter®96 Non-Radioactive Proliferation Assay, Promega) was used to assess cell density on these surfaces. *S. aureus* (ATCC, 25923) was used for bacterial growth studies on agar plates. All experiments were completed in triplicate and data was analyzed using student t-tests.

Results: SEM images (Fig. 1) of untreated and treated PHB scaffolds exhibited a significant change in surface roughness at the nanoscale. Untreated PHB membranes had more sharp surface morphology while treated PHB showed more round surface nanofeatures. More importantly, our preliminary results indicated that NaOH-treated PHB surfaces reduced bacterial adhesion compared to untreated counterparts (Fig 2). Furthermore, cell culture results demonstrated that treatment with NaOH promoted the growth of human osteoblasts on PHB membranes compared to untreated ones (Fig 3).

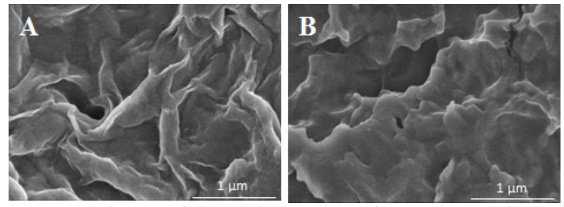

Figure 1: SEM images of untreated (A) and 1 N for 10 min treated (B) PHB membranes. NaOH treatment created more rounded nanoscale surface features. Scale bars = 1 µm.

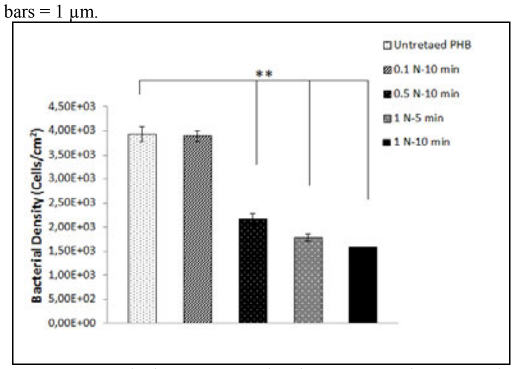

Figure 2: *S. aureus* colonies on untreated and NaOH-treated PHB membranes at 1 day. Values are mean +/- SEM; n=3. **p<0.05 compared to the untreated PHB membrane.

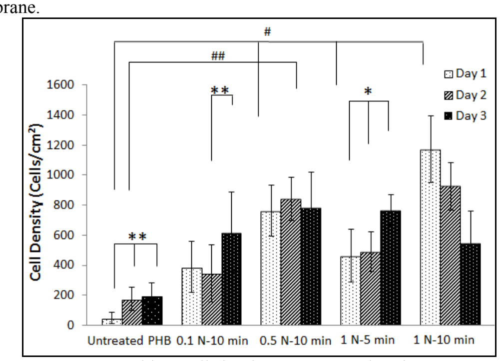

Figure 3: Human osteoblast cell density on untreated and NaOH-treated PHB membranes. Values are mean +/- SEM; n=3. #p<0.005, ##p<0.05 compared to the untreated PHB membrane; *p<0.005, **p<0.05.

Conclusions: In this work, the surface of a PHB membrane was modified by an alkali treatment using different NaOH concentrations for different time points. Results showed enhanced osteoblast density while improving antibacterial properties on the NaOH-treated compared to untreated PHB. For these reasons, NaOH-treating of PHB membranes has great potential for use as a biodegradable GBR membrane and, thus, requires further investigation.

Acknowledgement: The authors would like to thank Northeastern University for funding.

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