Anti-bacterial Silk Membrane for Wound Healing Applications

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Statement of Purpose:
In the United States, approximately 6.5 million patients suffer from chronic wounds caused by diabetes, circulatory problems, burn injuries and many other conditions resulting in treatment costs exceeding $25 billion per year.[1] Through recent advancements in developing better skin graft materials, healing damaged tissue has improved and patient survival rate has increased, although no approach has been successful to regenerate the same anatomy as healthy skin, neither to its full functionality nor in terms of aesthetics. Better skin substitutes which promote healing of wounds, as well as fight with infection are required for skin tissue engineering applications. Over the last decade, the use of nanophase materials to enhance cellular functions and decrease bacterial growth have been of great interest in biomaterial design. In fact, studies showed that cell adhesion and proliferation onto silk fibroin surfaces are highly affected by nanophase surface topography, chemical composition and wettability properties of the materials.[2] Therefore, in this study, we modified the surfaces of a previously investigated material, silk fibroin, using a NaOH alkaline treatment to obtain a biologically-inspired nanofeatured surface morphology to promote skin cell proliferation and fight against infection.

Methods: A silk fibroin solution was prepared from B. mori cocoons according to previously established protocols with slight modifications [30]. Firstly, Bombxy mori silk cocoons were cut into small pieces and boiled in 0.05 M Na_2HCO_3 for 30 minutes which was then rinsed with distilled water (diH_2O) three times. The obtained silk fibroin fibers were dried overnight at 40^oC, dissolved in a LiBr solution (9.3 M) at 60 °C for 4 hours, followed by dialysis through a cellulose membrane (12-14000, MWCO) across distilled water for 4 days. The obtained silk solutions were centrifuged twice at 13,000 g, diluted to a final concentration of 2 w/v% and stored at 4^oC. Afterwards, the dried membranes were treated with a methanol solution for 1 hour to make them non-water soluble. To create a nanofeatured surface topography, silk membranes were treated with 0.1, 0.5 and 1 N NaOH solutions for 5 or 10 min. For cell growth studies, Human epidermal keratinocytes (Invitrogen, C0015C, population numbers 5-7) were seeded onto silk membranes at 3500 cells/cm^2 and cultured 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 to perform the bacterial growth studies on agar plates. All experiments were completed in triplicate.

Results: SEM images (Fig 1) of untreated and treated PHB scaffolds exhibited a significant change in surface roughness. upon interacting the silk fibroin membranes with a NaOH solution, the surfaces expressed more nanophase suface features, mimicking the nanophase topography (Fig 1.) Our preliminary results indicate that silk membranes treated with NaOH showed enhanced adhesion and proliferation of skin cells compared to their untreated counterparts (Fig 2).

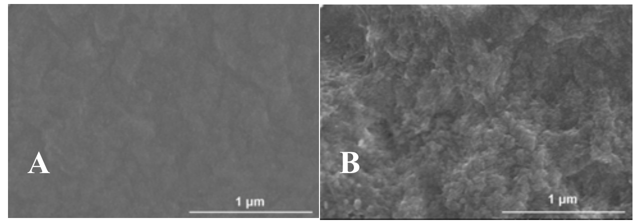

Figure 1: SEM images of untreated (A) and 1 N for 10 min treated (B) silk fibroin membranes. NaOH treatment increased nanorough topography. Scale bars = 1 µm.

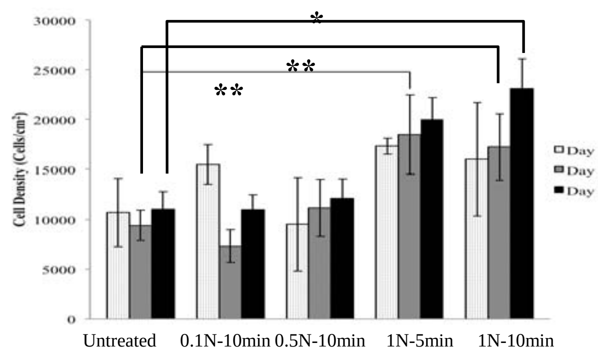

Figure 2: 3 day human epidermal keratinocyte proliferation results on untreated and NaOH treated silk fibroin membranes. NaOH treatment increased keratinocyte cell density on silk fibroin membranes. Values are mean +/- SEM; *$p<0.005$, **$p<0.05$ compared to untreated silk fibroin membrane for the same time point.

Conclusions: In this study, a novel alkaline treatment method to synthesize nanofeatured surface morphology on silk fibroin membranes was demonstrated. The relative simplicity of surface modification technique for the silk fibroin membrane makes it an attractive alternative to the currently-used wound healing materials. Due to these reasons, nanofeatured silk fibroin membrane is a promising material for wound healing applications and requires further investigation.

References:

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