Immobilizing CD47 Polypeptide on Implantable Biomaterials

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Introduction: The CD47 polypeptide is produced in the human body and goes undetected by the immune system. This protein has been found to accumulate around tumor cells allowing them to be camouflaged, enabling their ability to grow undetected from the patient's immune system. The question to be answered by this study is, "If the CD47 polypeptide could adhere to the surface of an implantable biomaterial, would it allow the device to function as planned while remaining undetected by the immune system?" The first objective of this study was to determine whether or not the use of succinimidyl 4-[N-maleimidomethyl] cyclohexane-1-carboxylate (SMCC) would serve as a linkage molecule to attach the CD47 polypeptide to a natural polymer such as silk fibroin and collagen coated polyester biomaterials. This led to the second objective which was to determine the level of CD47 that could be applied to the silk fibroin surface in order to change the immune response.

Materials and Methods: The sample of silk fibroin fabric was supplied by the College of Textiles pilot laboratory. The CD47 polypeptide was purchased from (Novoprotein, Summit, NJ), and the SMCC was obtained from (Fisher Scientific). The silk fabric was first scoured to remove any surface contaminants using Triton X-100 for 20 minutes near the boiling point. It was then immersed in a 0.2% aqueous solution of sulfo-SMCC for 30 minutes at room temperature. Two different concentrations of CD47 solutions were prepared by dissolving 25μL and 75μL of CD47 in PBS buffer solution. The high and low CD47 concentration treatments were obtained by immersing the SMCC treated silk in one of the two solutions at pH 7.2-7.5 for 30 minutes at room temperature. The samples were then left to dry at room temperature overnight.

Results: After the CD47 had been applied to the silk fibroin samples, the silk fibers were viewed by scanning electron microscopy (SEM) so as to confirm whether or not there was any visible attachment of the CD47 to the silk. The untreated control sample of silk showed a smooth fiber surface, while the treated samples of silk fibers showed attached particles on the surface (Figure 1).

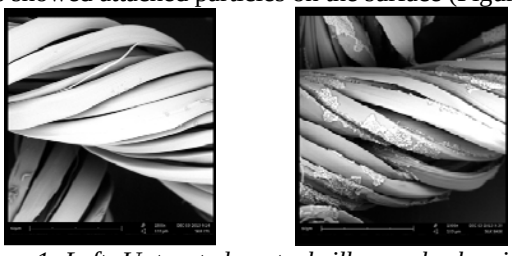

Figure 1: Left: Untreated control silk sample showing a smooth fiber surface. Right: Treated silk sample showing accumulations of particles on the fiber's surface.

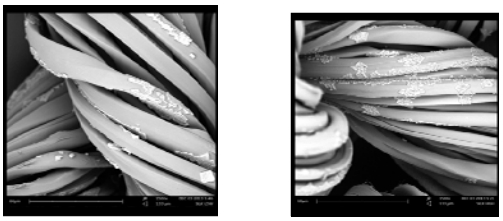

Figure 2: Left: Fiber surface of silk sample with low concentration of CD47. Right: Silk sample with high concentration of CD47 showing more accumulations of particles on the fiber surface.

We then used fluorescence microscopy to visualize the presence of the dyed CD47 particles and to determine whether or not they had been successfully attached to the fiber's surface.

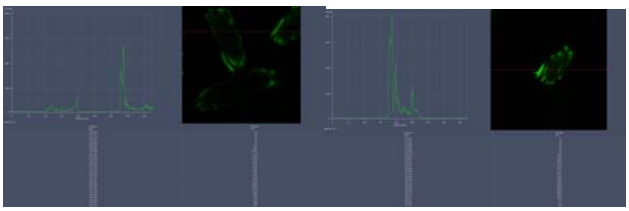

Figure 3: Left: Fluorescent image and intensity scan of silk fibroin sample treated with the low concentration of CD47. Right: Fluorescent image and intensity scan of silk fibroin sample treated with the high concentration of CD47.

Conclusions: The results obtained from SEM confirm that we have successfully modified the surface of the silk fibroin fibers. It can therefore be concluded that the CD47 polypeptide was successfully immobilized onto the surface of the silk fiber samples, so the question of whether it can adhere to an implantable biomaterial is still a possible option. Fluorescent microscopy added further confirmation by showing that the low concentration samples had a lower fluorescent magnitude and intensity compared to the high concentration samples. The next step will be to quantify the extent and uniformity of the CD47 immobilization on the silk fibers. The use of an *in vitro* study will help determine whether the presence of the CD47 will change the immune response.

References:

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