MACROPHAGE POLARIZATION DUE TO INTERLEUKIN-4 COATED POLYPROPYLENE MESHES

Rahul Rege1, Daniel Hachim D.1,2, Bryan N. Brown1,2,3
1McGowan Institute for Regenerative Medicine, 2Department of Bioengineering, 3Department of Obstetrics, Gynecology and Reproductive Science, University of Pittsburgh

INTRODUCTION
Pelvic Organ Prolapse (POP) is a condition that affects more than 250,000 women a year in the United States, costing more than $1 billion [1]. POP affects 30-50% of menopausal women. These numbers are on the rise. In this condition, pelvic organs fall out of place from their normal position in the body. Symptoms include pain, discomfort, and urinary incontinence [2].

Polypropylene mesh is commonly used in surgeries for the treatment of POP. This mesh provides mechanical support to hold organs in place. However, up to 40% of patients suffer from complications due to surgery [1]. These complications are caused by chronic foreign body reactions caused by immune system response to the synthetic material of the mesh. These reactions cause erosion of the soft tissue surrounding the mesh. This puts the patients in even more pain and discomfort. Often times, multiple surgeries are required to remove and/or replace the mesh [1].

The chronic foreign body reactions that cause surgery complications are a result of macrophage polarization at the tissue-implant interface. Macrophages are large phagocytic cells that are found at sites of infection and foreign material in the body [3]. These macrophages range in phenotype from the M1 phenotype to the M2 phenotype. The M1 phenotype causes more of an inflammatory response. This is the primary cause of foreign body reactions. On the other hand, M2 macrophages are more pro-remodeling. They favor tissue repair and can help integration of mesh within the body [3].

In vitro studies have shown that a cytokine protein called interleukin-4 (IL-4) can stimulate macrophage polarization to the M2 phase. IL-4 promotes the M2 phenotype, while suppressing the inflammatory response of the M1 macrophages. These studies give hope that IL-4 can be used in vivo to suppress the foreign body reactions due to polypropylene mesh [3].

Currently surgical mesh has no protein coating on it. There is nothing to subdue inflammatory responses to the M1 macrophages. However, IL-4 coated meshes provide a possibility of improving surgery treatments by reducing complications. The key step is being able to successfully load and release IL-4 from the mesh.

OBJECTIVE
The objective of this experiment was to quantify the effectiveness of IL-4 protein loading with different numbers of coating layers. The key was to compare the amount of IL-4 that can be loaded and released from polypropylene mesh. Triplicates of the three following conditions were compared: 20 bilayers, 40 bilayers, and no IL-4 bilayers.

SUCCESS CRITERIA
This experiment would be considered successful if it could be confirmed that it is possible to coat polypropylene meshes with IL-4. Another criteria of the experiment was quantifying the amount of IL-4 loaded and how long it could be released based on the number of layers of coating.

METHODS
Polypropylene meshes of size 1cm by 1cm were irradiated with plasma using maleic anhydride to produce a negatively charged surface. To coat the polypropylene mesh, a layer-by-layer (LbL) procedure was performed. The mesh was dipped in a solution of chitosan, a polycation. This was followed by a washing step. The mesh was washed with deionized water for 1 minute. Mesh was then dipped in dermatan sulfate, a polyanion, followed by another washing step. The ionic bonding reactions were used to make the layers attach to the mesh. This process was repeated to add 10 bilayers in order to form a core layer. IL-4 was incubated with dermatan sulfate and the process was continued to add the desired number of bilayers.

To measure whether IL-4 was successfully loaded and released, release assays were conducted. An enzyme-linked immunosorbent assay (ELISA) kit (Peprotech) was used where IL-4 loaded (20 and 40 bilayers) and coated (no IL-4) meshes were assayed after 72 hours of release in 37°C 1X phosphate buffered saline (PBS) containing 0.5 U/mL Chitosanase and Chondroitinase ABC. 100uL aliquots in triplicate were used for each condition. A cumulative release assay was also conducted to measure the amount of time the meshes were able to continue releasing IL-4. Positive control of 1.0 ng/mL IL-4 and negative control of 1X PBS were used.

Data from ELISA assays were obtained as absorbance values. Means and standard errors of these assays were analyzed by one-way ANOVA to determine the statistical differences, with statistical significance for p < 0.05.

RESULTS
The values calculated and plotted were the means of each condition, with standard error. As seen in figure 1, 20 bilayers of IL-4 coated on a polypropylene mesh was significantly greater than having no IL-4. In addition, 40 IL-4 bilayer coated mesh had even more statistically significant release of IL-4.

From the cumulative release assay, as seen in figure 2, 40 IL-4 coated mesh was able to release almost three times as much IL-4 as 20 bilayer IL-4 mesh (0.9 ng/cm² as compared to 0.3 ng/cm², respectively). The 40 bilayer one also was able to release the IL-4 for a longer time. It did not plateau its release in 8 days, as did the 20 bilayer one.

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DISCUSSION

From the results, it can be concluded that the success criteria of the experiment were met. IL-4 was successfully loaded and released from the polypropylene meshes in vitro. The amount of IL-4 that could be released increased as the number of layers of IL-4 increased. In addition, greater number of bilayers of IL-4 lead to longer release times. More IL-4 was able to be released before plateauing effect occurred.

These results illustrate the potential to release IL-4 at the tissue-implant interface. This opens up the possibility of reducing surgery complications by suppressing the inflammatory response of M1 macrophages. It also allows for recruitment/polarization of macrophages to M2 phase.

One limitation of this study was the plateauing time for 40 bilayers of IL-4 was not measured. In addition, it is necessary to see how the coated meshes interact in vivo. It would be of interest to determine if the coating of IL-4, along with the polyions, changes mesh properties and affects its role of providing mechanical support. It is also essential to measure the amount of M1 and M2 macrophages present at the implant interface compared between uncoated mesh and IL-4 coated meshes. The overall goal of this research is to reduce the number of patients with foreign body reactions from pelvic organ prolapse in order to reduce pain and discomfort.

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REFERENCES