Development of a Cytokine-Loaded Layer by Layer Coating on Polypropylene Meshes to Modulate Macrophage Polarization at the Tissue-Implant Interface

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Statement of Purpose: Implantation of polypropylene mesh is a common surgical procedure for hernia repair and for pelvic organ prolapse (POP) [1]. However, mesh implantation is commonly associated with a chronic foreign body reaction and a pro-inflammatory (M1) macrophage polarization phenotype at the tissue-implant interface [2]. It has been hypothesized that polarization of tissue macrophages surrounding the implant to a pro-remodeling M2 phenotype using IL-4 will lead to a better integration of the implant into the host tissue. A conformal coating with the ability to provide local delivery of IL-4 has been developed. This coating is based on the layer by layer (LbL) method [3], which produces a nanometer thickness coating on the surface of plasma treated polypropylene meshes.

Methods: Gynemesh® Polypropylene (PP) meshes were treated with an adapted radio frequency glow discharge (RFGD) method [5], using maleic anhydride (MA) to produce a negatively charged surface (PP^-). LbL coating was performed using chitosan (CH) as polycation and dermatan sulfate (DS) as polyanion. In the extracellular matrix, dermatan sulfate has been described to enhance IL-4 activity in-vivo [4]. To construct the coating, treated meshes were immersed interchangeably in either CH or DS concentrated solutions, by means of opposite electrostatic interactions. Cycles were repeated with intermediate washing steps until desired number of layers (up to 60 layers). Loading of IL-4 was performed by incubation of IL-4 into the dermatan sulfate solution prior to LbL coating. A $PP^-[CH/DS]_{10}[CH/DS^{IL4}]_{20}$ coating was done for further characterization. RFGD treatment and LbL coating on PP meshes were characterized with x-ray photoelectron spectroscopy (XPS), attenuated total reflectance – Fourier transformed infrared spectroscopy (ATR-FTIR) and alcian blue staining. IL-4 loading and release assays were performed with immunostaining and ELISA, respectively. Finally, IL-4 activity was evaluated in an in-vitro murine bone marrow-derived macrophage polarization culture. Macrophages were fixed with 2% PFA then stained for arginase-1. Intensity of staining was analyzed using Cell Profiler Image Analysis Software (Broad Institute, Cambridge, MA).

Results: XPS spectra revealed success in MA RFGD treatment to generate negatively charged carboxylic acid surfaces given the appearance of an additional O-C=O peak at 288 eV and an oxygen peak at 532 eV in each element spectra. Also, the LbL coating success is revealed by the presence of 3 types of carbon (O-C=O, C-O and C-C at 288, 286 and 284 eV, respectively) in the carbon spectrum, and the appearance of peaks in the nitrogen (399 and 401 eV) and sulfur (168 eV) spectra, indicative of the glycosaminoglycans (GAGs) used to construct the coating. These findings are consistent with ATR-FTIR measurements. Both XPS and ATR-FTIR measurements were performed at several points across the surface of mesh samples, revealing similar spectra throughout the mesh. Additionally, alcian blue staining revealed the presence of a uniform and conformal coating made of GAGs, which is only present in LbL coated meshes. Confocal microscopy images of immunostained meshes and ELISA assays have confirmed the loading of IL-4 into the coating and its release from the mesh surface, respectively (fig. 1a). Finally, an in-vitro culture assay showed that only coatings loaded with IL-4 were capable to polarize macrophages to a pro-remodeling M2 phenotype (IL-4), given the higher expression of arginase-1, which was similar to M2 positive controls (fig. 1b).

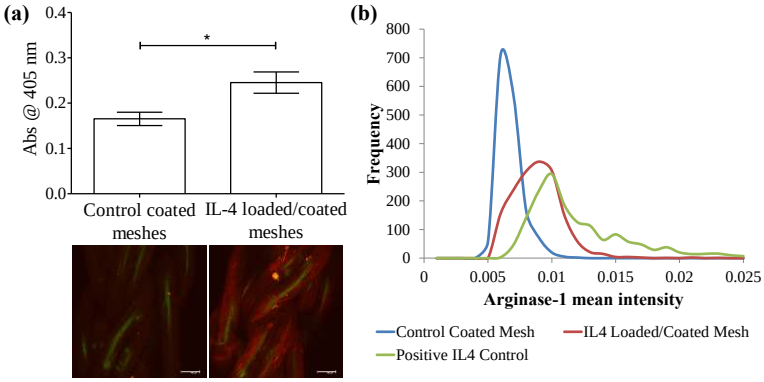

Fig. 1. (a) IL-4 ELISA (top) and immunostaining (bottom) of control coated meshes (left) and IL-4 loaded/coated meshes (right). Bars represent the mean +/- SEM, * significant with $p < 0.05$. **(b)** Arginase-1 cell profiling of cultured macrophages exposed to control coated mesh (blue), IL-4 loaded/coated mesh (red) and IL-4 positive control (green).

Conclusions: A uniform and nanometer thickness conformal coating has been successfully developed on plasma treated polypropylene meshes. This coating is suitable for the loading and release of active IL-4 and can shift macrophage polarization to a pro-remodeling M2 (IL-4) phenotype.

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