INTRODUCTION
There are currently more than 350,000 cases of hernia repair and pelvic organ prolapse surgeries in the United States every year [1,2]. On top, the numbers of cases are increasing annually. A common procedure for these surgeries include an implantation of a synthetic polypropylene mesh [3]. These polypropylene meshes provide mechanical support to the failing organs [3]. Although, this procedure usually results in healing the patient, 10 % of the patients experience complications with the synthetic meshes that are implanted [2]. One of the causes of these complications is the chronic foreign body reactions. Chronic foreign body reactions result in degradation and erosion of the host tissue surrounding the foreign substance [4]. Patients experience pain and discomfort with these conditions. Once a patient experiences chronic foreign body reactions, it may take multiple surgeries in order to remove the mesh from the body.

One of the key factors controlling the chronic foreign body reactions inside the body are macrophages. Macrophages are a type of white blood cells known to help fighting off infections and remodeling tissues [4]. Macrophages range in spectra of phenotypes from M1 phenotype to M2 phenotype. The M1 phenotype is known to help fight off infections and cause inflammatory reactions. On the other hand, the M2 phenotype is known to reduce inflammation and help rebuild tissues. Studies have shown that the M1 phenotype can be stimulated by LPS found in bacterial cell wall, and interferon gamma [4]. The M2 phenotype can be stimulated by interleukin 4 (IL-4) in vitro [4].

In our lab, we attempt to reduce the occurrence rate and severity of the foreign body reactions caused by the synthetic polypropylene meshes by loading the meshes with IL-4 protein. With the IL-4 protein, the macrophages within the host tissue surrounding the mesh will be polarized to M2 stage, causing less inflammation and resulting in tissue repair. This will help reduce the chronic foreign body reactions. The IL-4 protein is loaded on to the synthetic meshes by using the Layer-by-Layer method. The Layer-by-Layer method uses electrostatic energy in order to build layers of coatings on top of one another. The mesh is submerged alternatively in two chemicals where one chemical has a positive charge and the other chemical is negatively charged. The IL-4 protein is incubated in the negatively charged chemical to be loaded onto the meshes.

OBJECTIVE
The objective of this project is to quantify the effectiveness of the protein loading on to and release from the synthetic mesh used in surgeries according to different number of bilayers of coatings. In particular, the amount of IL-4 loaded and released onto the polypropylene mesh using the Layer-by-Layer method between 20 bilayers, 40 bilayers, and control coated mesh without the protein will be compared.

HYPOTHESIS/SUCCESS CRITERIA
We hypothesized that with the Layer-by-Layer coating method, we will be able to load IL-4 onto the mesh and that we will able to load more protein with increasing number of bilayers. We are verifying our hypothesis by confirming the protein loading with the release assay. Then we are quantifying the amount of IL-4 loaded by measuring the cumulative amount released at different time points using ELISA.

METHOD
Polypropylene synthetic meshes are cut into 1 cm by 1 cm square pieces for consistency. 3 square pieces are cut out for each of the groups, consisting of control, 20 bilayers, and 40 bilayers. Then the meshes were given an initial negative surface charge by radio frequency glow discharge using solid maleic anhydride. Then the meshes were immersed alternatively in two chemicals. Chitosan was used as the positive chemical and dermatan sulfate was used as the negative chemical. All of the meshes were coated with 10 bilayers of core coating without the IL-4 protein. From this point, the control meshes were coated with 20 more bilayers of the same chemicals without the protein. For the other two groups, the dermatan sulfate was incubated with IL-4 protein from this point. The dermatan sulfate chemical is known to enhance IL-4 activity. The 20 bilayers group meshes and the 40 bilayer group meshes were coated with 20 and 40 bilayers of chemicals with the protein respectively. The meshes were completely submerged in the chemical for 10 minutes. Once the one layer was complete, the meshes were washed with deionized water for one minute and dried with blowing air. Then the meshes were submerged in the other chemical for 10 minutes for the next layer to coat on top of the other layer. Before the next layer was coated the meshes went through the washing phase in between. After the set number of coating layers were complete, the meshes were kept in lyophilizer to maintain the protein.

Various tests were performed on the coated meshes. X-ray photoelectron spectroscopy was used for surface characterization of the meshes. It detects relative amounts of elements on the surface. The elements used to study were oxygen, nitrogen, carbon, and sulfur. Higher presence of these elements would indicate better coating. To confirm that the coatings were uniform throughout the meshes, an Alcian blue staining was used. The stain detects the presence of glycosaminoglycans, which are components of chitosan. A uniform color of stain over all areas of the mesh would indicate a uniform coating. In order to show that the IL-4 protein was successfully loaded onto the meshes, the immunostaining technique was used. The meshes with IL-4 would fluoresce with different colors when compared to the pristine mesh and the control meshes. The release assay and the enzyme-linked immunosorbent assay (ELISA) were performed to quantify the amount of protein loaded onto the different meshes.
With the data collected from these tests, a one way analysis of variance (ANOVA) tests were performed. The alpha values were set at 0.05 for the study.

RESULTS

From the XPS graphs generated, the Layer-by-Layer coated meshes had significantly greater peaks for nitrogen and sulfur compared to the pristine mesh. The two peaks seen in the nitrogen graph indicate presences of amines and amides. The strong peak in the sulfur graph indicates a carbon-sulfur-oxygen link presence on the surface of the mesh.

The meshes showed a uniform coating of the bilayers from the Alcian blue staining assay. The coated meshes had a uniform blue color while the pristine mesh did not change colors.

The control mesh with coatings without the protein was compared against the IL-4 loaded mesh with immunolabeling technique. The control mesh showed only a green fluorescence. The green fluorescence is the polypropylene auto-fluorescence. The IL-4 loaded mesh showed a strong red fluorescence along with the green. The red fluorescence indicates the presence of IL-4 protein.

The amount of IL-4 loaded onto the meshes and the rate of release of the protein from the meshes were measured using the release assay along with ELISA. The total amount of IL-4 loaded on the control mesh was less than 0.05 ng/cm², which can be considered as zero. The 20 bilayers mesh was able to load around 0.30 ng/cm², and the 40 bilayers mesh loaded more than 0.90 ng/cm² of the protein. The values cannot be determined exactly for reasons discussed later. The cumulative amount of IL-4 released from the three meshes at different time points can be seen in Figure 1. The bottom line, the middle line, and the top line indicate coated control mesh, 20 bilayers mesh, and 40 bilayers mesh respectively. The control mesh is constantly at zero at all time points indicating no protein release. The 20 bilayers mesh is increasing in the amount of IL-4 released up to 4 days then seems to plateau in value. The 40 bilayers mesh is increasing in value up to our last data point of 8 days.

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REFERENCES