INTRODUCTION
Pelvic organ prolapse is when internal pelvic organs move or fall out of place within the body due to lack of support from the surrounding tissue. This can occur after surgery or childbirth and can cause extreme discomfort and pain. Currently every year in the United States approximately 200,000 women undergo inpatient procedures for prolapse.1

Current treatments use a surgical mesh implantation to act as a structural support aid.2 However, due to the design of the mesh and the biomaterial used, increasing immune system complications have resulted.3 These complications can potentially require additional surgery with increased risk.

One way to improve the design of the mesh fibers is to observe how the body responds to the different biomaterials being used. Macrophages are a good indicator of recovery behavior because of their unique abilities to change phenotypes over time. There are two different phenotypes, M1 and M2; the M1 phenotype is pro-inflammatory and M2 phenotype is a pro-remodeling, anti-inflammatory response that promotes tissue repair. Once the body is injured the immune system sends white blood cells to the site and within those cells contain macrophages in the M1 form. Over time the macrophages transition from M1 to M2 based on the stage of recovery of the host. Using the knowledge of the location of each macrophage along with the phenotype it is in, can provide crucial information with regards to the recovery process.

One way to observe this macrophage activity is visually, in the form of utilizing antibodies. Antibodies are formed in the presence of an antigen, or a foreign substance that enters the body. Each antibody has a unique response to specific antigens, and each phenotype of macrophage mentioned contains those unique antigens. Primary antibodies have specific affinities for unique macrophage phenotypes. Using antibodies made to bind to these antigens, they will bind to the specific phenotype of the macrophage of interest. Once the primary antibodies have bound, a secondary antibody containing a fluorescent dye can attach to the primary. After exposing the secondary antibody to a specific wavelength of light, an image can be produced that would show the lit up macrophages. These images can then be compared to other images produced from different samples.

OBJECTIVE
The goal of this project is to observe the immune system response to the mesh implantation. By using primary and secondary antibodies to optimize immunofluorescent imaging, a picture depicting the response can be produced. By optimizing antibodies for each phenotype, M1 and M2, they can be individually isolated alongside the pan-macrophage markers. These surface markers, once optimized, will display the locations of all the macrophages regardless of the phenotype present. Having an overlay of these three parameters, allow for the classification of how the host is responding.

HYPOTHESIS/SUCCESS CRITERIA
By producing a picture with optimal saturation of fluorescent levels, this can indicate the location and phenotypes of the macrophages present and determine the success of the experimental approach.

METHOD
Immunofluorescent Staining (IF) was performed to attach antibodies to the macrophages on spleen tissue samples from a rabbit. The tissue samples were taken from rabbits at different days of recovery after a mesh implant was placed between the layer of muscle and skin. As the wound healed over time with the mesh inside, and the immune system responding, samples from equally spaced days of recovery were taken. IF is the process of preparing the samples and adding the antibodies at specific dilutions for binding. It is a two day procedure, first washing the samples in chemicals meant to prepare the sample for the best attachment affinity. The primary antibodies were added and allowed to sit overnight before adding the secondary antibodies with the fluorescent dye last. Once washed and cover-slipped, they can be placed under a microscope, exposed to different wavelengths of light and images can be captured.

To find the antibodies used, research papers were studied to find any past experiments using antibodies for macrophage labeling in rabbits. Of these ideal antibodies, they were either purchased, or already provided in the lab, and used as preliminary points of interest for the optimization procedure.

To optimize each antibody for the specific macrophages, the dilutions had to be adjusted. By changing the dilutions, different amounts of antibodies were exposed to the tissue samples. Too many antibodies exposed to the sample have shown to over attach to the macrophages and in some cases other proteins available, which supersaturates the image. This over florescence is too bright to accurately analyze. Having too little antibodies result in very minimum binding, sometimes having no binding occur on macrophages of interest. Having fewer or greater number of primary antibodies attached also impacted the amount of secondary antibodies that could bind. This decreased or increased the amount of fluorescent dye on each macrophage. As a result this adjusted the exposure levels of the images produced. Starting with equally spaced intervals of dilution levels gave a general assessment of the activity taking place and the binding affinities. Intervals around 1:50, 1:100, 1:150, and 1:200 were performed. Once a range of antibodies showing ideal images were observed, the dilutions
were more precisely adjusted by using dilutions within a closer range to the clearest image produced.

RESULTS

Antibodies were researched from past papers performing similar procedures within rabbits to find which performed effectively. Due to the limited resources of antibodies made for studies in rabbits, the testing pool of options were less than ideal. This is because antibodies have to be created for use in specific animals, whereas rabbits are not the popular choice for host immune system responses. Table 1 shows the possible antibodies that were selected to be optimized.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primary antibodies selected for optimization.</th>
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<td>M1 Marker</td>
<td>M2 Marker</td>
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<tr>
<td>iNOS</td>
<td>CD206</td>
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<td>CD86</td>
<td>CD163</td>
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Studies are still being conducted on the M1 and M2 antibody optimizations. The IF approach did not work for any of the primary antibody dilutions consistently enough for definitive results. However using a staining technique that stains the nuclei of the macrophages and the primary antibodies, they showed that the binding of primary antibodies were successful. This indicates that binding at the different dilutions is working even though the IF imaging does not show the fluorescence. This is the case for the M1 marker HLA-DR, and the RAM11 pan-macrophage marker, which labels the areas on the surface of the macrophage available for binding, as depicted in Figure 1.

DISCUSSION

Fluorescent images providing the location and phenotype of macrophages present could provide crucial information about how the immune system is responding. This could provide a success criteria for comparing different biomaterial designs for pelvic organ mesh implantation, by providing a consistent way of measuring the healing process over time. Surgical mesh was used in rabbits in vivo. This required the antibodies selected to have been made for use in rabbits. Since rabbits were used, the antibodies needed to have been prepared specifically for rabbits. If the antibody recognizes any familiar protein structures within the rabbit that is not a macrophage they could bind to the similar antigenic sites and invalidate the data. This posed great difficulty finding potential antibodies for each phenotype to use since most immune system research is conducted in mouse or rats. By starting with a limited number of possibilities to work with, it constricted the feasibility for the perfect optimization.

The IF imaging was not successful, but using other staining techniques confirmed the binding of antibodies did occur. This could indicate the IF procedure used needed to be optimized as well. The IF procedure for preparing the tissue for antibodies to effectively attach was not effective as it ideally should perform. By changing the IF approach and testing more antibodies at different dilutions, the images produced could aid in the biomaterial design of a mesh implant that could help translate to human clinical trials.

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REFERENCES