INTRODUCTION
The most common cause of abdominal surgery is abdominal wall hernia defects [1]. Each year, there are approximately twenty million hernia repair surgeries performed worldwide, of which almost 700,000 surgeries are performed in America [2]. While the anatomy of this highly prevalent disease is well-known, recurrence of hernias and post-surgical complications such as chronic pain, inflammation, and infection have been reported in over half the patients treated [2]. If left untreated, herniated organs can become incarcerated and blood deprived which may result in infection, sepsis, and ultimately death [1].

Surgical meshes used to treat abdominal wall hernia defects act as an artificial wall - providing mechanical strength and acting as a buffer that helps prevent recurrence of the hernia. In 1958, Dr. Usher introduced the first artificial Marlex mesh and since then over 70 different types of mesh materials have been tested and used for repairing abdominal wall hernia defects [3]. Currently, the most common treatment option is the polypropylene (PP) mesh [4]. However, PP is permanent and has been associated with many post-surgical complications due to isolation of mesh implant and the host tissue inflammatory response resulting in chronic inflammation and pain. Many studies have shown that biodegradable mesh made from poly-4-hydroxybutyrate (P4HB) has comparable repair strength and successful transfer of load bearing from mesh to the repaired abdominal wall has been demonstrated [5]. P4HB is a natural polymer that is produced by microorganisms for the purpose of regulating energy metabolism [6]. The P4HB mesh is a fully biodegradable mesh made from polymer produced by Escherichia coli K12 bacteria via transgenic fermentation techniques [6]. P4HB fully degrades in vivo within approximately 78 weeks into carbon dioxide and water, which are broken down by the body very quickly via the Krebs cycle and beta-oxidation [7].

There are three phases to the natural healing process that occurs after surgery or injury. The first phase is the inflammatory phase with incursion of macrophage precursors that starts at day 1, peaks at day 3 and persists until the healing process is complete [1]. The next phase is the connective tissue phase, which is followed by the differentiation phase when tension resistance reaches its highest levels [1]. Macrophages undergo specific differentiation depending on micro environmental signals received during these phases and they can either be classically activated into M1 type or the alternatively activated to M2 type macrophages. The M1 macrophages are pro-inflammatory while the M2 macrophages reduce inflammation, are immune regulators and they promote tissue remodeling. Determining whether a mesh material activates M1 or M2 type macrophages can help explain post-surgical complications. Previous studies have shown that biodegradable meshes prompt more favorable M2 cell response as compared to polypropylene mesh [7].

OBJECTIVE
The purpose of this pre-clinical study was to evaluate the host foreign body immune response to P4HB mesh and compare it to that of the widely used polypropylene mesh. The main focus was studying the types of macrophages activated and obtaining quantitative data. SUCCESS CRITERIA
The success criteria for this study was set at an approximate increase of 15% in the M2 immune cell activation up to 200μm away from the site of implantation which would significantly prevent inflammation and promote healing.

METHOD
For the in vivo part of this study, approximately 1.5 centimeter square of mesh (either P4HB or PP) was implanted in the abdominal wall thickness of Sprague–Dawley rat model. My role in this study was to compare the immune system response to each type of mesh material and to determine which mesh material promotes the anti-inflammatory immune cell response with a special focus on M1 and M2 type macrophages. Explaining the four post-surgical samples of each mesh material along with surrounding tissue for the various designated time points (3, 7, 14, 21, or 35 days) took approximately 3 days. The next step was to prepare the tissue samples obtained for staining by starting with tissue fixation using immersion. The explanted tissues were immersed in 10% formalin solution for 6 hours at room temperature. Next, the tissues were dehydrated by immersing them in 90% ethanol twice for thirty minutes each. The dehydrated tissues were then embedded in paraffin and approximately 5μm sections were cut and floated in a water bath. Finally, slides pre-coated with gelatin, were used to mount the tissue sections.

The next step was staining the tissues using antibodies specific for markers of macrophage types. For instance, the CD-68+ marker was used to identify the non-induced or Pan–Mφ macrophages. The M1 macrophages were identified using the CD-86+ marker and the CD-206+ marker was used to identify the M2 macrophages. This step was very important as it would help determine the immune cell response for each mesh material. However, the task of optimizing staining posed challenges due to the reaction of primary and secondary antibodies causing higher background fluorescence. In order to reduce background fluorescence, the dilution of proteins present in blocking solutions was key. This step helped minimize non-specific binding of antibodies.

The final step in this process was analyzing the stained tissue samples using a microscope. All the images taken were then uploaded to the Cell Profiler software so that the obtained data could be quantified. Cell quantification around the mesh material was done at four distances away from the mesh: 0-50 μm, 50-100 μm, 100-150 μm and 150-200 μm. After the data was quantified, we were able to compare the data for P4HB mesh to the control (Polypropylene mesh).

RESULTS
Significant differences were observed between the two mesh types. The absorbable P4HB mesh demonstrated a higher ratio of M2 over M1 macrophages especially with the earlier time points; day 3 and day 7, compared to polypropylene mesh.
This increase in the M2 (anti-inflammatory) response at the earlier time points is likely due to the increase in the expression of M2 related proteins such as Ym1, Arg1, and Fizz1 and the secretion of cytokines that were polarized to M2 type in animals treated with P4HB mesh. Figure 1 shows how the M1 and M2 macrophages were quantified at the various distances from the site of implant. While the figure here shows the different types of immune cells around the implant, the main goal of this study was to quantify only the M1 and M2 type of macrophages.

**Immune Cell Activation around Implant**

![Figure 1](image)

**Figure 1.** Immune cell activation around the site of mesh implant at various distances. Ring 1 is 50 μm, Ring 2 is 100 μm, Ring 3 is 150 μm and Ring 4 is 200 μm away from mesh.

Figure 2 below shows the M2/M1 ratio for both mesh materials for different time points (Days 3, 7, 14, 21 and 35) at each of these distances (rings) from the mesh. The four bars on each graph for P4HB and PP represent data collected from explanted tissues of the four animals that were treated with each of these mesh types.

**Macrophage Activation (M1 / M2)**

![Figure 2](image)

**Figure 2.** M1 vs. M2 macrophage activation at the site of implantation at Days 3, 7, 14, 21 and 35. Standard error bars are presented. P4HB shows a higher M2 activation than PP.

The X-axis of the graphs show the mesh type that was tested while the Y axis represents the M2 vs M1 ratio of macrophages activated at that time point for each ring. The figure also shows standard error bars. The error bars represent the differences between these biological replicates. Preliminary results show a 33% increase in M2 type macrophages for P4HB mesh over Polypropylene mesh.

**DISCUSSION**

The results of this study showed that P4HB does stimulate more anti-inflammatory immune cell response as compared to polypropylene mesh. We had originally expected that there would be an approximate 15% increase in the M2 macrophage cell activation but the P4HB mesh performance far exceeded this expectation. As mentioned earlier, M2 macrophages are anti-inflammatory as opposed to M1 macrophages, which are pro-inflammatory. The results of this study echo the results of other previous studies that biodegradable meshes do in fact activate the M2 anti-inflammatory cells [4,5,7]. This can prove to be very useful in treating hernias as most post-surgical complications with hernia repairs are associated with inflammation at the site of implant. While polypropylene mesh is widely used for hernia repairs, the fact that almost 50% of the patients treated develop some post-surgical complications has highlighted the need for a better treatment option.

However, there are certain limitations to this study. The statistical analyses for cell quantification that need to be done are very complex. Other biodegradable mesh materials also need to be tested to compare performance. The anti-microbial qualities of the mesh materials should also be tested so that infections and sepsis can be prevented. This study focused on M1/M2 type immune cells, but other immune cells also need to be quantified and the results obtained in this study need to be confirmed with in vitro models as well. Despite some challenges and requirements for further testing, the results of many studies have shown that biodegradable meshes such as P4HB can in fact be the best potential solution for treating hernia defects.

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**REFERENCES**


