INTRODUCTION
The world of regenerative medicine is heavily dependent on the extracellular matrix (ECM). The extracellular matrix is the non-cellular component that is present in all tissues and provides structural support. The ECM has been found to include many biologically active components including: collagen, laminin, fibronectin, glycosaminoglycans, growth factors, cytokines, and the recently discovered Matrix Bound Nanovesicles (MBVs) [1, 2]. These components allow the ECM to promote many regenerative processes by catalyzing angiogenesis, stem cell recruitment, and modulation of macrophage activation. Although the ECM has been shown to promote a regenerative response when added to an area of injury, the mechanism for promoting this response is still unknown.

Matrix Bound Nanovesicles were recently discovered in the extracellular matrix and can be classified as a type of extracellular vesicle (EV) [2]. Extracellular vesicles are secreted from cells and can perform actions that catalyze intercellular communication and transport. EVs carry cargo including: lipids, growth factors, mRNA, microRNA, nucleic acids, and proteins. The contents of extracellular vesicles, found in extracellular fluid, have been shown to regulate diverse physiologic and pathologic processes, such as angiogenesis, macrophage phenotype, cell differentiation and fate, and apoptosis. The biological effect of MBVs, however, have not been fully investigated or shown.

To investigate the effect of Matrix Bound Nanovesicles in catalyzing the regenerative response, their ability to modulate macrophage activation was considered. Macrophages are phagocytic immune cells found as either stationary cells in tissue or as mobile white blood cells. Macrophages can be activated to either a proinflammatory (M1) state or a proregenerative (M2) phenotype [3]. It is important to note that the promotion of a regenerative response does not require that all macrophages present are activated to an M2 phenotype. The M1/M2 ratio of macrophages should be less than one for the proregenerative response to predominate [3].

Matrix Bound Nanovesicles, like other extracellular vesicles, carry biologically functional cargo including proteins, microRNA, and growth factors. MBVs are cell derived and can only be isolated from the extracellular matrix after enzymatic digestion of the matrix [2]. It is hypothesized that the cargo included in MBVs, allow the nanovesicles to act as a mechanism by which the extracellular matrix is able to promote a regenerative response.

After Matrix Bound Nanovesicles were first discovered by the Badylak Laboratory, a preliminary experiment was done to see if treating macrophages with MBVs would mimic the response of macrophages treated with the extracellular matrix [2]. When stained for certain M1 and M2 markers the macrophages treated with MBVs showed the same response as those treated with the parent ECM [2].

OBJECTIVE
After this preliminary experiment showed promising results it was decided to further investigate the effect of Matrix Bound Nanovesicles on the immune response. More specifically, my project was to determine if MBVs are able to recapitulate the effect of the corresponding parent extracellular matrix on the immune response of murine bone marrow derived macrophages.

HYPOTHESIS/SUCCESS CRITERIA
Due to the results of the preliminary experiment it was hypothesized that the Matrix Bound Nanovesicles would be able to recapitulate the effects of the extracellular matrix. For this to be considered true the gene expression of the macrophages treated with MBVs would have to be the same or extremely similar to the gene expression of the macrophages treated with the corresponding ECM.

METHODS
To study the effects of Matrix Bound Nanovesicles on macrophage activation we decided to look at gene expression, which would be visualized through quantitative PCR and immunolabeling.

The macrophages were treated for twenty-four hours with either a control or testing group. The controls included: Interferon-γ and Lipopolysaccharide (M1), Interleukin-4 (M2), a pepsin digest, collagenase, and maturation media. The testing groups included: Matrix Bound Nanovesicles isolated from either the urinary bladder matrix (UBM) or small intestinal submucosa (SIS) and extracellular matrix hydrogels made from either UBM or SIS.

After the twenty-four hour treatment period, the macrophages were either harvested as cell lysates with Trizol or fixed to cell culture plastic with paraformaldehyde. The cell lysates were taken and RNA extraction protocol was performed. The RNA was then used to synthesize cDNA to use for qPCR. The cDNA was analyzed with qPCR for approximately 30-40 transcription factors, surface and metabolic markers. The fixed cells were then stained to determine the presence of several M1 or M2 markers. After immunolabeling, the fixed macrophages were imaged using a fluorescent microscope. They were stained for a pan macrophage marker F4/80, two M1 markers, TNFα and iNOS, and two M2 markers, Arginase 1 and Fizz 1.

RESULTS
Shown below are heat maps that show the gene expression of either M1 and M2 associated surface markers and transcription factors or M1 and M2 associated metabolic markers. All results are compared to macrophages treated with
maturation media. The scoring system is based off of fold changes from the maturation media treatment group. The different shades of yellow and purple show the differing degree of fold changes (i.e. the darker the shade the more significant the fold change). Yellow denotes up regulation of gene expression due to treatment and purple denotes down regulation of gene expression due to treatment.

**Figure 1:** The gene expression of M1 and M2 associated surface markers and transcription factors.

In the next column the immunolabeling results are displayed. If the cells are illuminated green then the staining is positive for the marker to the left.

**Figure 3:** Staining for M1 and M2 associated markers. (A) Maturation media, (B) Pepsin, (C) Collagenase, (D) M1, (E) M2, (F) UBM, (G) MBVs UBM, (H) SIS, (I) MBVs SIS

**DISCUSSION**

As shown in all three figures the macrophages treated with Matrix Bound Nanovesicles mimicked the gene expression of the macrophages treated with extracellular matrix almost exactly. This allows us to conclude that in terms of gene expression, MBVs are able to recapitulate the effects of ECM on bone marrow derived macrophages.

There is still future work to be done with this project including the western blots to determine protein expression and functional assays. Once the effect of MBVs is further determined they can be used clinically to treat injuries previously to hard to reach with the ECM. The limitations of this study is that the experiments were completed in vitro with murine cells rather than human. Clinically this means that when applied to the human body there may be different effects.

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**REFERENCES**

