Background. Tissue engineering and regenerative medicine aims to use scaffolds, cells, and signals to replace tissue lost as a result of trauma or pathology [1,2]. Recognizing the vital role of a tuned microenvironment in tissue repair, biomaterials are now being designed with the addition of bioactive molecules to mimic the native environment by simultaneously providing both structural support and cell-instructive signals. These design considerations support the recruitment of endogenous cells to create a pro-regenerative microenvironment.

In addition to the rapid development of synthetic scaffolds, the use of xenogeneic decellularized extracellular matrix (ECM) has offered an elegant alternative for efficient tissue repair, owing primarily to its inherently instructive signals [3,4]. ECM biomaterials have shown success in tissue repair applications, at least in part by modulating the immune response [4]. However, despite decades of commercial use and preclinical studies, the mechanisms by which ECM can facilitate tissue repair are not well understood. A general class of signalling molecules called **damage-associated molecular patterns** (DAMPs) are released during injury (e.g., free nucleotides, heat-shock proteins (HSPs), high-mobility group box (HMGB) proteins, cytoskeleton components, and ECM fragments). Of particular importance, ECM-derived biomaterials have also been shown to contain and release DAMPs, leading to improved therapeutic outcomes via immune modulation [4]. For example, previous work has demonstrated that HMGB1 is present in ECM derived from porcine urinary bladder matrix (UBM), skin, and small intestine submucosa (SIS) and its presence increases the pro-inflammatory response [3]. Another DAMP, prostaglandin 2 (PGE-2), has also been shown to regulate the immune response [5]. However, this work is still in its infancy and ongoing research of ECM-derived DAMPs is important. Combining the ability of DAMPs in regulating immune responses with biomaterials for tissue repair represents a novel approach to facilitate regenerative processes. **Therefore, the work proposed aims to determine the role of DAMPs in ECM scaffold-mediated inflammation and tissue remodeling.**

Significance. The immune system is crucial for development, regeneration, and repair. In turn, inflammation and the immune response are key regulators of biomaterials-mediated tissue regeneration [1,2]. Temporal cellular presentation of DAMPs is a protective mechanism, inciting inflammation to clear cellular debris and fend off foreign invaders. A well-coordinated inflammatory response is necessary for wound healing and tissue repair. However, the continued presence of DAMPs can exacerbate the inflammatory response, thus contributing to disease development and fibrosis. Although the roles of DAMPs in autophagy and cancer have been widely studied, little is known about their interactions with biomaterials. **The proposed study will determine the role of DAMPs in tissue repair processes and will inform new biomaterials strategies for inductive tissue repair.**

Approach. The role of DAMPs in modulation of the immune response, particularly exogenous DAMPs released from tissue-derived biomaterials is not clear. **The experimental plan will methodically address the hypothesis that DAMPs are necessary for immunomodulatory and tissue regeneration outcomes with ECM biomaterials.**

<u>Aim #1:</u> To measure the quantity of HSP70, PGE-2, and CRT from commercial ECM. Since HMGB1 has been isolated from ECM scaffolds, I will measure HSP70, PGE-2, and calreticulin (CRT) from three sources of ECM by Western blotting and ELISA-based methods. These DAMPs have postulated roles in immune activation, tissue remodeling, and immunomodulation [3,5]. UBM, skin, and SIS are common tissue sources for surgical applications of ECM and can be used for this work [4]. Further evaluation of the materials will be dependent on the DAMPs identified.

Aim #2 To characterize the macrophage and dendritic cell response to HSP70, PGE-2, and CRT *in vitro.* A collagen type I 2D substrate at two different concentrations (1 mg/mL and 5 mg/mL) will be used to study the effect of the DAMPs on macrophages and dendritic cells. Primary human immune cells will be isolated by positive selection and seeded on collagen substrate with varying concentrations of soluble DAMPs. Flow cytometry will be used to study the phenotypic changes of the immune cells and reactive oxygen species (ROS) levels. The redox status of the microenvironment influences whether DAMPs have an inflammatory or anti-inflammatory role. A commercially available ROS-sensitive dye (DCFDA, Invitrogen) will be used to study the ROS activity in the cells. Gene expression will be studied using qRT-PCR analysis of inflammation markers (*TNFα, IL-15, IL-18, CXCL12, and CXCR4*) and markers to identify the macrophage phenotypes and dendritic subset. Information from qRT-PCR will inform which proteins are most relevant and regulated to analyze with Western blotting and ELISA. Beyond this *in vitro* system, *in vivo* studies investigating the effect of DAMPs-supplemented collagen scaffold on a critically sized skin defect could provide further insight into DAMPs role in the immune response and tissue remodeling. Outcomes could include percent and rate of wound closure, immunolabeling for immune response markers, histology of remodeled tissue, and PCR to compare gene expression profiles.

Potential pitfalls and alternative strategies: The decellularization techniques used in the first aim may impact the retention of DAMPs. The relative quantities of DAMPs will first be compared to the source tissue prior to decellularization techniques. Alternative approaches would be to use more mild decellularization techniques. Using different sources of commercially available ECM could also be another option. The project could also focus on other widely studied DAMPs, such as serum amyloid A (SAA) or S100A8 (MRP8, calgranulin A). It is possible that the DAMPs may not produce a reproducible immune response. In this case, a cell line, such as THP1 cells, which are commonly used to assess immune responses, could be used to minimize inherent donor variability from primary cell isolates. It is feasible that DAMPs may work in concert, so combinations of DAMPs could be tested to measure if additive effects are observed with multiple DAMPs.

Broader Impact and Conclusions. Commercially available ECM biomaterials have varying decellularization and sterilization processes that contribute to the content of intracellular remnants (e.g. DAMPs) [4]. This is particularly important because DAMPs regulate immune function and ultimately tissue repair. The desired immune response may differ from patient to patient, thus some decellularization and sterilization processes may be favorable over others. Although ECM has been used clinically and is easily accessed, it has not been widely accepted. With the proposed research, further ECM characterization will help give physicians more confidence when choosing between ECM biomaterials. Success of the project will be based on the knowledge added to DAMP characterization in ECM biomaterials and the skills learned. The proposed work aligns with the research groups of Professors [redacted] and [redacted]. This project is a natural progression from my previous involvement in a project related to characterizing scaffolds for wound healing applications with a client from [redacted] and my current research using many of the proposed analysis techniques. In short, my proposed research will focus on characterizing DAMPs and their role in mediating immune response for improved biomaterial design. ***References.*** [redacted]