1

The Summer Undergraduate Research Fellowship (SURF) Symposium 6 August 2015 Purdue University, West Lafayette, Indiana, USA

Viewing the Extracellular Matrix: An imaging method for tissue engineering

Michael A. Drakopoulos, Sarah Calve Department of Biomedical Engineering, Purdue University

ABSTRACT

The field of regenerative medicine seeks to create replacement tissues and organs, both to repair deficiencies in biological function and to treat structural damage caused by injury. Scaffoldings mimicking extracellular matrix (ECM), the structure to which cells attach to form tissues, have been developed from synthetic polymers and also been prepared by decellularizing adult tissue. However, the structure of ECM undergoes significant remodeling during natural tissue repair, suggesting that ECM-replacement constructs that mirror developing tissues may promote better regeneration than those modeled on adult tissues. This work investigated the effectiveness of a method of viewing the extracellular matrix of developing embryos. Embryos were perfused with acrylamide, polymerized, and decellularized with sodium dodecyl sulfate (SDS) in phosphate-buffered saline (PBS). Cell nuclei were stained with a fluorescent DNA-binding compound to verify decellularization. Wholemount embryos were stained and used to verify that the overall structure of the ECM was not altered. Antibody staining was combined with fluorescent confocal microscopy to determine the presence of fibrillin-2 (FBN2), fibronectin (FN), tenascin-C (TNC), and collagen-VI (COL6). This study demonstrated that while the results of SDS decellularization depend on SDS concentration, embryonic stage, the protein under consideration, and the region of the embryo examined, FBN2 and FN tended to maintain their natural structure and to become more visible with treatment, while TNC and COL6 tended to be disrupted and removed. Future efforts to develop tissue-replacement constructs may benefit by using this method to examine, guantify, and mimic the structure and composition of specific proteins of developing ECM.

KEYWORDS

Extracellular matrix, confocal microscopy, tissue engineering, regenerative medicine, decellularization, antibody staining, embryo, development, fibrillin, fibronectin, tenascin, collagen