Surface Characterization by SIMS and SEM of Decellularized Porcine Extracellular Matrix Scaffolds

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Statement of Purpose: Regenerative medicine has been proposed as a solution to the shortfall of available donor tissues for patients in need of transplant. Much research has gone into the creation of various scaffold designs for use in regenerative medicine applications. One of the most successful scaffold based approaches has been the decellularization of tissues and organs of both xenogeneic and allogeneic origin. The extracellular matrix (ECM) which comprises these materials is complex and dense with signaling information embedded within the structure of its component proteins. Characterization of these materials is difficult due to their inherent complexity. Time-of-flight secondary ion mass spectrometry (ToF SIMS) has been used previously to characterize the surfaces of biomaterials, protein films, and various types of cultured cells. Here, ToF SIMS has been extended to the characterization of a set decellularized ECMs of porcine origin. For this work, ToF SIMS is used to characterize the differences between the surfaces of the following decellularized ECMs: small intestinal submucosa (SIS), urinary bladder matrix (UBM), and liver ECM (LECM). By applying principal component analysis (PCA) to the ToF SIMS data, the various materials were distinguishable and a series of mass fragments (from the PCA loadings) could be assigned to the differences between samples. An assessment of differences created by chemical cross-inking was performed by comparing UBM crosslinked with both carbodiimide and glutaraldehyle to non-crosslinked material. Finally, segments of decellularized porcine adipose tissue (DFAT) were analyzed. Spectra from DFAT were also compared to spectra obtained from the SIS, UBM, and LECM samples.

Methods: SIS and UBM were prepared by mechanical delamination of the tissue followed by an osmotic cell bursting rinse in saline and disinfection with 0.1% peracetic acid in 4% ethanol. LECM was prepared by dissection into slices and enzymatic cell removal followed by a detergent rinse. Crosslinking of UBM was done in 10mM carbodiimide or 0.625% glutaraldehyde for 8-10 hours at room temperature. DFAT was prepared from pork trimmings using 0.02% trypsin/0.05% EDTA followed by Triton-X 100 then 4% deoxycholic acid and finally 0.1% peracetic acid in 4% ethanol. SEM was performed using a JEOL 6330F field emission gun SEM with an accelerating voltage of 3 kV. ToF SIMS spectra were acquired on an ION-TOF ToF.SIMS 5-100 spectrometer using a 25 keV Bi+ ion source in the pulsed mode, at a pulse width of approximately 2 ns. PCA was performed on normalized spectra using in-house scripts developed at NESAC/BIO written for Matlab.

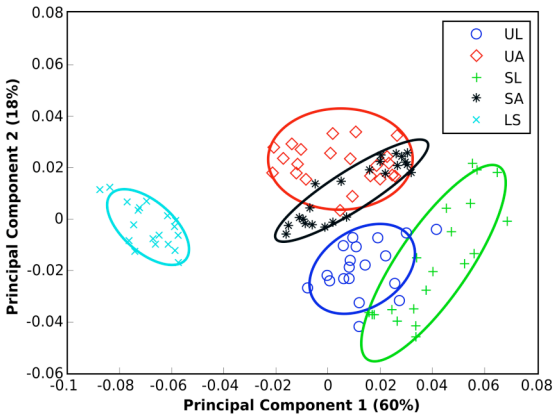

Figure 1: PC 1 vs. PC 2 derived from the normalized ToF SIMS data taken from the surfaces of UBM luminal and abluminal (UL and UA), SIS luminal and abluminal (SL and SA) and LECM (LS)

Results: SEM images of the surfaces of the UBM (both sides), SIS (both sides), and LECM scaffolds showed distinct structural differences in the fibrillar organization of all three samples types. Additionally, fibers in the crosslinked UBM surfaces appeared to group together differently depending on the method of crosslinking. ToF SIMS with PCA was used to distinguish between the surface chemistries of the three sample types (Figure 1). Additionally, variable surface chemistries were seen between the crosslinked UBM materials suggesting that crosslinking could have an effect on seeded cells stemming from surface chemistry. Lipid structures were seen on the surfaces of the UBM and SIS samples. Finally, the ToF SIMS spectra of DFAT was compared to the spectra obtained from UBM and SIS and differential lipid signatures were seen.

Conclusions: ToF SIMS has been used in previous studies to characterize inorganic and organic materials alike. This study represents the first application of ToF SIMS and PCA towards the analysis of the surfaces of decellularized ECM scaffolds. PCA was used to identify differences between spectra from SIS, UBM, and LECM. Additionally, crosslinked UBM scaffolds were differentially characterized showing surface chemistry differences attributed to the crosslinker used. Finally, lipid fragments were found on the surfaces of SIS and UBM and were compared with lipid fragments from the decellularized porcine adipose tissue matrices and found to have unique ToF SIMS fingerprints.