# Mass Spectral Imaging for Analysis of Tissue Scaffolds

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**Statement of Purpose:** Biologically relevant samples often contain significant topography. This is particularly true of tissue engineering scaffolds that are typically made of porous polymer materials. In order to design better scaffolds and understand how to optimize tissue growth within these scaffolds, it is of interest to understand how cells interact with these materials on a chemical level. The imaging mass spectrometry technique of time of flight secondary ion mass spectrometry (ToF-SIMS) is an ideal candidate for this. However it is very difficult to analyze materials with significant topography such as porous scaffolds using ToF-SIMS. Furthermore, due to the typical size of the pores in these materials (10 to 100 microns in diameter) one must be able to depth profile through large amounts of material. This presents additional challenges due to the limited extraction depth of the current instruments. In addition, with current instrumentation, depth profiling deep into materials (>~2 microns) can cause significant lateral shifts in the images due to changes in the location of the primary ion beam impacts due to the relative height change of the sample.

We are developing methods for dealing with topographically challenging samples in ToF-SIMS analysis. This will be done using the novel idea of embedding the samples to minimize the topography. By filling the voids in the samples, the surfaces will become more uniform in structure and flatter. This will reduce or eliminate artifacts created by surface topography and enable 3D depth profiling. The advent of the argon cluster sources has enabled deep depth profiles with ToF-SIMS. We have developed methods for compensating for the lateral shifts discussed above, which will enable deep depth profiles that will be required to adequately characterize porous materials where the pores can be 10 to 100 µm in diameter.

**Methods:** Scaffolds created by microsphere templating [1] are used in our initial studies. These scaffolds are formed by scintering monodisperse microsphere beads and backfilling the voids using the polymer of choice for the scaffold, curing the polymer, and then extracting the beads. The resulting pores are uniform in both size and distribution and the size of the interconnects. Sputter depth profiles for 3D analysis were carried out using Argon clusters as the sputter source and a Bi liquid metal ion gun (LMIG) for the analysis beam. A new strategy has been developed to adjust for lateral shifts during profiles.

In order to avoid issues with topography caused by the pores within the scaffolds, we have investigated embedding the scaffolds in OCT cryofixant (a technique used for ToF-SIMS analysis of tissue biopsy samples). Cell seeded versions of the scaffold are also used to test our technique.

**Results:** Initial studies were carried out on a scaffold created using 50 micron PMMA beads backfilled with PHEMA. For this work the PMMA beads were left in place to simulate embedding the scaffold and filling the voids. The sample was depth profiled using Argon 1000 clusters using a sputter rate of approximately 1 micron per sputter cycle for 39 cycles. We show that a 3D mass spectral map of the sample can successfully be reconstructed for at least 40 micron depth of the sample. Figure 1 shows a two color overlay with green representing the PHEMA scaffold by following m/z 59 ($C_2H_3O_2$) and red representing the PMMA beads by following m/z 55 ($C_3H_3O$).

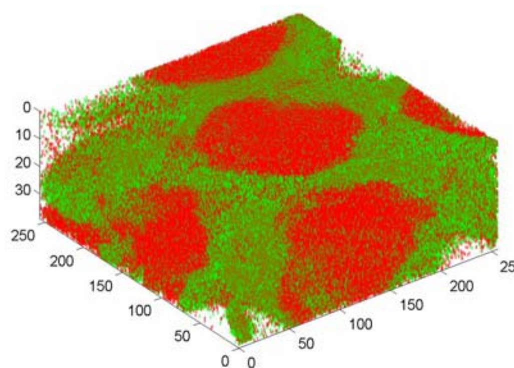


**Figure 1.** ToF-SIMS 3D reconstruction of PHEMA scaffold (green) with PMMA beads (red). (scale bars are in microns)

**Conclusions:** Our work with the polymer scaffolds has a two-fold result. We gain further insight into scaffold design for optimal tissue growth and, with the analysis of the cell-seeded scaffold, learn about the cell/scaffold interactions with potential information about the extracellular matrix that may result with different types of cells. We also gain further insight into various methods to potentially achieve a 3D ToF-SIMS analysis of biopsied tissue samples.

**References:** (1) Marshall, A.J. and B.D. Ratner, Aiche Journal, 2005. 51(4): p. 1221-1232. The authors thank Tom Long (UW BioE) for preparing the pHEMA scaffold sample.


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