# Imaging Mass Spectrometry As A Novel Method of Characterizing Glycosaminoglycans in Microvasculature.

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**Abstract:**

Glycosaminoglycans (GAGs), including Heparan Sulphate (HS) are linear polysaccharides which play an essential role in the permeability of microvasculature, and are responsible for the dysregulation of vascular permeability in diseases such as diabetes. *In situ* detection and analysis of Gags has largely been limited by the availability of appropriate methodologies. Current methods use specific antibody binding sites, which are poorly applicable to tissue partly due to steric exclusion. Time of flight secondary on mass spectrometry (ToF-SIMS) is a spatial technique with optimal precision, which has been shown to differentiate GAGs in purified samples (*Hook et al., 2021).* Here we present the development of the technique via HS knockouts in cell culture to renal biopsies, to achieve label free GAG detection. We demonstrate that both Orbi-trap-SIMS and ToF-SIMS can be used in a diagnostic manner in 3D culture and tissue samples, to spatially resolve the expression of both commonly associated ions for GAGs and HS in mouse glomeruli. Orbi and ToF-SIMS work in conjunction to provide a high mass resolution and high spatial resolution, respectively, allowing for extremely detailed spatial analysis of complex biological samples. SIMS data was collected, complementing electron microscopy and fluorescent staining of wild type, diabetic and Alport Syndrome mouse kidneys, allowing for direct comparison of the GAG content of glomeruli in healthy and diseased tissue. The diabetic and Alport Syndrome mice showed the expected reduction and increase in glycocalyx depth (by WGA), and associated basement membrane changes (by electron microscopy). We provide evidence that secondary ion mass spectrometry can be used as a novel method of *in situ* spatial analysis of common GAGs and HS in diseased tissue. While the signal-to-noise from most preparations is not yet high enough for true optical resolution analysis, cryogenic methods are likely to improve this. Further work aims to identify ions characteristic of other specific GAG families, and to use SIMS on human pathological biopsies. The technique, with correct reference samples and with increased sensitivity due to cryogenic preparation techniques, may be able to determine changes to GAGs that a spectrum of antibodies could never achieve in tissue.

**References:**

Hook, A.L., Hogwood, J., Gray, E. *et al.* High sensitivity analysis of nanogram quantities of glycosaminoglycans using ToF-SIMS. *Commun Chem* **4,** 67 (2021)