“High resolution chemical surface imaging for the characterisation of biomaterials”

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Statement of Purpose: A biomaterials mechanical properties are defined by its underlying chemical and structural relationships. Local variations in properties determine cell growth propensity. Novel surface chemical spectroscopy and imaging methods on nano- and micro-scale levels have been developed in response. Biocompatibility of biomaterials is dependent on functional groups expressed and local variations in molecular order through their contributions to a materials mechanical properties. Established averaging methods for the estimation of molecular order and chemical functional groups do not provide information of their spatial distributions within the biomaterials. An easily applied spectroscopy method that reveals local functional group variations at the nano- and micron scale on the surface of biomaterials would be transformative. One of the most widely employed electron microscopes is the Scanning Electron Microscope (SEM) in the Secondary Electron (SE) imaging mode. Here we demonstrate how SE Hyperspectral Imaging (SEHI) is applied to map; functional groups, polymer cross-linking, surface and bulk oxidation, and localised molecular variations within beam sensitive biomaterials. SEHI was used to analyse poly(glycerol sebacate) methacrylate (PGS-M) surfaces post industry standard autoclave sterilisation and also equivalent PGS-M surfaces resulting from the application of low-pressure Argon glow discharge. This analysis was performed to explore the application of Argon plasma as a potential biomaterial sterilisation method. **Methods/Materials:** The PGS-M materials were either enclosed in a gas semi-permeable bag and exposed to low-pressure argon (AR) glow discharge or placed into an Autoclave. A Scanning Electron Microscope (FEI Nova Nano 450 SEM) was employed to observe the surface morphology of the PGS-M surfaces post treatment. Surface charging and consequent damage to the sample was avoided by using a low accelerating voltage (1 KV). A typical vacuum pressure of 10^{-5} mbar at a working distance of 3 mm was applied. A voltage controlled deflector electrode and a through lens detector is integral to the FEI Nova Nano 450 SEM with the deflector electrode channelling the signal into the SE detector. A predetermined number of deflector voltages are set for the deflector electrode and an image is generated for each deflector voltage. Post-processing of the image series results in Spectra and hyperspectral images.

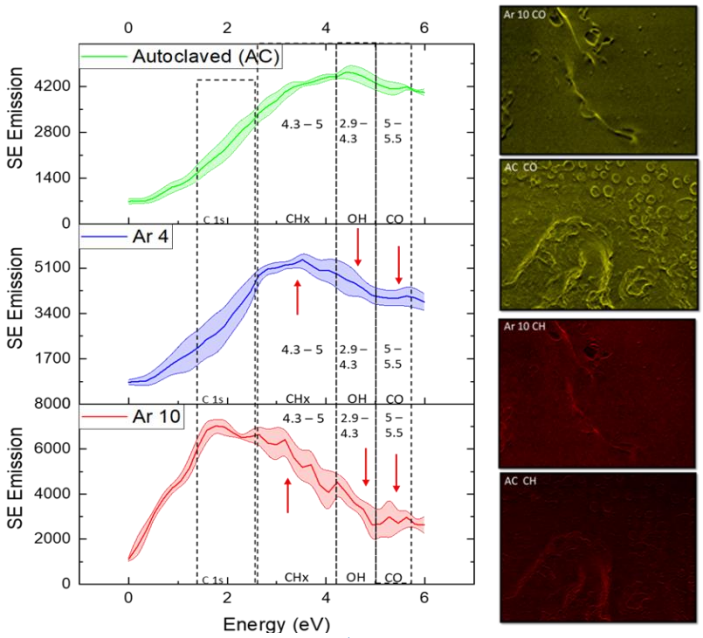

Figure 1: SE Spectra and SEHI images (CO /CH) of Argon plasma treated and Autoclaved PGS-M. Reproduced in CC-BY licence from DOI:10.1002/advs.202003762

Results: Figure 1 shows the SE spectra and resulting SEHI images of AC PGS-M, 10-minute Argon plasma treated PGS-M (Ar 10) and 4-minute Argon plasma treated PGS-M (Ar 4). Changes within the SE spectra for peak emissions associated with (CHx, OH and CO) chemical bonding were present within the test samples. An example of The ability of SEHI to characterise chemical functional groups is shown in the SE spectra for Argon plasma treated and AC samples observed emissions within the region of 5-5.5 eV, related to C=O bonding. The spectra shows significantly reduced emissions in the 5 – 5.5 eV range for post Argon plasma treatment. Argon plasma treatment is known to cleave away C-O-C bonds attached to the methacrylate within PGS-M. By cleaving away this bond, removal of methacrylate decreases the amount of C=O bonds present within the polymer. It can be observed that the decrease in C=O bonding is greater in the Ar 10 compared to the Ar 4 samples suggesting that the cleavage of methacrylate units is time and region dependent. Mapping the SE emission differences and evaluating the results it is evident that a decrease in CO emission is visible in Ar 10. X-ray photoelectron spectroscopy (XPS), a well-established surface analysis tool, was employed to corroborate the findings. It can be seen that SEHI offers efficient mapping at multi-length scales of functional groups with high image resolution.