

**Physicochemical characterisation of advanced hydrogels for oral biofluid sampling for the analysis of epigenetic and inflammatory biomarkers.**

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**Background:** The sampling of biofluids for the analysis of epigenetic and immunological markers provides valuable information for studying the onset of a disease, its progression and monitoring therapeutic interventions. However, the current sampling techniques for oral biofluids, specifically gingival crevicular fluid, is limited in both quality and volume of biomarkers obtained. A range of advanced biocompatible hydrogels have been designed and characterised to demonstrate their potential in standardising biofluid sampling technique, eliminate variability in biofluid volumes and concentrations of biomarkers.

**Methods:** Hydrogels were formulated from aqueous blend of polymers, plasticizers and salts. To make the prototype sampling devices, formulations were casted in silicone moulds made from 3D printed master moulds. The formulations were dried at 25°C for 48 hours and heat crosslinked at 80 °C. The rheological characteristics of the aqueous blends were compared. Characteristics of their swelling capacity in phosphate-buffered saline at time points 5 s to 24 h was analysed. Subsequently, texture profiles and surface properties were also evaluated using a texture analyser and scanning electron microscope and microCT imaging. Data were analysed separately as groups with either Welch's t-test (for two in a group) or one-way ANOVA followed by a Tukey post hoc test.

**Results:** The shear modulus of the aqueous blend of candidate hydrogels were between 300 to 1700 Pa. The swelling behaviour of the formulations were grouped into three categories based on their percentage increase in weight after 24 hours in PBS pH 7.4 (moderate swelling 100 to 1000 %, intermediate swelling 250 to 1000 % and super swelling 1000 to 3000 %). The brittleness of candidate formulations was exhibited by the distance at break during the texture analysis. The least brittle formulation had a distance at break of  $1.974 \pm 0.1282$  mm (n=5). The surface characterization revealed solid dense material with interspatial resolution < 7.4  $\mu$ m indicating the potential to uptake analytes and biomarkers of interest into their network.

**Conclusions:** Hydrogels formulated exhibited desirable characteristics for the sampling of oral biofluids for the analysis of epigenetic and inflammatory biomarkers with the potential to uptake specific volumes of biofluid and concentrations of biomarkers. Target product profile is currently under co-design with dental clinicians to identify key characteristics. Prototype devices will now be developed and tested for proof of concept.