Project: Effects of dentine extracellular matrix components (dECMs) on mesenchymal stem cells derived from human periapical lesions (PL-MSCs) - an in vitro study

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Aims: Firstly, to characterise dECMs isolated from extracted teeth using a clinically translatable irrigation regime; secondly, to characterise the cells extracted from human periapical lesions of teeth diagnosed with apical periodontitis.

Methods: Fifteen millilitres of 17% EDTA was collected following its administration, via conventional needle irrigation and 60 seconds passive ultrasonic activation, into the standardised root canals of ten extracted single rooted teeth. Solutions were then concentrated to 1 mL by way of centrifugation using a 3 kD filter. The total concentration of protein and up to 40 analytes in samples normalised to volume (1 mL) and total protein concentration (250 µg/mL) determined using the Bradford dye-binding assay, Raybiotech Quantibody Growth Factor Array and TGFβ, VEGF and BMP2 sandwich ELISAs. Primary PL-MSCs were isolated from inflamed periradicular granulomas of teeth diagnosed with apical periodontitis via an enzyme digestion technique. Fluorescence activated cell sorting (FACS) analysis was used to confirm that the cells isolated in this experiment expressed an immunophenotype that was characteristic of a MSCs lineage using monoclonal antibodies for CD105, CD90, CD73, CD45, CD34 and an IgG1 isotype control. Results were presented as medians ± interquartile ranges.

Results: From the collected samples of EDTA, the total protein concentration was 802 ± 328 µg mL^{-1}. The Quantibody array revealed the presence of 38 analytes above the minimum detection limit and 18 above the minimum quantification limit. The most abundant analytes included insulin growth factor binding protein-3 (1474 ± 1415 pg mL^{-1}), transforming growth factor beta-1 (1143 ± 750 pg mL^{-1}), insulin growth factor binding protein-1 (565 ± 221 pg mL^{-1}) and bone morphogenetic protein-4 (457 ± 317 pg mL^{-1}). Isolated primary cells from the granulation tissue of teeth diagnosed with apical periodontitis were found to display a high abundance of surface markers indicative of mesenchymal lineage (i.e., CD105, CD90, CD73) but not those indicative of haematopoietic lineage (i.e., CD45, CD34).

Outstanding Aims: Determine the dose dependant effects of dEMCs on the viability, proliferation, migration, and osteogenic differentiation of PL-MSCs.

Related Publications:

- **Virdee SS**, Bashir NZ, Camilleri J, Cooper PR, Tomson PL (2021) Exploiting Dentine Matrix Proteins in Cell-Free Approaches for Periradicular Tissue Engineering, *Tissue Engineering: Part B Reviews,* [*Online ahead of print]*.

- **Virdee SS**, Ravagi V, Camilleri J, Cooper PR, Tomson PL (2020) Current trends in endodontic irrigation amongst general dental practitioners and dental schools within the United Kingdom and Ireland: a cross-sectional survey. *British Dental Journal,* [*Online ahead of print]*.