# Mineral composition of hypothermally induced ankylosis in rat molars

A report submitted in Partial Fulfilment of the requirement for the degree of Doctor of Clinical Dentistry (Orthodontics)

By

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### 1.4.1 General

| | |
|---|---|
| H&E | Haematoxylin and eosin |
| VK/H&E | Von Kossa counterstained with haematoxylin and eosin. |
| IMVS | Institute of Medical and Veterinary Sciences |
| EPMA | Electron Probe X-ray Microanalysis |
| EDS | Energy Dispersive X-ray Spectrometry |
| WDS | Wavelength Dispersive X-ray Spectrometry |
| INAA | Instrumental neutron activation analysis |
| BSE | Backscattered electron imaging |
| PDL | Periodontal ligament |
| ID | Identification number |
| SE | Standard error |

### 1.4.2 Units of measurements

| | |
|---|---|
| µm | micrometres |
| px | pixel |
| wt% | percentage weight |

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## 3. SIGNED STATEMENT

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Albert Leung and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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## 4. SUMMARY

This study used Backscattered electron imaging (BSE) and X-ray Microanalysis to qualitatively and quantitatively investigate morphology and elemental composition of ankylotic adaptation in the periodontium following hypothermic insult to their maxillary first molars. This method has been shown in previous studies to induce aseptic root resorption along with ankylotic changes within the periodontal ligament (PDL). A secondary objective was to assess the pulpal changes that occurred concurrent with the changes in the periodontium.

Twenty-eight eight week old Sprague Dawley rats were divided into four groups of seven animals corresponding to one of four observation periods i.e.: $t_1$= 7 days, $t_2$= 14 days, $t_3$= 21 days, $t_4$= 28 days. At t=0 days, six animals in each group received a thermal insult as a continuous 20 minute application of dry ice ($CO_2$ at $-81^0$C) to the crowns of their upper right maxillary molar. The untreated left molars were used as controls. The remaining rat within each group did not receive the dry ice. All rats were given two sequential bone labels, calcein 5mg/kg and alizarin red 30mg/kg, administered intraperitoneally 8 days apart. The timing of the labels was such that all rats were euthanased 2 days after the last label. Following sacrifice, the maxillae were dissected out, fixed in ethanol and embedded in methylmethacrylate. Ten microns thick, undecalcified maxillary first molar coronal sections through the furcation were obtained. For every 3 out of 10 sections: the first was left unstained and undecalcified; the second stained with Von Kossa/haematoxylin & eosin; and the third decalcified and stained with haematoxylin & eosin. Unstained sections were viewed under fluorescence, while transmitted light

microscopy was used for the other sections. Following initial analysis, the unstained, un-decalcified sections were de-coverslipped and carbon coated. These sections were investigated with scanning electron microscopy and Energy Dispersive X-ray Spectrometry (EDS). Quantitative spot analysis and element mapping was performed on alveolar bone, ankylotic areas, cementum and dentine. A linear mixed effects model was employed to investigate any interaction between the four tissues of interest.

A focal pattern of ankylosis was observed at days 14 and 21 in three rats. No ankylosis was observed in the control teeth. SEM revealed a focal type of ankylosis with central nodules of mineralized tissue forming within the PDL. Bridging between bone and dentine occurred with fine trabeculae which extended from the central mineralized nodule. Bridging was progressive and was more extensive at day 21 compared to day 14. At day 28, no ankylosis was observed. EDS analysis revealed that the ankylotic tissue was composed of major constituents Calcium and Phosphorous along with trace elements of Mg and Na. This was comparable to the surrounding alveolar bone, cementum and dentine. There was no statistically significant difference in the Ca/P ratios, Mg when ankylotic material was compared to bone. There was a trend towards elevated Na levels in ankylosis but this was not statistically significant relative to bone. Mg in dentine was lower than for all the other tissues and Na was higher in dentine when compared to bone and cementum.

In the pulp, hypothermic injury demonstrated alteration of the odontoblast layer, reduction in cellularity, vascular alterations and tertiary dentin formation. At the 28 day observation period, the cellular and vascular changes had returned to levels comparable

to the control teeth, indicating successful pulpal healing and regeneration. Marked tertiary dentine deposition was also observed at days 14, 21 and 28. Pulp chambers were visibly smaller due to tertiary dentine formation, however no pulp necrosis was observed. Thus the aseptic root resorption model, using a continuous 20 minute application of dry ice, suggested the occurrence of reversible pulpal tissue alterations compatible with an inflammatory repair process.

The observation of ankylosis initiating as centralised nodules within the PDL suggest that the origins may be a consequence of osteogenic potential from PDL stem cells. The null hypothesis that a single, prolonged thermal insult on a rat molar does not have an effect on mineralized tissue formation and that ankylotic tissue is similar to bone was rejected.