Abstract #1: Survey of Irrigation Protocols Used by Dentists in British Columbia

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Objectives: The study aimed: 1) to investigate the type, frequency and order of use of irrigants and irrigation techniques used in the chemical cleaning of the root canal, and 2) to compare how the aforementioned treatment modalities are used by general practitioners and endodontists.

Methods: The survey included general dentists (GP) and endodontists (ENDO) in the province of British Columbia, Canada. Following a telephone call, a two-page questionnaire was faxed to 150 GPs and to all 42 endodontists in British Columbia, containing 49 questions about the type of irrigants used in the case of vital pulp (VP) and non-vital pulp (NVP), the concentration of sodium hypochlorite (NaOCl), the volume of various irrigants, the type and size of the irrigation needle, depth of the irrigation needle tip in the canal, and the initial and final irrigant used during endodontic treatment. The data was analyzed using one way ANOVA with post Hoc Bonferroni adjustment.

Results: The overall response rate was 79.2%. Different irrigants in treating NVP were used as follows: sodium hypochlorite: ENDO 95.74% and GP 94.22% (P= 0.657); EDTA: ENDO 76.24% and GP 36.23% (P=0.000); chlorhexidine: ENDO 34.06% and GP 14.66% (P=0.005); and RC-Prep: ENDO 31.97% and GP 71.98% (P=0.000). There were no significant differences among the two groups of dentists with regard to other solutions such as MTAD, Smear Clear, citric acid, alcohol, saline and water. Group ENDO used higher concentrations of NaOCl than group GP (P=0.000).

Conclusions: NaOCl was the most common irrigant used by both groups, while EDTA was used mainly by endodontists.

Abstract #2: The Crystal Structures of Chondroitin and Dermatan Sulfate-Cathepsin K Complexes

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Objectives: Cysteine proteases such as cathepsin K (catK) are involved in bone resorption via a process of degradation of triple helical collagen. Crucial to this activity is the formation of a complex with glycosaminoglycans (GAGs) such as chondroitin sulfate (C4-S) and its epimer dermatan sulfate (DS). Biochemical analysis has revealed that these GAGs promote collagenolysis at equimolar concentrations with catK but inhibit it at higher concentrations. Here we use X-ray crystallography to determine the nature of the catK:GAG complexes.

Methods: C4-S and DS were digested with Hyaluronidase enzyme and purified via gel filtration. 8.4 kDa sized fractions of either GAG were mixed in a1:1 molar ratio with purified recombinant catK and concentrated for crystallization using the sitting drop vapour diffusion method. Crystals of catK:C4-S and catK:DS complexes were allowed to grow over two weeks and then flash frozen in liquid nitrogen for data collection. Cystallographic data was processed with MOSFLM and analysed with the CCP4 programmed suite and COOT.

Results: Both GAGs bind to and interact with identical residues on catK in an area distinct but continuous with the previously published GAG binding site. The entire GAG binding region is rich in Arg and Lys residues creating an extensive positively charged surface for binding to the negatively charged GAGs. Four disaccharide units bind to each catK molecule adopting a linear strand in contrast to the cosine curve-shaped conformation seen in the previously published C4-S (17 kDa size) bound catK structure.

Conclusions: These results indicate the presence of multiple GAG binding sites on the surface of catK. The length of the GAG chain could potentially determine which binding site on catK it occupies.

Abstract #3: Patient Experiences of Continuous Positive Airway Pressure and Oral Appliances

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Objectives: There are two therapies most commonly used to treat Obstructive Sleep Apnea (OSA): Continous Positive Airway Pressure (CPAP) and Oral Appliances (OA). Both can be used as the first line of therapy for mild to moderate OSA. The aim of this study was to understand the experience of CPAP and OA users, as well as the factors that influence a patient's choice of treatments.

Methods: Our study had a total of 22 participants. There was one interview conducted with a CPAP user, 1 focus group of CPAP users (n=3), 1 of OA users (n=3), and 2 with a mix of CPAP and OA users (n=15). The interview and focus groups were analyzed together and are referred to collectively as the focus groups.

Results: Participants expressed six benefits they hoped to obtain from sleep apnea treatment: improved health, eliminated/reduced apnea, reduced daytime fatigue, uninterrupted/improved sleep, terminated/reduced snoring and benefits to bed partner. Participants identified several factors that could influence their decision about whether to use CPAP or OA: effectiveness, transportability, stigma/embarrassment and cost. Additional factors that were mentioned infrequently were: impact on bite, power supply and bed partner's preference. Participants stressed the importance of the treatments' effectiveness for their choice of devices, as well as in their satisfaction with the device and their continued use of it. Participants preferred a device that is small and easy to pack for traveling. Both treatments were perceived by participants as expensive. A patient's choice was generally not influenced by his or her bed partner.

Conclusions: The focus groups yielded insights into patient experiences of using CPAP and OA for the treatment of sleep apnea and illuminated some of the factors impacting patient choice for one or the other. This information could be used to increase a treatment's appeal to potential users as well as to develop patient-centered treatment strategies.

Abstract #4: A Culturally Sensitive Oral Cancer Prevention Program for South Asians

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Objectives: This was a mixed method study based on three phases of the PRECEDE-PROCEED model: (a) *Epidemiological assessment*: To determine the incidence and survival rates of oral cancers among South Asians (SA) and the general population; (b) *Social assessment*: A qualitative ethnographic approach was used to understand the reasons SA chew smokeless tobacco (ST); (c) *Ecological and educational assessment*: a questionnaire was constructed using qualitative research findings and some pilot data was collected.

Methods: All oral cancer cases diagnosed from 1980 to 2006 were identified through the British Columbia (BC) Cancer Registry; surname lists were used to establish ethnicity. Incidence and survival rates were calculated using BC Vital Statistics data. For social assessment, an interpretive realist ethnographic approach was used; data was collected with participant observations and semi-structured interviews (n = 62). Pilot testing of the questionnaire was done using a convenience sample.

Results: Oral cancer rates among SA men and women were 1.33 and 1.61 times higher than the general population, while the survival rates were less among SA in both genders. ST was readily available in SA shops and grocery stores, and was dispensed in plastic pouches, which had no labels or health warnings. Participants focused on the benefits of ST chewing. Themes related to perceived benefits included: a) a way to manage demanding work, b) an aid to mental health, and c) a more acceptable and healthier practice than smoking tobacco. The mean age of chewers was 20.5 ± 3.8 years, 66.6% consumed alcohol and chewed ST, and 72.2% did not have dental insurance coverage.

Conclusions: ST chewing is common among SA in BC. There is a need for a culturally sensitive oral cancer prevention program to identify high risk patients and to develop health promotion programs to spread awareness about the ill effects of chewing ST. ST should be brought under Canadian tobacco control policies.

Abstract #5: Transforming Growth Factor Beta Signalling Modulators in Periodontal Disease

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Objectives: Transforming growth factor beta 1 (TGF-β1) plays a crucial role in the protection of many organs from inflammation. TGF-β1 is expressed as a latent cytokine and needs to be activated in order to function. Epithelial integrin αvβ6 and thrombospondin-1 (TSP-1) serve as activators of TGF-β1. Mice deficient in β6 integrin (β6-/-) develop periodontal disease with apical migration of junctional epithelium, inflammation and bone loss. The aim of the present investigation was to determine whether TSP-1 jointly with αvβ6 integrin participates in TGF-β1 activation and protection of periodontium from inflammation. In addition, we hypothesized that TGF-β1-mediated immunoprotection is mediated via Smad3, a key signalling molecule in the TGF-β pathway.

Methods: Mandibles and maxillae of the 3-6 month-old wild-type (WT) and β6 integrin, TSP-1 and Smad3 deficient mice and from β6 integrin and TSP-1 double knockout mice were dissected and processed for histology. Sections stained with hematoxylin and eosin were analyzed by light microscopy. Inflammation and junctional epithelial migration distance on the root surface apical to the cemento-enamel junction were recorded.

Results: Unlike WT mice, the β6-/- mice consistently showed inflammation of the gingiva and apical migration of the junctional epithelium. Similar changes were also noted in the β6 integrin and TSP-1 double knockout mice. However, no abnormalities of periodontal structures were observed in either TSP-1 or Smad3 knockout mice.

Conclusions: The αvβ6 integrin, but not TSP-1, plays a critical role in protection of the periodontium from inflammation and apical migration of junctional epithelium. Effects of αvβ6 integrin are mediated by signalling molecules other than Smad3; possibly they are mediated by Smad2.

Abstract #6: Gene Expression Analysis of Macrophage Polarization by Surface Roughness

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Objectives: Macrophages, one of the first cell populations present on newly implanted devices, can polarize into different phenotypes, which either affect the inflammatory response (classical pathway) or promote immunosuppression and wound healing (alternative pathway) through the release of respective cytokines. Macrophages have been found to be abundant on rough SLA surfaces, which exhibit an improved performance compared to implants with smooth surfaces. The goal of this study is to examine if surface roughness affects the macrophage phenotype.

Methods: Murine macrophages (RAW 267.4) were cultured on polished and rough (SLA) substrata. Starting at 6h and up to 5d, RNA was isolated and reverse transcribed. The expression of genes typical of the phenotypes (Arg-1, IL-10, TNF-α) was quantitatively analysed by qRT-PCR. On tissue culture plastic (TCPS) control samples, macrophages were primed with IL-4 or LPS towards the alternative and classic phenotypes, respectively.

Results: LPS-stimulated macrophages showed upregulation of the proinflammatory cytokine TNF-α and a high upregulation of IL-10, whereas macrophages stimulated with IL-4 showed a strong upregulation of Arg-1, a cytokine associated with the alternative activation pathway. Expression of Arg-1, TNF-α and IL-10 did not vary as a function of the surface topography on non-stimulated macrophages within the studied time frame of 5d. However, it was observed that on the polished and rough SLA substrata, expression of IL-10 and TNF-α was downregulated with time, while Arg-1 expression showed a slight upregulation between 3d and 5d.

Conclusions: Within a time frame of 5d surface topography alone does not seem to effect the expression of the cytokines Arg-1, TNF-α and IL-10. However, upregulation of Arg-1 after 3d showed that the macrophages on the substrata did show changes in phenotype. Furthermore, control experiments with LPS and IL-4 have shown that the macrophages can be primed into different phenotypes.

Abstract #7: 3D Modeling of Brain-Face Relations During Human Embryo Development

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Objectives: As the face develops, the forebrain not only acts as a supportive structural framework but may also exert an influence from several potential signaling sites for facial morphogenesis such as ectodermal placodes. The purpose of this study was to produce 3D ribbon models and measurements to illustrate the dynamic spatial and morphologic relationships between potential signaling sites and features of the developing face in stage 16 to 18 Kyoto (Japanese) and Carnegie (Caucasian) embryos.

Methods: Photographs of normal transverse serial sections of stage 16 to 18 Carnegie and Kyoto embryos were reconstructed into 3D ribbon models using WinSURF software. ImageJ software was then used to take linear measurements of important areas of change and their relationships. Potential signaling sites in facial morphogenesis, including the adenohypophysis, olfactory epithelium, and trigeminal ganglia, were represented in reconstructions of coronally sectioned embryos.

Results: Models and measurements showed advanced brain development but delayed primary palate and upper lip development in Kyoto embryos as compared to Carnegie embryos that may be related to the greater prevalence of cleft lip in the Japanese population. In both embryos, the maxillary prominences grew forward to contact and fuse with the medial nasal region that simultaneously narrowed and elongated. We also showed the forward movement of the face beneath the prosencephalon at the time of telencephalon outpocketing. Representation of potential signaling sites demonstrated the large volume occupied by ectodermal placodes in the developing embryo.

Conclusions: 3D ribbon models illustrate the complex relationships between the brain and face during primary palate development. These can also be measured to quantitatively analyze relationships that may be important in normal formation of the primary palate and upper lip.

Abstract #8: Regulation of Moonlighting Proteins by Matrix Metalloproteinase Processing

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Objectives: Matrix metalloproteinases (MMPs) are an important family of extracellular proteases involved in homeostasis as well as pathologies such as arthritis, cancer and periodontitis. Protease function is defined by the functions of the substrates modified. Clinical trials of MMP inhibitors were fraught with side-effects and failed, probably due to inhibition of the processing of unforeseen substrates of MMPs. Thus, we are elucidating the substrate degradome (repertoire) of MMP family members in order to fully understand their roles.

Methods: We have developed new proteomics methods to identify protease substrates. These quantitative degradomics screens reveal changes in substrate levels when a proteome exposed to the protease of interest is compared with an unexposed proteome. This has been achieved by adding exogenous protease compared with a control (buffer or inactive protease) to a proteome or secretome (all secreted proteins) *in vitro*, in cell culture systems using exogenous, endogenous or transfected protease and using tissues from MMP knockout vs wild-type mice. Once novel substrates are identified, validation and elucidation of the biological role of MMP processing is necessary.

Results: Our studies have vastly expanded the MMP degradome and helped overturn the dogma that MMPs are dowdy matrix degraders, since we have identified substrates that are receptors, growth factor binding proteins and chemokines. It has become obvious during proteomic analyses of secretomes that particular intracellular proteins lacking conventional signal sequences are not only consistently found outside of the cell, but are cleaved by MMPs. Several of these "moonlighting" proteins have ascribed extracellular functions that are distinct from those performed inside the cell, whilst many have unknown extracellular roles. Intracellular proteins that we have confirmed as MMP substrates include HMGB1, a major mediator of sepsis, and tRNA synthetases that are cytokines, modulating angiogenesis and inflammation.

Conclusions: Extracellular moonlighting functions may be relatively common for intracellular proteins. MMPs may play a role in the regulation of these functions.

Abstract #9: Risk Factors for Oral Cancer Development in British Columbia: A Population-Based Approach

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Health professionals have used reliable risk-prediction tools for breast and lung cancers. The establishment of a risk-predictive model can have important public health implications. There is a lack of such a model in oral cancer.

Objectives: 1) to review path reports from 1980-2005, 2) to collect patient and lesion information, and 3) to locate outcome data.

Methods: We manually selected and scanned the reports of mucosal biopsies between 1980 and 1996 and electronic data was queried from the Pathology database between 1997 and 2005. An Excel database was developed with the patients' demographics and lesion characteristics. Patients' personal health numbers were used to identify outcomes through links to the Pathology database and the BC Cancer Registry.

Results: There were 32,053 mucosal biopsies identified from 1980 to 2005 and 2,451 (7.6 %) were dysplasia or cancer. The incidence of mucosal biopsies has increased ~ 4.5 times (0.25, 1980 vs. 1.1, 2005, per 1000 persons in BC). When the increment of the dentists in BC is examined, there is no increase in biopsy number per dentist (0.6 biopsies/1000 dentist/year). Surprisingly, the incidence of precancerous and cancerous lesions has increased ~ 24 times (3.6, 1980 vs. 86, 2005, per 1,000,000 persons in BC). Among the 2,018 precancerous patients, we have identified 106 (4.3%) cases that progressed to cancer within 3 years on average. There was no difference in age and gender between progression and non-progression. Not surprisingly, there is a statistically higher portion of progressing lesions involving the tongue and floor of mouth (67% vs. 50%, $P = 0.03$).

Conclusions: This is the first step in the examination of this unique, 30-year population-based material. With the analysis of this enriched archive material (patient and lesion information and archive paraffin tissue), we will be able to build a population-based risk model for oral cancer.

Abstract #10: Identification of Cathepsin V Exosites Necessary for Elastin Degradation

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Objectives: While human cathepsin V demonstrates a potent elastolytic activity, human cathepsin L, which shares a 78% amino acid sequence identity, exhibits only minimal proteolytic activity towards insoluble elastin. This suggests that the remaining portion of their amino acid differences may play an important role in gaining or losing elastin degradation ability. Since the active sites of both enzymes share very similar specificity, it is likely that cathepsin V possesses certain distinct structural features that aid in binding and orientating elastin for proper hydrolytic peptidolysis.

Methods: Previously constructed wild-type cathepsin V and L expression pPIC9 vectors served as templates for constructing all chimera vectors by joining different parts of their cDNA together by polymerase chain reaction (PCR). Mutants were subjected to Elastin-Rhodamine degradation and HPLC profiling to determine their elastolytic activity.

Results: In this study, a total of 11 chimera variants of cathepsins V and L were generated to identify potential elastin-binding domains in cathepsin V. The evaluation of the elastolytic activity of these variants revealed two exosites that contributed significantly to the elastolytic activity of cathepsin V. Both exosites are distant from the active site of the protease. The replacement of either exosite 1 or 2 with the analogous residues present in cathepsin L led to a 75% or 43% loss in the elastolytic activity. The replacement of both exosites resulted in an elastolytically inactive variant despite retaining its full peptidolytic activity.

Conclusions: The elastolytic activity of cathepsin V is facilitated by two exosites remote from the active site of the protease. The identification of exosites may contribute to the design of inhibitors that will only affect cathepsin V's elastolytic activity while not interfering with the normal protease function of the enzyme.

Abstract #11: Misexpression of the RA Inactivating Enzyme CYP26A1 Inhibits Jaw Development

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Objectives: My aim was to study the effects of reducing retinoic acid (RA) levels in the embryonic face on jaw morphogenesis. I increased the levels of one of the RA catabolic enzymes, *CYP26A1*, in the facial prominences in order to lower endogenous RA levels.

Methods: Human *CYP26A1* was cloned into an avian retrovirus and injected into the face of differently staged chicken embryos. Embryos were collected at 48 and 72 hours for gene expression and cell dynamic studies or at 12 days for skeletal analysis. Micromass cultures from the mandibular and frontonasal mass prominences were also made to study the effect of *CYP26A1* over-expression on cartilage differentiation.

Results: RA target genes, *RARβ* and *MEIS2,* show decreased expression in virus-injected embryos, which was consistent with a decrease in RA levels. Almost all embryos had an inhibition of cartilage and bone differentiation and clefts formed in 50% of maxillary and frontonasal injected embryos. However clefts were not observed in stage 20 embryos demonstrating stage specificity. Just as loss of RA inhibited skeletal development, so did an excess of RA activity. Micromass cultures of frontonasal mass or mandibular mesenchyme treated with RA did not form cartilage. Transfection with the retinoic acid response element (RARE) reporter confirmed that RA activity had been increased. The direct effects of CYP26A1 enzyme on cartilage differentiation in culture and its ability to rescue the effects of RA will be reported.

Conclusions: Increased expression of *CYP26A1* appears to be an effective way to decrease RA levels based on target gene expression. There do not seem to be any spatial differences in the skeletal response to *CYP26A1,* however the clefting phenotypes suggest that adequate RA levels are necessary in order for outgrowth and fusion to take place.

Abstract #12: Enhancing Learning Through Development of Dental Applied Learning Experiences (DALEs)

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Objectives:
To enhance student learning by (a) providing a direct link between the biomedical sciences and the practice of Dentistry, and (b) increasing student accountability for this information through the creation of DALEs with global learning objectives.

Methods:
1. All the 1st year 2nd term dental version-PBL medical cases were reviewed and global dental learning objectives were identified.
2. The global dental learning objectives were organized to create 4 DALEs, which link the dental relevance to each of the first year 2nd term medical blocks. These DALEs will be integrated into the 2nd term of the 2010-11 academic year.
3. Tutor and student surveys were created to collect information on the effectiveness of this pilot project. These results will be compared with previous years' surveys.

Results: "Since assessment strategies are perhaps the most powerful force driving the learning process and develop an appropriate standard to judge achievement" (Hubball & Levy, 2004), students' learning of the global objectives will be assessed through their performance in the DALEs, in a DENT 410 exam and in subsequent overview exams. Studying for these exams and preparing for the DALEs increases students' accountability for this information. Since this project will not be completed until the end of term 2, no current data is available. However, previous surveys (May 2008-May 2010) indicate that students acknowledge the importance of applying the medical information to their clinical experiences and feel that a list of dental learning objectives would enhance their learning.

Conclusions: We anticipate that having a clear link between medical-dental knowledge and its application to the practice of dentistry, as well as being repeatedly accountable for this knowledge through varied assessments will enhance student learning.

Abstract #13: Matrix Metalloproteinases in Scarless Wound Healing

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Objectives: Wound healing in skin often results in scar formation characterized by excessive accumulation of extracellular matrix, and in severe forms causes considerable morbidity. Interestingly, wound healing in oral palatal mucosa is fast and rarely results in scarring. Therefore, understanding the mechanisms that promote oral scarless wound healing may provide novel approaches to prevent scar formation in skin. We hypothesize that matrix metalloproteinases (MMPs) play a significant role in scarless oral mucosal wound healing since they are capable of processing extracellular matrix and modulating inflammation. The goal of the study is to compare the expression profile of MMPs in skin and gingival wounds using human and pig wound healing models.

Methods: Experimental wounds are created in human oral mucosa and in the oral mucosa and dorsal skin of red Duroc pigs. Wound biopsies are collected at various time points after wounding and expression of MMPs will be assessed by gene expression profiling, RT-PCR, Western blotting and zymography. Immunostaining will be used for localization of the MMPs in the tissues.

Results: Preliminary results suggest that oral wound healing is associated with fast and robust regulation of MMPs. In addition, regulation of MMP expression in oral mucosa appears to be significantly different from that in the skin.

Conclusions: Rapid controlled processing of wound extracellular matrix may play a key role in scarless palatal wound healing. In addition, MMPs may regulate the inflammatory reaction that plays a central role in scar formation. Comparing expression of MMPs in scar forming skin and scarless oral mucosal wound healing may provide novel therapeutic targets to prevent scar formation in skin.

Abstract #14: Effect of Saliva Substitutes on Enamel Measured by Quantitative Light Fluorescence

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Objectives: Little scientific evidence exists to support the use of over-the-counter xerostomia products in xerostomic individuals. Previously, the demineralizing effects of xerostomia products *in vitro* have been reported. The purpose of this randomized controlled experimental study was to examine how the xerostomia products Biotene® Oralbalance® and MouthKote Oral Moisturizer® affect the mineral content of human enamel *in vitro*.

Methods: 104 caries-free extracted human teeth were selected and prepared, followed by baseline quantitative light fluorescence (QLF) imaging and exposure to an erosive solution of lactic acid (pH of 4.5) and/or Biotene®, MouthKote®, or Oral-B® fluoridated rinse. Mineral loss was determined with respect to mean fluorescence loss (ΔF, %), maximum fluorescence loss (ΔQ, %), and lesion area (WS, %/mm^2). Within-group and among-group comparisons were made employing independent sample t-tests, paired sample t-tests, and ANOVA for multiple comparisons with Bonferroni post hoc adjustment, or their non-parametric equivalents. For all tests, the threshold for statistical significance was set at $P<0.05$. The statistical software SPSS 17.0 was used for data analyses.

Results: The xerostomia products produced significant demineralization in extracted human teeth with prior demineralization ($P=0.000$) and without previous pre-demineralization ($P=0.000$). There were substantial statistically significant differences in mineral loss among all groups. The amount of demineralization (Mean±SD) was higher in the MouthKote group 2 (-27.19±6.70) and group 4 (-11.45±2.94) than in the Biotene group 1 (-11.15±3.05) and group 3 (-7.38±0.44) respectively. The Oral-B fluoridated rinse aided in re-mineralization albeit not to baseline levels.

Conclusions: Biotene® and MouthKote® induced substantial mineral loss in pre-demineralized and unaltered enamel of extracted human teeth. MouthKote® induced greater demineralization than Biotene®. Oral-B rinse induced re-mineralization in all experimental groups except for group 2 (lactic acid/MouthKote®) where further dissolution of enamel was observed.

Abstract #15: Comparative Analysis of Recurrent and Non-recurrent Keratocystic Odontogenic Tumours

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Keratocystic odontogenic tumours (KCOT) have a higher local recurrence rate. There is no good predictive marker for recurrence. The current literature on predicting outcomes remains inconclusive due to either small case numbers or incomplete analyses of demographics, clinical-pathological features, and molecular markers.

Objectives: 1) To identify KCOTs in the pathology database together with the collection of demographics, clinical-pathological features, and outcomes; 2) To determine the status of proliferation-related markers between recurrent KCOT (rKCOT) and non-recurrent KCOT (nrKCOT).

Methods: Using the key-word search in the database of the BC Oral Biopsy Service (BCOBS), a population-based pathology service, we identified all KCOT from 2000 to 2009. The hematoxylin and eosin stained slides of all cases were reviewed and the demographic data and outcome status were collected. A unique tissue microarray was fabricated for the analysis of proliferation-related markers (Ki67, CyclinD1, p-EGFR, p27, and MMP-9) using immunohistochemistry.

Results: From 2000 to 2009, there were 108 KCOT cases in the BCOBS (34 rKCOT cases and 74 nrKCOT cases). To have at least a 5-year follow-up for the nrKCOT, we include all rKCOT and 30 of the nrKCOT identified from 2000-2005. There is a significant difference in age between rKCOT and nrKCOT (46.4 ± 23.1 vs. 33.7 ± 17.1 years, P=0.03). There is no difference in gender and location; however, the premolar location is twice more frequent in rKCOT than in nrKCOT. When comparing histological features, rKCOT cases showed more basal cell proliferation (P=0.03), which is complemented by higher expression in Ki67, Cyclin D1 and MMP9 markers.

Conclusions: This is the largest collection of rKCOT and the most comprehensive comparison between rKCOT and nrKCOT in the literature. Ki67, CD1 and MMP9 might serve as objective markers in prediction of recurrence.

Abstract #16: Translation Methodology Revised for Oral Health-Related Quality of Life

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Objectives: Translating methods to culturally validate existing sociodental indicators involve a rigorous methodology, which can be costly to some researchers who need to either develop or translate an indicator sensitive to their target ethno population. As a result, researchers may be inclined to use one of the many free-of-charge online translator tools available. Such alternative tools pose, however, unknown and potentially detrimental effects to the validity of the chosen measure as a culturally relevant and psychometrically sound instrument. Our project sought to explore the value of using online translator tools to develop oral health-related quality of life measures using the Chinese language as an example.

Methods: Six online translating tools were employed to translate the original English versions of the Geriatric Oral Health Assessment Index (GOHAI) and the Oral Health Impact Profile (OHIP) to traditional Chinese and back translate to English. Two bilingual translators were consulted to check for the accuracy of the Chinese version produced.

Results: The online tools provided translations with no grammatical meaning in Chinese for most of the questions as they offer literal translation. The questions provided in Chinese would be almost impossible to be understood at face value. Two bilingual translators unaware of the study did a content validation exercise on the questions provided by the tools in an attempt to produce a more meaningful version, which was independently compared to the official translations currently in use.

Conclusions: As a free-of-charge translator, the selected tools have to be used with caution, particularly in languages that are not alphabet-based such as Chinese. However, with minimal impact on resources, financially deprived researchers can still use these tools once complemented by a content validation exercise using bilingual professionals. This method can be useful in languages that as of yet have no translated version of a given health measurement. The measure produced should still be tested for its psychometric properties accordingly.

Abstract #17: Screening for Novel Inhibitors of Cathepsin K

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Objectives: Cathepsin K (catK) has recently received considerable attention because of its major contribution to the digestion of extracellular matrix in bone remodeling, particularly in the breakdown of the collagen network. In fact, overexpression of catK is found in diseases such as arthritis, osteoporosis and invasive breast cancer. Therefore, it is important to screen for potential inhibitors of catK.

Methods: Potential inhibitors were tested *in vitro* by using the collagen assay and the gelatin assay. These two assays will not only determine if the molecule can inhibit catK but will help determine whether the molecule will inhibit by interfering with the active site or the formation of the catK/C4-s complex.

Results: A couple of molecules that we screened showed a positive result in inhibiting catK by potentially disrupting the catK/C4-s complex. We believe this complex is required in the degradation of type 1 collagen as our lab has shown that C4-s enhances the degradation of collagen by catK.

Conclusions: We have found a couple of molecules that inhibit catK *in vitro* via a novel mechanism of inhibition. These molecules are unique as they specifically inhibit the collagenolytic activity of cathepsin K without interfering with the enzyme's proteolytic activity towards non-collagenous substrates.

Abstract #18: Potential Molecular Mediators of the RA-Noggin Beak Duplication Phenotype

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Objectives: The aim here is to profile the expression differences seen in the maxillary prominence of chickens that have been treated with Noggin and retinoic acid (RA). We have shown previously that this treatment leads to a transformation of identity, essentially duplicating the facial midline on the side of the upper beak. We are using this model to understand the molecular basis for midline specification. The objectives of this study are to use an unbiased microarray approach to pinpoint downstream target genes and then to validate these results with QPCR and in wholemount *in situ* hybridization (WISH). Ultimately these genes will be studied with knockdown and overexpression experiments.

Methods: Two beads soaked in Noggin+RA, Tris+RA, Noggin+DMSO or Tris+DMSO were implanted into the presumptive maxillary prominence at stage 15 (2.5 days of incubation). Following 16h, maxillary tissues from 10-15 embryos were dissected and pooled together. Three biological replicates were collected for each treatment. Microarray analysis using the Affymetrix Chicken Genome chip was carried out. Intensity values were imported into Genespring X and analyzed for chip quality and then for expression differences with Anova and post-hoc testing. Genes with 2 or more fold expression differences in at least one of the treatment groups were validated with QPCR and WISH of treated chicken embryos at 6 and 16h post treatment.

Results: QPCR is currently ongoing for 59 genes. WISH will be carried out on a subset of these genes including *TBX22, PI15, MAB21L2, BAMBI, ID1, ALX1, CYP26A1, DLX5, DLX2, PITX2, PRRX1* and *POSTN*. Thus far WISH data on *PI15, TBX22* and *MAB21L2* are consistent with the microarray data.

Conclusions: A gain in *PI15* and *TBX22* expression and loss of *MAB21L2* may be mediating the specification of an ectopic midline in the Noggin+RA treated embryos.

Abstract #19: Wnt5a Induces Matrix Degradation in Facial Mesenchyme via Non-Canonical Pathways

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Objectives: Data from mouse knockout studies and human syndromes suggests that Wnt5a is required for mandibular development. To separate the early effects of Wnt5a on outgrowth from those on specification of skeletal mesenchyme, my goal was to culture chicken mandibular mesenchyme and to determine the effects of Wnt5a on skeletal differentiation.

Methods: High density micromass cultures from stage 24 mandibular mesenchyme were incubated in either Wnt5a-Conditioned Media (CM) or control media. Adobe Photoshop was used to quantify the areas stained with Alcian Blue and Alkaline Phosphatase. The proportion of the culture stained with blue or red was analyzed using one-way ANOVA and posthoc testing (Statistica). For luciferase assays, the SuperTopflash luciferase reporter and Renilla luciferase plasmid were transfected into mesenchymal cells at the time of plating and cultures grown for 48h. The Firefly/Renilla ratio was measured in a luminometer.

Results: Wnt5a-CM caused a striking reduction in the amount of cartilaginous matrix after 6 days. Surprisingly, initial stages of differentiation (2-4 days) were unaffected. Sections confirmed the loss of Alcian Blue and showed a decrease in type II collagen. To determine whether the matrix was enzymatically degraded, we carried out RT-PCR to amplify Matrix Metalloproteinase-13. There was no expression at 3 days but strong induction at 4 days. Luciferase assays were then carried out and Wnt5a did not induce canonical signalling. To test whether Wnt5a can antagonize canonical signalling we combined Wnt5a-CM with either Wnt3a-CM or LiCl both of which stimulate canonical signalling. Wnt5a completely inhibited the effects of both reagents.

Conclusions: These results show that Wnt5a works via non-canonical pathways to promote enzymatic removal of the sulphated proteoglycans in cartilage matrix. Our data suggest that during normal development Wnt5a maintains a balance of non-canonical and canonical activity in the face and that it may play a role in cartilage maturation.

Abstract #20: Satisfaction and Maintenance with Mandibular 1- or 2-Implant Dentures

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Objectives: Mandibular dentures retained by one implant offer potential as a more economical treatment compared to the more common approach of two or more implants. We aimed to test the hypothesis that there are no differences in patient satisfaction or in the number or timing of maintenance events for implant attachments when comparing mandibular overdentures retained by one or two implants after three years.

Methods: 86 edentulous subjects were enrolled in the Vancouver Implant Prosthesis (VIP) clinical trial and were assigned randomly to receive one or two Straumann implants in the anterior mandible to support their existing lower dentures using a standard ball and gold matrix attachment. Subjects reported their satisfaction on a Visual Analog Scale (VAS), 0 to 100mm, with their lower dentures at baseline, one year, and three years. In addition, the dates and types of treatment to the attachment system, namely adjustments, reattachments, and replacements of the patrix or matrix component, were collected from patient records.

Results: After three years, 1-implant subjects (n=23) had no significant difference (p=0.128) in their overall VAS satisfaction with a median of 91.0 (mean 77.5) compared with 2-implant subjects (n=21) with a median of 92.0 (mean 84.4). Satisfaction was slightly lower compared to one-year post-insertion, but still significantly improved compared to baseline scores. We also found no significant difference in the time to the first adjustment, reattachment, or replacement of attachment components (Wilcoxon p<0.05), or the frequency of these different events (Chi square p<0.05), although 1-implant subjects experienced a non-significant tendency for more frequent reattachments of the matrices.

Conclusions: The study found similar satisfaction and implant maintenance for 1- and 2-implant overdentures at three years of loading. One-implant overdentures still appear to be an economical and successful alternative to 2-implant overdentures.

Abstract #21: Effect of Flowable Composite Intermediate Layer on Interfacial Fracture Toughness

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Clinical motivation: Flowable composites (FC) are advocated as intermediate layers between adhesives and restorative resin composites (RC). Their effect on interfacial fracture toughness (K_{IC}) has not been assessed.

Objectives: To test the null hypothesis that the interfacial K_{IC} of dentin RC is not affected by a FC layer.

Methods: Equilateral triangular prisms (6x6x6 mm) were obtained by wet grinding from extracted human molars. A flat dentin surface was exposed by wet grinding before bonding. Clearfill SE Bond was the bonding system used. Clearfill Majesty Flow, Filtek Flow, or Tetric Flow was used as a liner. The control group had no liner. A hybrid RC *(*Z100) was packed incrementally and light cured on top of the flowable RC in a mould to obtain 6x6x6x12 mm test specimens. After demoulding, the specimens were cured for an additional 120s and then stored in 37°C water for seven days. Interfacial K_{IC} was determined using the notchless triangular prism (NTP) specimen K_{IC} test with a computer-controlled (System IX) universal-testing machine (Instron 4301). Results were analyzed using one-way ANOVA. Fractured samples were examined under a light microscope.

Results: The null hypothesis was accepted since no statistical significance difference was detected between the groups. It seemed, however, that the presence of FC resulted in changes in the fracture propagation path.

Conclusions: Under the conditions of this study, a layer of FC did not affect the interfacial K_{IC} of dentin-RC interface but resulted in a change in the fracture propagation path.

Abstract #22: Dental Hygiene Baccalaureate Degree-completion Education in Canada

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Objectives: This study aimed to explore and analyze the learning experiences and outcomes of completing a dental hygiene baccalaureate degree from the perspectives of diploma dental hygienists who had advanced their education to the baccalaureate degree.

Methods: This study employed a qualitative phenomenological design using a maximum variation purposeful sampling strategy. After completion of a pilot study, several dental hygiene national and provincial associations helped recruit participants through an email broadcast to their members. Semi-structured interviews were conducted with 16 dental hygienists who initially earned their dental hygiene diploma in Canada, subsequently practised dental hygiene for a minimum of two years, and then completed their dental hygiene baccalaureate degree from a Canadian university. Interviews were audio-recorded, transcribed verbatim, and coded for data analysis involving pattern recognition and thematic development.

Results: Findings indicated that the participants experienced a broader education as they were exposed to a wider scope of knowledge within and outside of dental hygiene theory, and experienced a more independent learning environment with a stronger focus on literature review and critical thinking compared to their experiences in their dental hygiene diploma education. Perceived outcomes associated with completing this degree include an increase in: confidence, literature review and critical thinking ability, evidence-based decision making and integration into practice, comprehensiveness of clinical care based on an expanded knowledge base, credibility from professional colleagues and clients, and an appreciation for lifelong learning. Participants also commented that they had more career opportunities available to them outside the private clinical practice setting.

Conclusions: Participants concluded that completing a dental hygiene baccalaureate degree positively influenced their self-perception and their dental hygiene practice.

Abstract #23: RAW 264.7 Macrophage-Mediated Depletion of Hydrogen Peroxide in Cell-Culture Media

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Objectives: Macrophages have traditionally been known to produce hydrogen peroxide in order to defend their host against foreign cells and act as secondary messengers. The current study was conducted in order to determine whether RAW 264.7 macrophages were capable of depleting exogenously added hydrogen peroxide.

Methods: Conditions for ideal experimentation using Amplex Red fluorescence as a hydrogen peroxide detection reagent were investigated using different cell culture media and microplates. The ability of RAW 264.7 cells to produce and/or deplete extracellular hydrogen peroxide was determined using different cell concentrations, timelines, incubation temperatures, number of passages, along with the H_2O_2 inducer LPS, and catalase inhibitor 3AT.

Results: When using an Amplex Red protocol to detect hydrogen peroxide, PBS medium and black microplates were found to be the most appropriate materials for detection. In addition, direct contact of Amplex Red with RAW 264.7 macrophages was found to interfere with the detection reagent's ability to detect exogenous hydrogen peroxide. Most importantly, Raw 264.7 macrophages were not found to produce significant amounts of intra or extracellular hydrogen peroxide, even when stimulated with LPS. In contrast, the RAW 264.7 macrophage was found to deplete any hydrogen peroxide in the surrounding medium. This depletion was found to be time, cell concentration, temperature and passage number dependent. The use of a catalase inhibitor, 3AT, had no effect on reducing the ability of RAW 264.7 cells to deplete hydrogen peroxide from its surrounding environment, and was even found to lower the detection capacity of Amplex Red.

Conclusions: RAW 264.7 cells deplete exogenous hydrogen peroxide, and is time, cell concentration, temperature and passage number dependent. H_2O_2 depletion is not affected by the H_2O_2 inducer LPS, nor the catalase inhibitor 3AT.

Abstract #24: Remote-controlled Titania Nanotube Drug Delivery System

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Objectives: The purpose of this study was to determine the optimal size of nanotubes for sustained and prolonged drug release in order to develop a titania nanotube drug delivery system. The feasibility of remote-controlled drug elution from this system using UV light was also evaluated.

Methods: Anodized titania nanotubes (diameter: 100 nm, length: 0.1-3 µm) were prepared on a Ti sheet (1×1 cm^2). One wt% tetracycline only and a mixture of 0.25wt% poly-lactic acid (PLA) and 1 wt% tetracycline were loaded on nanotube specimens to evaluate the effect of PLA on drug-release rate. Drug-loaded nanotube specimens were immersed in distilled water and stored at 37°C for 1, 5, 10, 30, 60, and 180 minutes. The concentration of extracted tetracycline was measured by an ELISA microplate reader. A remote control test was performed by UV light to estimate the effect of UV irradiation on accelerating drug release. All data were analyzed by one-way ANOVA test. The surface structures of nanotubes were observed by FE-SEM.

Results: The results of the drug elution test showed that the behavior of drugs released by tetracycline-loaded titania nanotubes indicated an initial burst at the beginning of the incubation time. However, titania nanotubes storing the mixture of PLA and tetracycline showed the behavior of sustained and prolonged drug elution. The remote control test by UV irradiation showed that the concentration of tetracycline released by the UV irradiated group was statistically higher than that released by the non-UV irradiated group ($p < 0.01$).

Conclusions: In summary, the mixture of PLA and tetracycline showing the behavior of sustained and prolonged drug release is expected to be suitable for a drug delivery system. Also, the remote-controlled drug release system based on titania nanotubes is expected to be helpful for more effective antibacterial and osteogenic acceleration of titanium implants.

Abstract #25: The Effect of Microtubule Disassembly on Palatal Fusion

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Objectives: Microtubule-disrupting drugs have been reported to cause craniofacial defects including cleft lip and palate. However, the mechanisms underlying the failure of palatal fusion remain unknown. In the present study, we investigated the effect of nocodazole, a microtubule de-polymerization drug, on palatal fusion.

Methods: We performed palatal-organ culture to investigate the effect of nocodazole on palatal fusion. A BrdU assay was conducted to examine proliferation of the medial edge epithelium (MEE). Hypertrophy was assessed by measuring cell surface area of MEE. Immunofluorescence was performed to observe epithelial cell differentiation markers, microtubule and actin cytoskeleton, integrin receptors and E-cadherin. The contribution of ROCK and PI3K kinases in the effect of nocodazole was investigated by pharmacological inhibitors.

Results: Nocodazole caused the failure of palatal fusion due to the induction of a thickened midline epithelial seam (MES) in the secondary palate. No changes in either proliferation or differentiation of MEE were observed. Cell surface area of MEE was significantly increased in nocodazole-treated palatal tissue compared to controls. Microtubules were completely disrupted by nocodazole. Remodeling of actin filament, an important contributor to cell hypertrophy, was also observed. The distribution and expression level of integrin receptors were altered by treatment with nocodazole. The cell-cell adhesion molecule, E-cadherin, was still retained in the hypertropic MEE. ROCK and PI3K inhibitors (Y-27632 and LY294002) abrogated the nocodazole-induced MEE hypertrophy.

Conclusions: Palatal fusion was inhibited by microtubule disassembly secondary to nocodazole treatment. Nocodazole caused a thickened MES composed of hypertropic MEE. There was no contribution of either MEE proliferation or altered epithelial differentiation to the thickened MES. Weakened cell-extracellular matrix and cell-cell interaction may result in multi-layered hypertrophic MEE. RhoA/ROCK and PI3K signaling pathways may play a pivotal role in hypertrophied MES formation through actin filament remodeling.

Abstract #26: Prediction of Local Recurrence during Follow-ups Using Fluorescence Visualization

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Background: Oral cancer has substantial morbidity and mortality. Recurrence is observed in 30% of surgically treated high-risk lesions (HRLs), severe dysplasia, carcinoma *in situ* and cancer. Reactive tissue damage seen post-surgery may mask the cancerous lesions and make early identification of recurrence difficult. A new approach to identify early signs of recurrence is vital in disease management. New advances using fluorescence visualization (FV) have been promising.

Objectives: 1) To review and examine the FV measurements and images of HRLs post-treatment, and 2) To examine the relationship between FV alteration and recurrence.

Methods: In the BC Oral Cancer Longitudinal Study, we recruited ~400 HRLs with surgical treatment as the primary modality. Patients who 1) attended follow-up appointments within 6 months of surgery, 2) had direct FV images assessed and recorded in length (anterior-posteriorly) and width (superior-inferiorly) (i.e., FV follow-ups), and 3) attended at least two follow-up appointments within the first year of treatment were eligible for this study. The 'plateau' of the FV measurement during the FV follow-ups is defined as no change in length and width in a time period equal to or greater than 6 months.

Results: A total of 198 patients were identified and 24 (12%) patients had lesions recur at the previous surgically treated site. There was no difference in gender, age, ethnicity, smoking habits, anatomical site, diagnoses, and follow-up time between the recurrence and non-recurrence groups. For those (N=190) with at least 6 months between first and last FV follow-ups, the frequency and duration of 'plateaus' are statistically higher and longer in the non-recurrence group in both length and width measurement.

Conclusions: The stability of FV alteration during follow-ups has a potential to become a useful marker in predicting local recurrence of HRLs.

Abstract #27: How the Turtle Makes its Palate without Palatal Shelves

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Objectives: Here we study for the first time the embryonic palate of the turtle *Emydura subglobosa.* We hypothesize that differences in expression profiles in the early embryo may explain the architectural differences in the turtle hard palate. The objectives are to describe normal morphogenesis of the turtle secondary palate and to examine expression of genes that are known to be important for palatal morphogenesis in mammals.

Methods: Eggs were obtained from the Toronto Zoo and staged according to Werneburg et al. (*Dev Dynam* 238: 2770, 2009). Radioactive *in situ* hybridization was performed on serial sections with turtle-specific probes. Neighbouring sections were used for cellular proliferation, cell apoptosis assays and routine histologic staining. Other embryos were stained for bone and cartilage in wholemount to determine the ossification sequence of maxillary bones.

Results: Stage 4 embryos had a fused primary palate and the maxillary prominences were positioned at the lateral edges of the oral cavity similar to other amniotes. Gene expression patterns for *Ptc, Fgfr2, Bmp7* and *Twist1* were similar to those of chicken, while *Msx2* was expressed more broadly. Higher proliferation was noted on the medial sides of the maxillary prominences. By stage 5, maxillary bones had begun to differentiate but palatine bones had not started ossification. Strikingly, there were no palatal shelves at any stage. In other reptiles including snakes, birds and alligators, palatal shelves bud off the maxillary prominences on the medial side similar to mammals. Despite not forming palatal shelves, a palatine bone still differentiated beneath the nasal cavity at stage 6.

Conclusions: The failure to form palatal shelves was not explained by differences in molecular signalling at early stages. Since the turtle palatine bone forms without palatal shelves, these studies reveal a previously undescribed mechanism for hard palate formation.

Abstract #28: The Differences Among Age Groups of the Early-staged Oral Cancers

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Oral squamous cell carcinoma (OSCC) is a disease with known risk factors, e.g., being older and a heavy smoker. However, the incidence of oral cancer in younger populations with no obvious risk factors is increasing world-wide. Research to understand this shift may have a significant impact on oral cancer control.

Objectives: To determine the characteristics of OSCC in different age groups and to compare differences among groups.

Methods: One hundred and fifty patients of early-staged OSCC (stage I & II) with at least 6 months' follow-up time were identified from the BC Oral Cancer Longitudinal Study. To better define the differences based on a patient's age at initial OSCC diagnosis, we have arbitrarily selected those ≤ 40 years (Young group, N = 19), between 50-65 years (Conventional group, N = 41), and ≥ 80 years (Old group, N = 9). Demographics, smoking habits, clinicopathological features, treatment, and outcome data were collected.

Results: Most young patients were Caucasian (68%), male (58%), and nonsmokers (61%) and their OSCCs were frequently located at the tongue (95%) with a high local recurrence (58%). When comparing Young and Conventional groups, we found significant differences in ethnicity (Caucasian, 68% vs. 93%, $P = 0.02$), smoking habits (ever smoked, 39% vs. 83%, $P = 0.002$) and the anatomical site (tongue, 95% vs. 44%, $P = 0.0002$). When comparing Young and Old groups, we found in addition to the predilection of tongue in the Young (95% vs. 44%, $P = 0.007$), the Old group had more female patients (42% vs. 78%, $P = 0.03$). There were no significant differences in grade of differentiation, treatment modalities used, and local and regional failure.

Conclusions: Nonsmokers and tongue-located cancer characterize the Young group of early-staged OSCC. An attempt to characterize the difference using molecular tools among different age groups is ongoing.

Abstract #29: Role of Small Leucine-rich Proteoglycans in Wound Healing

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Objectives: Scar formation in skin is an unwanted condition that forms as a result of the wound healing process. Clinically, scars range from fine lines to devastating hypertrophic scars that cause great morbidity. By comparing scarless oral mucosal wound healing and scar-forming skin wound healing, our findings suggest that relative abundance of TGF-β1 and β3 isoforms in the pericellular extracellular matrix (ECM) is critical for scarless wound healing. Small leucine-rich proteoglycans (SLRPs) are important components of the pericellular ECM and modulate the abundance and activity of TGF-β isoforms. We hypothesize that SLRPs, produced by fibroblast cells, generate a distinct microenvironment in the oral mucosal ECM that promotes scarless wound healing.

Methods: Using an *in vivo*-like cell culture model, we will generate oral mucosal fibroblast (OMF)- and skin fibroblast (SF)-derived 3D ECMs and characterize their TGF-β and SLRP composition and quantity. Cell functions and expression of TGF-β target genes of OMF and SF seeded on the 3D ECMs will be analyzed using cell and molecular biological methods. To find out the importance of SLRPs for cell functions, the SLRP composition of the ECM will be modulated using the siRNA technique.

Results: We expect that SLRPs present in the 3D ECM derived from OMF will alter SF function towards the OMF phenotype that is associated with scarless wound healing.

Conclusions: Our research will help understanding of the molecular mechanisms in scar formation and contribute to the development of novel anti-scarring therapies and improve the quality of life for those who suffer from scars.

Abstract #30: Critical Role for αvß6 Integrin in Enamel Biomineralization

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Objectives: Tooth enamel has the highest degree of biomineralization of all hard tissues in the body. During the secretory stage of enamel formation, epithelial ameloblasts deposit an extracellular matrix whose proteins are cleaved by enamelysin (MMP-20) and kallikrein-4 (Klk-4), resulting in almost total enamel matrix protein degradation. During this process, the ameloblast plasma membrane has direct contact with the matrix and its mineralizing crystallites. The receptors that mediate ameloblast-matrix adhesion and organization are not well characterized. Integrins mediate cell-matrix adhesion and signaling in most cell types. Therefore, we hypothesized that epithelia-restricted αvß6 integrin plays a role in the organization and interaction of ameloblasts with the enamel.

Methods: Sections of 12-day-old wild-type mouse mandibles were used to investigate normal expression of αvß6 integrin in ameloblasts of developing incisors using immunofluorescent microscopy and *in situ* hybridization techniques. Tooth histology from ß6 integrin-deficient (ß6-/-) mice was analyzed and compared to wild-type mice by light and electron microscopy. Enamel surface characteristics and mineralization were studied by scanning electron microscopy and micro-computed tomography. The ameloblast cell layer of incisors was extracted and analyzed by Western blotting for enamel protein expression. Attrition of molars was investigated using scale-based cusp height measurements.

Results: Wild-type mouse ameloblasts expressed both ß6 integrin mRNA and protein. Interestingly, ameloblasts of the ß6-/- (knockout) mouse incisors accumulated an abnormal amelogenin-rich extracellular matrix facing the forming enamel and between ameloblasts. However, levels of MMP-20 and Klk-4 were not altered, suggesting that accumulation of amelogenin was attributable to either altered synthesis or endocytosis. The maxillary incisors of the ß6-/- mice lacked yellow pigment and the mandibular incisors appeared chalky and rounded. Molars of ß6-/- mice showed severe attrition.

Conclusion: Integrin αvß6 is expressed by ameloblasts where it plays a crucial role in regulating amelogenin deposition and subsequent enamel biomineralization.

Abstract #31: Characterization of Elastolytic Activities of Cathepsins Expressed in Macrophages

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Objectives: Atherosclerosis is characterized by a thickening and loss of elasticity of the arterial wall. The elasticity of the arterial wall is lost when the elastin matrix undergoes remodeling via elastin-degrading proteases. Cathepsins are papain-like cysteine proteases known to have elastolytic activities, and they have been identified in macrophages present in plaque areas of diseased blood vessels. Here, we characterize the elastolytic activities of cathepsins B, K, L, and S expressed in mouse macrophages, and the effect of inhibitors towards their elastolytic activities.

Methods: Peritoneal macrophages were isolated from mice injected i.p. with 4% Brewer thioglycollate medium. Four days after injection animals were euthanized and macrophages were collected by peritoneal lavage with PBS. After washing, 400,000 cells were plated onto a 24-well plate. Then, DQ-elastin (200ug/well) was added with multiple inhibitors, and they were incubated for 24 hours. The fluorescence in supernatants was determined using excitation at 485 nm and emission at 530 nm and is represented as mean ± SD in percentage fluorescence from three independent experiments.

Results: Macrophages were incubated for 24 hours at 37°C in the presence of DQ-elastin with and without protease inhibitors. Using K17 and E64 permitted the estimation of all elastolytic cathepsins expressed in macrophages. The elastolytic activity was reduced by approximately 60-70%. E64d was used to estimate intracellular elastin degradation, and it reduced elastolytic activity by 70%. With a cathepsin S inhibitor, a 20% reduction in the elastolytic activity was observed. CA074-OMe and CA074-OH were employed to investigate intra or extracellular cathepsin B elastolytic activity. Both inhibitors reduced the activity by approximately 20%. GM6001, a general matrix metalloproteinase (MMP) inhibitor, suppressed elastin degradation by 20%.

Conclusions: Cathepsins B and S contribute each approximately 10-20% and cathepsins K and L together contribute 40% to elastin degradation. MMPs contribute 20% towards elastin degradation.

Abstract #32: Cathepsin S in Experimental Collagen-induced Rheumatoid Arthritis

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Objectives: Rheumatoid Arthritis is an autoimmune disease in which the cysteine protease, cathepsin S, may play a crucial role in the inflammatory and joint destructive processes of the disease. It has been shown that cathepsin S is pivotal for antigen presentation and that the protease is capable of degrading the major cartilage proteoglycan, aggrecan, efficiently at neutral pH. In this study, we applied a specific cathepsin S inhibitor to collagen-induced arthritic mice in a therapeutic and prophylactic treatment regime to evaluate whether the inhibitor can reduce inflammation and joint destruction.

Methods: Collagen-induced arthritis (CIA) in mice is characterized by chronic inflammation of the joint, leading to the destruction of cartilage and bone. Eight-week old DBA/1J mice were injected with a mixture of Type II collagen and Complete Freund's Adjuvant. In the therapeutic study, mice were fed with a high fat diet that contained the cathepsin S inhibitor for 4 weeks after onset of arthritis. While in the prophylactic study, mice were fed 4 days prior to induction of arthritis with continuing treatment post-induction. The mice were clinically scored throughout the treatment period. At the endpoint, limbs were isolated, sectioned and stained accordingly with toluidine blue, hematoxylin and eosin stain to evaluate the inflammatory and erosive status of the disease.

Results: In the therapeutic study, there was no significant decrease of inflammation in CIA joints. The clinical scores showed no significant differences between the control and treatment group, as well as there were no significant difference histologically. In the prophylactic study, however, the clinical scores showed a significant reduction of inflammation in CIA joints. The histological findings will be further discussed in the near future.

Conclusions: Whereas the therapeutic treatment of CIA with a cathepsin S inhibitor does not affect disease progression, a prophylactic administration of drug significantly reduced the inflammation.

Abstract #33: Fatigue Testing of Controlled Memory Wire Nickel-titanium Rotary Instruments

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Objectives: Although nickel-titanium (NiTi) rotary instruments are very popular in endodontic treatment, instrument separation is still a challenge in the clinic. To improve the fracture resistance of NiTi files, manufacturers have introduced either new alloys or new manufacturing processes to produce NiTi files with improved resistance to fracture. This study aimed to examine the fatigue behavior using a strain-life approach on NiTi instruments from a novel controlled-memory NiTi wire (termed CM wire).

Methods: Instruments examined included ProFile, and two other files which were produced both from traditional NiTi and the experimental CM wire: TYP, TYP CM, NEYY and NEYY CM (size 25/.04). The files were subjected to rotational bending at the curvature of 35 and 45 degrees, and the number of revolutions to fracture (N_f) was recorded. The fractured surface of all fragments was examined by SEM. The crack-initiation sites, the percentage of dimple area to the whole fracture cross-section area, and surface strain amplitude, were analyzed.

Results: Files made of CM alloy had superior flexural fatigue resistance as compared to the other files made from conventional NiTi alloy at both curvatures. The new alloy yielded an improvement of 2 to 7 times in N_f. Most CM instruments showed more than one crack origin, significantly more than in conventional NiTi files.

Conclusions: The findings of this study suggest that NiTi rotary files made from the newly developed CM alloy have better flexural fatigue resistance than files of the same design and size made from the conventional NiTi alloy.

Abstract #34: Dentin and Enamel Structure Revealed by Scanning Electron Microscopy

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Introduction: The Scanning Electron Microscope (SEM) allows imaging of surface ultrastructure of biological materials. Dual beam SEMs allow surface milling of a structure using a gallium ion beam, coupled to imaging using an electron beam. Cycles of milling and imaging generate image stacks that reveal 3D structure at the nanometer scale. FIB-SEM has not yet been applied to tooth structure.

Objectives: Analysis of the three-dimensional ultrastructure of human teeth, especially dentin and the dentin-enamel interface, using FIB-SEM.

Methods: Primary human teeth were processed in a number of ways to optimize imaging; for this study they were unfixed, stored dry, fractured mechanically and coated with iridium before imaging. The gallium ion-beam cut 20nm slices, and a variety of modalities were used for electron beam imaging using a Helios Dual Beam FIB-SEM. Serial images for 3D reconstruction were processed using Amira software.

Results: Images of dentin/dentinal tubules will be presented. Collagen fibrils were visible in the walls of the tubules, showing the 50-60nm periodicity characteristic of the collagen molecule. Three-dimensional series were composed: for dentin, showing dentinal tubule structure; and for dentin-enamel interface, showing ramifications of fine dentinal tubules close to the interface.

Conclusions: The FIB-SEM is a powerful tool to analyze tissue structure; sample preparation, image processing and ion-beam-milling techniques need to be optimized for dental structures.

Abstract #35: Release of Amphiregulin in Lipopolysaccharide-Mediated Loss of Epithelial Barrier

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Objectives: Lipopolysaccharide (LPS) is a bacterial virulence factor implicated in periodontal disease onset. Amphiregulin (AREG) is an epithelial growth factor receptor ligand normally seqestered at cell-cell contacts in stable epithelial barriers. We used a histiotypic model of junctional epithelium to test if LPS-induced loss of epithelial barrier was mediated by altered AREG expression and localization.

Methods: Three epithelial cell lines (MDCK1, IEC-6, PLE) were cultured in Transwell™ chambers and transepithelial electrical resistance monitored as an assay of barrier formation. Stable epithelial barriers were treated with LPS for six days and assayed at time points for loss of barrier. Media from apical and basal chambers collected at 48 hr intervals was analyzed for AREG and tumor necrosis factor-α (TNF-α) secretion by Elisa. Fixed cultures were immunostained for AREG and E-cadherin as markers of cell-cell junctions.

Results: Chronic LPS, exogenous AREG or TNF-α treatment was found to progressively reduce epithelial barrier from 2 to 6 days. Elisa analysis of conditioned media showed that LPS-treated cultures contained a concentration dependent total increase in AREG, which was significantly elevated ($p<0.05$) and increasing from 4 (59.4%) to 6 (88.8%) days of treatment. A smaller but significant ($p<0.05$) increase in TNF-α secretion was found at 2 (36.0%) and 4 (39.7%) days but diminished by day 6. Increasing LPS concentration and treatment duration correlated to both the extent of barrier loss and total AREG secretion. Also, LPS-induced AREG was predominantly secreted into the basal culture chamber whereas induced TNF-α secretion was equally distributed between apical and basal directions. Immunostaining of day 6 cultures showed that LPS treatment was associated with the loss of intracellular AREG and E-cadherin at cell-cell contacts.

Conclusions: LPS-reduced cell-cell barrier function at the onset of periodontal disease may be mediated by loss of normal AREG localization and secretion.

Abstract #36: Antimicrobial Efficacy of Chlorhexidine in the Development of Multispecies Biofilms

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Objectives: Detailed information on the nature of the physiological and metabolic phases of biofilm development is important in combating resistant, disease-associated biofilms. The aim of this study was to conduct a kinetic study of the susceptibility of multispecies biofilms at different phases of growth to root canal irrigants.

Methods: The multispecies biofilms were grown from plaque bacteria on collagen-coated hydroxyapatite discs in brain heart infusion (BHI) broth for time periods ranging from two days to several months. Fresh nutrients were added weekly for the first three weeks, followed by a nutrient deprivation phase during which fresh BHI medium was added only once a month. Biofilms of different ages were subjected to 1-, 3-, or 10-minute exposure to 2% chlorhexidine (CHX) or CHX-Plus. After treatment, the volume ratio of dead bacteria in biofilms was assessed by confocal laser scanning microscopy using a LIVE/DEAD viability stain. Biofilm structure was visualized using scanning electron microscopy.

Results: The thickness of biofilms increased from 57μm (2d) to 155μm (3w) during biofilm development. It reached a steady state under nutrient-limiting conditions, with the thickness of 190μm (6w) to 201μm (12 w). The proportion of killed bacteria in mature biofilms (3w) was lower than in young biofilms (2d, 1 & 2w) after treatment with both CHX products ($P<0.01$). The resistance of mature biofilms under the nutrient-limiting phase (6-12w) to chlorhexidine remained stable and was similar to 3-week old biofilm. CHX-Plus showed higher levels of bactericidal activity at all exposure times than 2% CHX ($P<0.01$).

Conclusions: Bacteria in mature biofilms and nutrient-limited biofilms are more resistant to CHX killing than bacteria in young biofilms. The results emphasize the importance of standardization of factors such as biofilm age when studying the comparative effectiveness of disinfecting agents against biofilm bacteria.

Abstract #38: Antibacterial Effect of a Novel Root Canal Irrigant

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Objectives: Periapical healing depends on successful elimination of microbes from the infected root canal. Current endodontic treatment is not able to predictably eliminate all bacteria; therefore, further improvements in disinfection are necessary. This study aimed to assess the efficacy of a novel root canal irrigant (Qmix) against *Enterococcus faecalis* and mixed plaque bacteria in planktonic phase as well as in biofilms.

Methods: A clinical strain of *E. faecalis* and mixed plaque bacteria were suspended in water and exposed to Qmix (pH=7.5), 2% Chlorhexidine (CHX) and BioPure MTAD for 5 seconds, 30 seconds and 3 minutes. Following the exposure, samples were taken, serially diluted and grown aerobically and anaerobically on Tryptic soya agar (TSA) plates or on blood agar plates for 24 and 72 hours, respectively. After the incubation, bacterial colonies were counted. *E. faecalis* and mixed plaque biofilms were grown for three weeks on collagen-coated hydroxyapatite (CHA) discs. The biofilms were subjected for 1 and 3 minutes to Qmix, 2% CHX and BioPure MTAD. The amount of dead bacteria in biofilms was analyzed by confocal laser scanning microscopy (CLSM) using viability staining.

Results: Qmix was the most effective solution against *E. faecalis* and plaque bacteria in planktonic state. It eliminated all bacteria for 5 seconds. CHX and BioPure MTAD were not able to kill all plaque bacteria after 30 seconds of incubation, and after 3 minutes *E. faecalis* was still alive. Qmix was superior also in the elimination of biofilm bacteria. It killed four times more bacteria than CHX and up to thirteen times more bacteria than BioPure MTAD after exposure to the solutions for 1 and 3 minutes.

Conclusions: Qmix was able to eliminate all bacteria in suspension in only 5 seconds. In addition, it killed four to thirteen times more biofilm bacteria than Chlorhexidine and BioPure MTAD, respectively.

Abstract #39: Evaluation of Canal Instrumentation Using GT Series-X™ versus Prosystem GT™

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Objectives: To compare the canal center displacement during root canal instrumentation of human teeth of two nickel-titanium rotary file systems using a split-mould design (endodontic cube).

Methods: Mesial roots of 31 mandibular molars with separate canals and curvatures ranging from 15 to 40 degrees were randomly divided into two groups of 32 canals. After access cavity preparation and working length determination, each tooth was embedded in composite resin using the endodontic cube as a mould. Each endodontic cube consisted of five brass pieces having horizontal grooves as indexes for precise reassembly. Tooth-resin complex slices of 1.5 mm were made using a Buehler™ saw. After reassembly of the sections, canals were randomly assigned to receive either GT-Series-X™ or ProSystem-GT™ instrumentation. Instrumentation to size 30/06 for both rotary systems was carried out according to manufacturers' instructions. Digital photographs were taken of all the sections before and after instrumentation and AutoCad™ software was used to measure canal enlargement and movement of canal centers by superimposing the images of the instrumented and non-instrumented canals. Data was analyzed using the *t*-test (p<0.05).

Results: The overall mean canal center displacement of the ProSystem-GT™ was 0.08 ± 0.074mm while for GT-Series-X™ it was 0.064 ± 0.063mm. Also, based on different canal regions, the canal center displacement above the curve (coronal third) of ProSystem-GT™ and GT-Series-X™ was 0.11±0.061 and 0.08±0.052, respectively. The canal center displacement at the curve region of ProSystem-GT™ and GT-Series-X™ was 0.06±0.0 and 0.05±0.027, respectively. Finally, the canal center displacement below the curve region of ProSystem-GT™ and GT-Series-X™ was 0.05±0.052 and 0.03±0.045, respectively.

Conclusions: Both rotary NiTi systems tended to move the original canal toward the furcation, but the ProSystem-GT caused significantly greater canal center displacement than the GT-Series-X. However, there were no significant differences between the regions of canal center displacement with either the GT-SeriesX or the ProSystem-GT.

Abstract #40: Screening Improves Time Delay to High-Risk Lesion Diagnosis

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Background: With 50-60% five-year survival rates, oral cancer has a poor prognosis, and is often diagnosed at its later stages. Detection of lesions at earlier stages results in better prognosis.

Objectives: 1) To characterize the experiences of patients with high-risk oral lesions (HRLs) from initial identification to diagnostic workup, and 2) To determine the factors that impact on diagnostic time delay, according to patient experiences.

Methods: A survey-type questionnaire was used to collect data on patient experiences leading to the diagnosis of an HRL. Patients attending the BC Cancer Agency with HRLs and diagnosed within 12 months of interview were invited to participate in this study.

Results: Among 114 patients interviewed, 67 (59%) patients had self-identified lesions (SIG) and 47 (41%) were identified by healthcare professional screening (PSG; 87% by dental professionals). Higher rates of invasive squamous cell carcinomas (SCCs) were identified by SIG as compared with PSG (75% vs. 43%, P=0.0008). Among those (N=52, 46%) who have a time lag greater than or equal to three months from the initial lesion identification to first diagnostic workup, there was a significant difference between SIG and PSG (55% vs. 32%, P=0.02). The most common symptoms for SIG were pain (78%) and non-healing ulcers (60%), which prompted these patients to seek healthcare attention and the majority of patients (77%) in PSG reported no symptoms. Surprisingly, 54% (28/52) of patients who experienced time lags indicated that they were not aware of oral cancer.

Conclusions: Oral cancer screening by healthcare professionals, in particular, by dental professionals, can provide a critical role in the identification of oral lesions at risk of cancer progression. Promotion of oral cancer awareness for both patients and healthcare professionals may facilitate earlier diagnostic workup of oral lesions, and consequently, better prognosis.

Abstract #41: Connexin-43 Regulates Key Genes Involved in Scar Formation

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Objectives: Excessive scar formation is a common unwanted outcome of wound healing. In scars, fibroblasts are responsible for excessive deposition of the collagen-rich extracellular matrix (ECM), the hallmark of scars. During wound healing, fibroblasts communicate via cell-cell adhesions mediated by gap junctions (GJ). Connexin-43 (Cx43) is the most abundant GJ protein expressed by fibroblasts. Interestingly, blocking its function accelerates wound granulation tissue formation *in vivo*, but little is known about the mechanisms involved and the role of Cx43 in scar formation. We hypothesized that Cx43 regulates expression of key genes involved in tissue repair and that blocking its activity promotes scarless wound healing.

Methods: In order to identify the gene expression changes that associate with GJ-mediated cell-cell communication, fibroblasts were cultured in low density (cells have few GJs similar to cells in normal tissue) and high density (cells have many GJs similar to wound fibroblasts), and expression of Cx43 and key anti-fibrotic and pro-fibrotic genes were analyzed using real-time PCR, Western blotting, zymography and immunostaining. To find out whether blocking of Cx43 affects fibroblast phenotype, high-density cultures were treated with a peptide that specifically blocks Cx43 function (GAP27) or with Cx43 siRNA to downregulate its expression, and gene expression was analyzed as above.

Results: In high-density cultures, expression of Cx43 was upregulated, and there was an increase in the proportion of Cx43 molecules that were phosphorylated and associated with cell-cell contacts compared to low-density cultures. This was associated with altered gene expression of key wound healing molecules by fibroblasts. Blocking of Cx43 in the high-density cultures resulted in upregulation of anti-fibrotic genes and downregulation of pro-fibrotic genes.

Conclusions: Cell-cell communication mediated by Cx43 regulates key genes involved in wound healing and scar formation in fibroblasts. Blocking of Cx43 regulates expression of genes that may promote scarless wound healing.

Abstract #42: Investigating the Sustainability of Community Dental Clinics for the Underserved

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Objectives: In British Columbia, community dental clinics have emerged to enhance access to dental care for underserved, low-income populations. However, little evidence is available on how well these clinics achieve this objective. Some clinics are volunteer-based and limited to emergency pain-relief whilst others provide comprehensive dentistry at reduced fees. The objective of this study is to investigate the financial sustainability of the community clinics that provide comprehensive dentistry.

Methods: Five community dental clinics were selected if they: 1) were not-for-profit; 2) provided comprehensive dentistry; 3) had paid professional staff; and 4) operated regular, full-time hours. Aggregate patient and procedural data from electronic files along with financial data for a complete year were collected and analyzed.

Results: The five clinics together employed about 31 full-time "equivalent" dental professionals and annually provided about 64,000 dental services during 23,000 patient visits for total revenues of approximately 4 million Canadian dollars. On average, two-thirds of the expenses related to salaries, one-fifth related to administration and capital costs (rent, utilities, etc), and the remaining costs related principally to clinical supplies and dental laboratory fees. Two of the clinics received 25% of their annual income from a local health authority, and this permitted the clinics to treat patients who could not afford to pay for dental treatment. Clinics operating without support from a health authority or other government agency depended mostly on payments from patients either directly or through private dental insurance, which reduced their ability to help patients with low incomes and without insurance.

Conclusions: Community dental clinics depend on government subsidies to address the treatment needs of communities with low incomes and no dental insurance.

Abstract #43: Curcumin Affects Proliferation, Migration and Apoptosis in Fibroblasts

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Objectives: Fibroblast proliferation and migration play a key role in pulmonary fibrosis. Preliminary studies revealed that curcumin, an antioxidant from the Indian spice, turmeric, can protect mice from bleomycin-induced pulmonary fibrosis. However, little is known about how curcumin relates to apoptosis in fibroblasts.

Methods: Bleomycin-stimulated fibroblasts were exposed to different concentrations of curcumin. Thiazolyl Blue Tetrazolium Bromide was used to study fibroblast proliferation. A cell wound healing assay was employed to study fibroblast proliferation and migration. Western blotting was used to evaluate the expression of cathepsins K and L, TGF-β1, bcl-2, bax, and caspase-3. Flow cytometry analysis was used to analyze cell cycle distribution.

Results: At various concentrations, curcumin can inhibit fibroblast proliferation and slow down the wound healing, especially at 30μm ($p<0.01$). Cell cycle distribution was changed with the treatment of curcumin. Curcumin stimulates a three-fold increase in cathepsin K and L expression, and a two-fold decrease in the expression of TGF-β1. The expression of caspase-3 and the ratio of bax/bcl-2 were increased two-fold in a curcumin dose-dependent manner. A potent cathepsin inhibitor was able to rescue cells from death.

Conclusions: Curcumin can inhibit bleomycin-induced fibroblast proliferation and migration, and affects the cell cycle. These effects are associated with an increasing expression of cathepsins L and K, a decreasing expression of TGF-β1, and an induction of apoptosis in fibroblasts.