Stem Cells and Dentistry

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Dental hygienists have an unprecedented responsibility to educate patients regarding stem cells and dental and oral regeneration. Stem cells are master cells that generate tissues and organs. In the oral cavity, stem cells generate all the structures involved in dental hygiene therapy, including enamel, dentin, cementum, gingival epithelium and periodontal ligament. Stem cells and related technologies will transform dentistry at a magnitude far greater than amalgam and dental implants once did, because stem cells, capable of generating tissues in native development, have the ability to regenerate tissues following trauma or disease. Imagine what the practice of dentistry will be like if the periodontium, including cementum, alveolar bone and periodontal ligament, can readily regenerate. This is no longer science fiction – biomolecules are being used to regenerate the periodontium in patients.

Stem cells are typically quiescent cells that reside in virtually every tissue and organ in the body. They are activated to participate in tissue turnover and homeostasis during aging, upon injury or in disease and play a central role in wound healing. Both the periodontal ligament and alveolar bone harbor stem cells. These periodontal and alveolar stem cells have the capacity to differentiate into bone and other cells, and participate in the healing of periodontal defects. Importantly, stem cells reside in the pulp of both deciduous and permanent teeth. Dental pulp stem cells are being explored for the regeneration of not only dental/oral structures, but for structures distant from the orofacial region. Dental stem cells may play important roles in future medical regenerative therapies.[1]

What can a dental hygienist do to educate patients on the coming revolution of stem cells and dental/oral regeneration? Patients will increasingly ask whether their extracted teeth and other dental tissues should be stored for stem cell "banking." Cryopreservation of stem cells has been a medical practice long before the discovery of dental stem cells. Following years of cryopreservation, a percentage of the stored stem cells retain their initial capacity.

Dental pulp stem cells are isolated by opening the pulp chamber and root canal of the extracted or exfoliated tooth to liberate cells out of the extracellular matrix. The isolated cells are then stored under ultra–low temperature to induce the arrest of cellular activities. While it should be the patient's own decision as to whether to "bank" their dental stem cells, dentists and dental hygienists have the newly added responsibility of educating their patients about the advantages and disadvantages of cell storage. On the plus side, the patient's own cells are stored for potential regenerative therapies for use that will likely not be limited to the regeneration of dental and oral structures. Autologous cells should not cause immune rejection or extrinsic pathogen transmission, risks that may occur with tissues from a different donor.

Others argue against storing dental stem cells, as there are no approved therapies at this time that utilize these cells. Conversely, proponents feel that it is only a matter of time before therapies will become available, justifying the need for storing these cells now. Those who promote the storage of dental stem cells further point out that more stem cells or stem cells of potentially higher potency are more likely to be present at a younger age, which supports the collection of dental stem cells from the pulp of deciduous teeth and from extracted premolars and third molars in children and adolescent patients. An analogy to what should be an amicable and dispassionate debate of cryopreservation of dental stem cells is perhaps the half glass of water: those who see it as half empty will probably opt not to store dental stem cells, whereas those who see it as half full probably would. Both parties are correct. The bottom line is that it should be the patient's decision whether to store dental stem cells, and dental professionals can assist their patients with understanding dental stem cells and the research regarding dental/oral/tissue regeneration. Dental professionals can gain important background information and new knowledge about the progress of dental stem cell research by staying current with published literature. Continuing education articles written for dental professionals about dental stem cells and dental/oral regeneration are also available.[2]

What can dental hygienists do as active participants, rather than bystanders, in the transformation of dentistry by stem cells and related technologies? The answer is simple – engage in research. A profession that fails to advance itself by new knowledge is not a profession that lasts. What will dental hygiene care be like for regenerated tissues and teeth? Dental hygiene evolved into a profession during a time when dental defects, including caries, gingivitis and periodontal disease, were repaired by scaling, root planing and restorations with amalgam and composites. What will be the new competency requirements for dental hygiene students and practicing dental hygienists in the era of dental stem cells and transformed dentistry when regeneration increasingly replaces repair? Answers to these questions can only be discovered in research. Abraham Lincoln once said, "The best way to predict the future is to create it." So, get involved.

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A Saliva–based Prognostic Test for Dental Caries Susceptibility

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Saliva has become the medium of choice for a variety of diagnostic tests that formerly employed blood or urine.[1] Current tests range from a simple measurement of alcohol to a complex, multi–analyte test for oral cancer. With solutions to stabilize DNA present in saliva, global genomics is possible with little more than "spit" and a postage stamp.

Among tests under development is a class that is not precisely diagnostic, but rather prognostic. We present here a prognostic test for caries susceptibility with the aim to provide scientifically based, individualized guidelines for preventing dental caries before they start. The remarkable decrease in the average number of caries in the U.S. over the last half century can be largely attributed to improvements in dental hygiene and nutrition. However, the complete eradication of caries by these methods is unlikely because inherent susceptibility remains that is due to host factors. The impact of these factors is very significant. Approximately 15% of all children under the age of 10 present with caries in their 6 year molars, despite living with benefits of regular oral health care. Approximately 30% remain caries free between the ages of 16 and 19 years–old (http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5403a1.htm).[2] If we knew in advance the degree to which each child is susceptible, procedures and treatments are available that realistically could prevent more than 90% of those remaining caries.

The caries susceptibility test, which we call the CARE test, is based on the types of oligosaccharides (sugar chains) attached to proteins in saliva. These oligosaccharides are analogous to, and representative of, one's blood types.

Oligosaccharide chains play important roles throughout the body for maintaining good health. However, they also appear to be the primary mechanism for attachment of pathogens to the host, often resulting in infection.[3] Different pathogens have different oligosaccharide requirements for attachment. Thus, an individual may be particularly susceptible to one pathogen whose preferred oligosaccharide is among that person's blood types, but not to another pathogen because of the absence of that preferred oligosaccharide.

The tooth pellicle is a coating of select salivary proteins with their attendant oligosaccharides. The primary function of these oligosaccharides is to provide lubrication to the tooth surface, thereby preventing excessive wear. If the pellicle is composed of oligosaccharides favored by oral cariogenic bacteria for attachment, it will likely lead to increased risk. Equally important is a caries prevention mechanism in saliva. The effectiveness of this system is also dictated by inherently produced oligosaccharides, which are attached to MUC7 mucin and other proteins called agglutinins. If these oligosaccharides are capable of binding with the cariogenic bacteria, they form protein–bacteria aggregates while still in the fluid phase of the saliva. Once aggregated, bacteria are prevented from attaching to the pellicle. If an individual does not make the types of oligosaccharides that promote this aggregation, caries susceptibility is further enhanced. The dental caries susceptibility test is based on the ratio of oligosaccharides that contribute to the 2 processes.

The CARE test typically uses whole, resting saliva (collected by drooling) and measures the specific oligosaccharides on small dots of dried saliva. The amount of each type of oligosaccharide is fed into a mathematical algorithm that was developed from the caries histories (DFT) from young adults. The test, when applied to the saliva of children, projects what the individual caries patterns in permanent teeth would be as young adults, if preventive measures are not employed. While the test can yield an estimate of the total number of caries that can be expected as the child matures, the algorithm has been modified to provide insight to the groups of teeth most susceptible.[4] This prognostication has the advantage of targeting specific tooth groups for preventive treatments on an individual basis.

The test stratifies children into 4 levels of susceptibility:

- Level 1 – no caries as a young adult
- Level 2 – caries on no more than 2 teeth
- Level 3 – 3 or more molars with caries
- Level 4 – 3 or more molars and/or premolars with caries

Levels 3 and 4 directly lead to targeted preventive strategies, such as which teeth should receive sealant applications. The 1 or 2 caries that are associated with level 2 typically do not appear until after age 14. Thus, we suggest these children are given special monitoring intended to identify the very early lesions when preventive measures are still effective. Overall, though the test output is limited to 4 levels and results in some preventive over treatment, this is not excessive and appears to be cost effective even in the short term.

As we look toward bringing the prognostic test to general usage while satisfying regulatory agencies, a new set of concerns must be addressed. Chief among these are to validate the prognostic value of the test in children and to calibrate the

test algorithm for all geographic locations it will be used. These goals are being pursued in a partnership between designers of the test and 1 or more dental insurers. This partnership provides the opportunity to focus on that portion of the population which will benefit most directly from the test, as well as the ability to pre–select individuals with a history of dental coverage. The latter is important because the caries restoration history can be reconstructed from claims records as a function of the age of the individual. This allows for validation of the prognostic value of the test by a so–called "retrospective prospective" study. Here, children at various ages are tested for their susceptibility level, which is then combined with their caries restorations to provide the historical record associated with each susceptibility level. These records, in the aggregate between 6 and 23 years old, will provide the benchmarks for prognostic validation at specific ages in future studies, as might be expected for approval by the FDA.

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Diagnostic Devices for Detecting Oral Cancer

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In the U.S., it is estimated that 34,000 Americans will be diagnosed with oral and pharyngeal cancer this year, causing over 8,000 deaths. Worldwide, oral cancer is the sixth most common malignancy, with more than 400,000 new cases diagnosed each year. Oral cancer is more prevalent than cervical cancer and Hodgkin's lymphoma. One American dies every hour from oral and pharyngeal cancers.[1] Unfortunately, diagnosis of oral cancer is established twice as often at a later stage, resulting in poor prognosis. In these situations, the overall 5–year survival rate is less than 50%.

Oral squamous cell carcinoma accounts for over 90% of oral cancers. Lesions often present as leukoplakia, erythroplakia or erythroleukoplakia. Risk factors for oral cancer include tobacco, alcohol consumption, infections (including human papilloma virus), mucosal diseases, exposure to ultraviolet light, ionizing radiation, arsenic or industrial chemicals, chronic irritation and immunosuppression. Other cofactors include chronic periodontal disease, poor oral hygiene, ill–fitting dentures, sharp teeth and edentulism.[2] Surprisingly, an estimated 25% of oral cancer victims do not fit the traditional profile of older users of tobacco and alcohol as they have no risk factors.

Early detection of oral cancer can be accomplished through a variety of approaches. The conventional oral examination (COE) is the main approach used by dentists and dental hygienists to identify oral abnormalities. Once identified, a scalpel biopsy and histologic examination of the lesion can be performed to determine the definitive diagnosis. However, it is difficult to visually diagnose premalignant and malignant pathoses. As well, not all clinicians routinely perform a COE.

To improve opportunities for diagnosing oral lesions, adjunctive diagnostic techniques have been developed and marketed among the dental community. These devices include toluidine blue (TB) staining, light–based detection systems, narrow emission fluorescence and brush biopsy.

TB has been used for over 40 years to detect mucosal abnormalities. TB is a metachromatic vital dye that tends to bind preferentially to tissues undergoing rapid cell division to sites of DNA change associated with oral premalignant and malignant lesions. It has been useful for demarcating the extent of a lesion prior to surgical removal. An overall sensitivity of 93.5% and specificity of 73.3% had been previously reported.[3] However, a recent meta–analysis reported a wide range of variation with respect to sensitivity and specificity.[4] In addition, no randomized clinical trials have been conducted to assess TB.

Light–based detection systems use chemiluminescent light to enhance visualization techniques. A pre–rinse of 1% acetic acid solution is used, followed by examining the oral cavity with a blue–white light source. Three systems are currently on the market including ViziLite Plus with TBlue (Zila Pharmaceuticals), Microlux DL (AdDent) and Orascoptic DK (Orascoptic, a Kerr Corporation). The ViziLite system combines a blue–white light energy source with TB staining. The Microlux DL system uses a blue–white light–emitting diode and a diffused fiber–optic light guide. The Orascoptic DK system is a 3–in–1, battery–operated, hand–held LED instrument that has an oral lesion screening instrument attachment. These light–based detection systems can enhance visualization of oral white lesions, but they cannot distinguish between oral malignancy, premalignant lesions, benign keratosis and other mucosal inflammatory lesions. No published studies were found for the Microlux DL or Orascoptic DK systems. Several studies of the ViziLite Plus with TB demonstrated improvement in specificity, reduction of the false positive rate by 55.26% and increasing the negative predictive value to 100%.[4]

Narrow emission fluorescence involves exposure of the mucosa to the blue light spectra using the VELscope® device (LED Dental). Tissue undergoing neoplastic change, such as dysplasia and invasive carcinoma, will demonstrate a loss of fluorescence. This system has been promoted as useful in assessing lesion margins enhancing surgical management. A summary of 2 studies evaluating VELscope indicated both sensitivity and specificity were high. However, these studies were of known lesions confirmed by biopsy. This system was not studied in relation to use as an adjunct for detection of new lesions.[4]

Recently, a new multispectral fluorescence device has been introduced, the Identafi™ 3000 (Trimira™). This system uses 3 distinct color wavelengths to distinguish lesion morphology purportedly reducing false positives. However, no published studies were found on this system.

Brush cytopathology using the OralCDx Brush Test system (Oral CDx Laboratories) involves the microscopic study of cell samples. A specialized brush that collects transepithelial cells are smeared onto a glass slide and sent to a laboratory for staining and analysis. A computer–based imaging system ranks the cells on the basis of degree of abnormal morphology followed by a cytopathologist who interprets the results. Reported accuracy, sensitivity and specificity results vary. Use of this test has been recommended for assessment of lesions the clini-

cian might not investigate further.

Although the opportunity exists to utilize adjuncts in detecting precancerous and cancerous lesions, there appears to be a lack of definitive evidence to imply that any of these systems improve the sensitivity or specificity of oral cancer screening beyond COE alone.[5] Ultimately, the scalpel biopsy and histologic examination remain the gold standard for achieving definitive diagnosis. Nevertheless, early detection of oral squamous cell carcinoma will only occur if dental professionals are looking for it.

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