Updates Available!: Time for Orthodontics' research approach to catch up with the rapidly evolving techniques and discoveries in Molecular Biology research ?

For years, orthodontic research into the mechanisms of orthodontic tooth movement has been confined to and dominated by the same singular basic tenet of bone remodeling.[1] To put it in an oversimplified way, applied force is mechanotransducer by cells in the periodontal ligament (PDL) through primary messengers (such as prostaglandins, cytokines, interleukins, growth factors, and hormones) and secondary messengers (for example, cyclic Cyclic Adenosine Monophosphate (cAMP), Cyclic Guanosine Monophosphate (cGMP) and phosphatidylinositols) which in turn activate osteoblasts which deposit bone and osteoclasts which resorb bone, all of which bring about tooth movement eventually.[1] Research has remained limited to investigating the levels of these messengers in gingival crevicular fluid or saliva of patients with different treatments[2,3] or identifying the cells they activate by immunohistochemical staining of PDL and associated tissues in animal experiments.[4] The mere elevation or depletion of these markers may not be definitely conclusive but often is taken to indicate a physiological occurrence one way or the other – for example, increased Tartrate-resistant acid phosphatase (TRAP) staining is indicative of higher number of osteoclasts, which invariably is taken to imply higher levels of bone resorption wherever they are detected. If only it was so simple in reality. Science, as it has done countless times in the past, has a way of shattering established paradigms, necessitating a re-calibration and reset of the entire way of looking at a certain area of research.

One such area is the role of receptor activator of nuclear factor kappa-B (RANK)-RANK ligand (RANKL) axis in bone remodeling.[5] Until now, it was unequivocally accepted that the RANK receptor on preosteoclasts and osteoclasts binds with the RANKL generated by the osteoblast, which serves to activate osteoclasts and enhanced bone resorption. However, in the last few years, evidence has been accumulating that osteocytes are the major sources of RANKL while the exact physiological roles of osteoblastic RANKL remain mostly unknown.[6,7] A study published in the September 2008 issue of Nature by Ikebuchi's team from Japan now shows that not only does osteoblastic RANKL combine with RANK to promote osteoclastogenesis but also vesicular RANK from osteoclasts combines with RANK to promote osteoblast differentiation and bone remodeling.[8] In other words, the RANK-RANKL signaling is bidirectional, with forward signaling stimulating osteoclast differentiation and reverse signaling activating Runt-related transcription factor 2 (Runx2) leading to osteoblast differentiation. The authors conclude that "our findings indicate that the role of RANKL is the accelerator of bone turnover rather than the stimulator of bone resorption."[9] Suppression of the reverse signaling in mice disrupted bone formation which shows that osteoblastic RANKL is essential for normal bone remodeling since it couples both resorption and formation and not drives osteoclastogenesis alone as previously thought.

The impact of this finding on the bone turnover mechanisms in any field cannot be overstated. To quote just one previously used example, the presence of TRAP does not indicate bone resorption alone, since the osteoclasts involved are reverse signaling and regulating osteoblast-mediated bone deposition as well! This necessitates re-examining of the entire cascade of events that trigger tooth movement after a force is applied and how experiments will now be designed to study these. Biological systems are characterized by complex, multidirectional, redundant/synergistic signaling, many of which remain undiscovered and unknown. Hence, deep thought and careful analysis have to be applied to develop a research hypothesis and determine what components will be investigated and how, bearing in mind the unknowns and factoring in the newly available developments.

Another research area of deep interest to orthodontists is white spot lesions, which undoubtedly profoundly affects our patients' smiles. Many studies in this area in orthodontics seem to investigate the role of *Streptococcus mutans*,[10-12] assuming that it is the main culprit for causing caries, even though caries has now shown to be of polymicrobial etiology with host-dependent pathogenicity of specific microorganisms.[13] The default assumption that *S. mutans* alone is the sole and most important cariogenic bacteria needs to be updated.[14] The presence of several other bacteria in enamel caries and white spot lesions in addition to *S. mutans* which was cariogenic has been adequately demonstrated.[15] In addition, saliva is a surrogate indicator and does not directly indicate microbial pathogenicity, in spite of which it is most frequently used to assess microbial levels. Bacterial types and levels vary

not only according to the region of the mouth but also according to specific surfaces of a single tooth alone. Hence, what matters is the presence of specific type, quantity and pathogenic activity of bacteria in the bio-film or plaque surrounding the brackets and the unique outcome variation this has in different patients, as determined by individual host factors.[16] Microbiological analysis in today's day and age is far advanced than doing salivary assay of a lone bacterial species, which can often be misleading. Treatments designed on such faulty assumptions may be the reason why they do not work as well as one thinks they should. Microbiome analysis through non-culture methods such as DNA sequencing, polymerase chain reactions, and 16S ribosomal RNA analysis of hundreds of species and strains of bacteria is being done in a cost- and time-efficient manner on a routine basis and is undeniably the future of research.[15] This has already resulted in the development of the Human Oral Microbiome Database (http://www. homd. org), which lists all bacterial species found in the human mouth, and can serve as a starting point for many new research projects pertaining to microorganisms relevant for specific issues being dealt by various dental specialties including orthodontics.

Another emerging relevant field for orthodontics is "metabolomics." The European Bioinformatics Institute defines metabolomics as "the large-scale study of small molecules commonly known as metabolites, within cells, biofluids, tissues, or organisms. Collectively, these small molecules and their interactions within a biological system are known as the metabolome."[17] The value of metabolomics lies in the fact that it depicts the real-time biochemical changes taking place in the body, whether in health or in disease. A recent study used metabolomics to assess risk factors for root resorption in patients undergoing orthodontic treatment, a malady worrying orthodontists right since the advent of orthodontics.[18] Metabolomic profiling of patients helped in identifying metabolites or biomarkers in saliva of patients susceptible to root resorption. This holds a lot of promise for future research to monitor treatment progress and institute potential remedial measures.

Similarly, there are also large-scale studies of genes, RNA, and proteins, which are known as metabolomics, transcriptomics, and proteomics, respectively. These methods are now being used to deliver "precision medicine" or personalized medicine based on individual biological characteristics, to deliver the exact type of treatment that will work in the medical specialties, especially in patients who have been refractory to commonly used, generic treatment methods. One can definitely begin to think of the day when "precision orthodontics" no longer remains a dream but becomes a reality. Incorporating these sophisticated tools into orthodontic research as they become available is the future forward way, lest one keep running around in circles trying to find solutions based on erroneous or out-of-date techniques. As rapidly does the technology develop, so do the approaches in molecular biology research evolve; propelled by the hitherto inaccessible biological frontiers, getting unlocked and made accessible by these very technological advances. It is time for orthodontics to be in lockstep with these rapidly changing times in basic research and incorporate the cutting-edge, innovative techniques in orthodontic research, all of which will highly enhance the quality of service we can provide to our patients.

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