Orthodontics in the Year 2047: Genetically Driven Treatment Plans

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The past 40 years have seen rapid biomedical advances leading to treatment modalities that could not have been predicted decades ago. Clinically relevant discoveries in orthodontics during that period have occurred mainly in materials science and appliance design. Although progress in those fields will continue to affect the orthodontic profession, advances in genetic testing, gene therapy, pharmacogenomics, mechanogenomics, and stem cell therapy are likely to produce the most dramatic changes in orthodontic treatment in the next 40 years.

It is impossible to accurately predict the most significant developments in technology, science, and medicine decades into the future. As the famous computational neurobiologist Terry Sejnowski recently noted, "The structure of DNA was discovered in 1953 and the human genome was sequenced in 2003. I once asked Francis Crick if back then he thought the human genome would be sequenced in his lifetime. He said it never occurred to him."[1] In fact, the publication of the human genome sequence by the International Human Genome Sequencing Consortium and Celera Genomics revolutionized biomedical research.[2,3] Technological advances including the automated "shotgun" method[4] allowed Celera to sequence the human genome in about nine months.[2] The initial "rough draft" of the billions of nucleotides in the human genome continues to be revised,[5] and future technology is likely to lead to faster and more accurate results.

The importance of advances in high-throughput genome sequencing was emphasized last year with the announcement of the Archon X Prize for Genomics—$10 million for the first team to successfully sequence 100 human genomes in 10 days or less. If the past is a predictor of the future, this prize is likely to be awarded within the next decade; eight years after the announcement of the $10 million Ansari X Prize for Private Spaceflight, it was won by aircraft designer Burt Rutan and Microsoft co-founder Paul Allen. In addition, the National Human Genome Research Institute has established the long-term goal of sequencing an individual's genome for less than $1,000 and is currently funding projects to overcome existing technical barriers.

With such significant resources directed toward high-throughput genome sequencing, it is likely that 40 years from now, health-care professionals will have their patients' genomes available for analysis. Although the information encoded by

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billions of nucleotides will affect orthodontic treatment in ways that we cannot currently imagine, examples of the impact of genetic testing, gene therapy, pharmacogenomics, mechanogenomics, and stem cell therapy may provide hints of what lies ahead.

Genetic Testing

The potential benefits of genetic analysis are best illustrated by the advances made in the past 15 years in our understanding of the genetic basis of Treacher Collins syndrome. Features of this disorder, which is sometimes encountered by orthodontists, include micrognathia, midfacial hypoplasia, microtia, conductive hearing loss, and cleft palate. In the early 1990s, the location of the mutation responsible for Treacher Collins syndrome was narrowed to a relatively small region on chromosome 5.[6] A few years later, the gene Treacle/TCOF1 was identified in this region, and mutations were found in individuals in five unrelated families.[7] Recently, the cellular mechanism underlying Treacher Collins syndrome was revealed through the generation of mice with a mutation in TCOF1.[8] Analysis of the mice demonstrated that insufficient amounts of TCOF1 led to reduced cranial neural crest formation and proliferation due to insufficient ribosome biogenesis. Of the 51 mutations associated with Treacher Collins syndrome, most have been shown to lead to the formation of a truncated protein that probably has no function.[9]

The mutations involved in Treacher Collins syndrome have been identified because of the autosomal dominant inheritance pattern and unambiguous clinical manifestations of this disorder, but subtler abnormalities in craniofacial form will likely be explained through similar mechanisms in the coming decades. The size of the mandible (as well as that of the maxilla) is partially regulated by the number of neural crest cells that migrate successfully into the first pharyngeal arch. Mutations in genes such as Treacle that lead to less pronounced changes in protein function or expression may be responsible for the milder cases of mandibular retrognathia commonly seen in orthodontic practice.

Mandibular prognathism has recently been mapped to regions on chromosomes 1, 6, and 19.[10] Even though this condition seems to involve multiple genes, genetic research into mandibular prognathism is probably less than 20 years behind research on Treacher Collins syndrome. Given the accelerating pace of scientific advances, in-office screening for the genetic basis of mandibular prognathism may be possible within the next few decades.

Although genetic screens for various diseases currently exist, future progress in identifying the functions of genes in facial development and the mutations that affect these functions could change orthodontic practice. Analysis of the genetic background of "responders" to growth modification, for instance, would allow orthodontists to apply appropriate treatment methods judiciously, thus reducing treatment time for the average patient. Rather than making an educated guess regarding a young patient's future growth, orthodontists will be able to use software that detects mutations in a patient's genomic sequence and provides a genetic growth prediction based on these variations.

Gene Therapy

Gene therapy involves the insertion of genes into an individual's cells or tissue to treat a disease. For gene therapy to be successful, the gene must be targeted to a specific cell type population; once the gene is within its targeted cells, its expression levels must be controlled.

Various strategies can now be used to achieve spatial and temporal control of the expression of any gene in several orthodontically relevant tissues. One example related to cranial sutural growth is the use of a fragment of the collagen Iα1 promoter to drive the expression of a fluorescent protein in the developing mouse calvaria.[11] This demonstrates how the expression of a gene of interest could be targeted to the osteoblasts lining the calvarial bones (Fig. 1). Another example related to mandibular condylar growth is the use of a promoter fragment of the collagen IIα gene (Fig. 2) and the 2.3kb promoter fragment of the collagen Iα1 gene fused to fluorescent proteins

(Fig. 3). If the promoter fragment were instead linked to a gene of interest, the expression of that gene would occur only in the areas where the particular promoter fragment drives expression.

Continued development of techniques that allow the temporal and spatial control of gene expression will be critical to the development of clinically relevant tools. At least three approaches are likely to be followed:

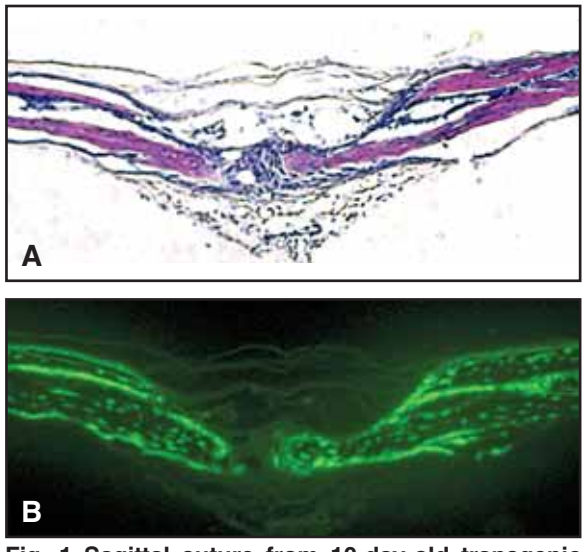

Fig. 1 Sagittal suture from 10-day-old transgenic mouse with 2.3kb fragment of collagen type I promoter fused to emerald (green) fluorescent protein. A. Hematoxylin and eosin stain. B. Fluorescent image, showing emerald fluorescence localized to parietal bone fronts and absent from sutural mesenchyme.

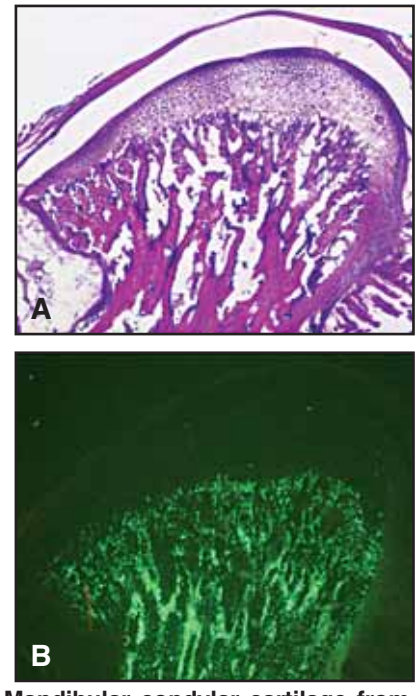

Fig. 3 Mandibular condylar cartilage from 21-day-old transgenic mouse with 2.3kb fragment of collagen type I promoter fused to emerald (green) fluorescent protein. A. Hematoxylin and eosin stain. B. Fluorescent image, showing emerald fluorescence localized to subchondral bone and absent from condylar cartilage.

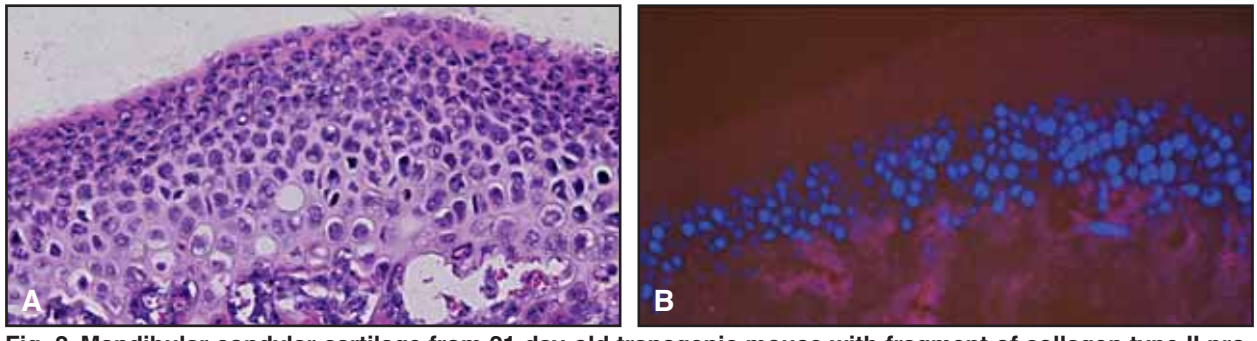

Fig. 2 Mandibular condylar cartilage from 21-day-old transgenic mouse with fragment of collagen type II promoter fused to cyan (blue) fluorescent protein. A. Hematoxylin and eosin stain. B. Fluorescent image, showing cyan fluorescence localized to deep layer of condylar cartilage and absent from subchondral bone.

1. Gene therapy for sutural growth disturbances. Mutations in FGFR2 have been linked to several human craniosynostosis disorders, including Pfeiffer, Apert, and Crouzon syndromes.[12] Analysis of transgenic mouse models harboring orthologous mutations, as well as mice lacking FGFR2, have revealed both the conservation of these signaling pathways and potential mechanisms of action. The primary role of FGFR2 in sutures appears to be in regulating the proliferation and differentiation of osteoblast progenitor cells.

Normal suture morphogenesis in the cranial vault relies on a balance between cell differentiation (into osteoblasts) at the osteogenic fronts and the proliferation of osteogenic precursors within the sutures themselves. A complex series of signaling interactions among the developing brain, the overlying dura mater, the growing ends of the calvarial bones, and the sutural mesenchyme uniting the bones are involved in determining whether sutures remain proliferative and "open" or closed through bony union. These signaling interactions allow growth at the sutures to accommodate the growing brain.[13-15]

Human mutations in FGFR2 appear to cause increased proliferation of the osteoprogenitor cells within the sutural mesenchyme. This proliferation leads to advancement and eventual fusion of the opposing calvarial bone fronts, resulting in synostosis. Patients with craniosynostosis usually require surgery to excise the fused calvarial bone and allow expansion of the cranial vault during growth. Multiple procedures are often necessary because of recurring fusion of the calvarial bones.

In 40 years, we will probably be able to address the underlying cause of this debilitating condition rather than treating its external manifestations. Although current gene therapy trials have been effectively halted as a result of several tragic outcomes, future technological advances will make current methodologies appear crude at best. In cases of craniosynostosis involving mutations in FGFR2, temporarily blocking FGFR2 signaling in the preosteoblasts within the sutural mesenchyme or providing a different antiproliferation signal to these cells would allow normal sutural growth without surgical intervention. One can imagine clinical scenarios akin to today's orthodontic treatment model in the management of these cases if genetic modulation were designed to degrade over several weeks. Monthly visits to assess the patency of the sutures would be followed by a decision on whether to continue or discontinue alteration of the sutural signaling.

2. Gene therapy for mandibular growth. Whether functional appliances can actually increase the length of the mandible has been a longstanding controversy in orthodontics. Studies in monkeys have shown that the use of functional appliances does cause remodeling of the TMJ[16] and perhaps lengthening of the mandible,[17] but the genes responsible for this growth induction have not been identified. In contrast, studies of rats by Hägg and colleagues have demonstrated that the use of functional appliances causes transient up-regulation of a number of genes (PTHrP,[18] Indian hedgehog,[19] Runx2,[20] collagen type X,[21] and VEGF[22]) in the mandibular condylar cartilage. In rats, however, the use of functional appliances does not appear to cause mandibular lengthening.[16] Because of limited access to human tissues, no studies have examined whether functional appliances can produce changes in human gene expression.

Most orthodontists would agree that the response to functional appliances varies widely among patients.[23] This variability has been attributed to differences in patient compliance and appliance design. Within the next 40 years, however, identification of the specific genes involved in patients' response to functional appliances will be able to help the orthodontist predict an appliance's chances of success in a given individual.

Successful gene transfer to the TMJ with the use of recombinant adeno-associated virus[24] and lentivirus[25] has been reported in animal models. If the next 40 years bring a clearer understanding of the genes responsible for mandibular growth and safe methods of transducing genes into tissues, gene therapy may become the standard of care for the treatment of mandibular-deficient malocclusions.

3. Gene therapy for orthodontic tooth movement. Tooth movement depends on the remodeling of alveolar bone, which is controlled by osteoclasts and osteoblasts. These have two different sources:

stromal cells (osteoblasts) and hematopoietic cells (osteoclasts). The formation of mature bone-resorbing osteoclasts from hematopoietic precursors requires interaction with cells from the osteoblastic lineage.[26] Periodontal ligament cells or osteoblastic cells are therefore said to be necessary to "support" osteoclastogenesis. The molecule mediating this interaction is the receptor activator of the NF-kappa B (RANK) ligand, or RANKL.[27] Osteoblastic cells express RANKL as a membrane-associated factor, induced by multiple stimulators of resorption, including PGE_2.[28] Osteoclastic precursors express RANK, the receptor for RANKL. RANKL is also a ligand for osteoprotegerin (OPG), which is produced by osteoblastic cells or periodontal ligament cells and acts as a decoy receptor for RANKL, preventing RANKL-RANK binding.[29] Excessive OPG expression can thus suppress osteoclastic formation.[30]

Two elegant studies by Kanzaki and colleagues have used gene therapy with OPG and RANKL to accelerate and inhibit orthodontic tooth movement in a rat model. Local RANKL gene transfer to the periodontal tissue accelerated orthodontic tooth movement by approximately 150% after 21 days, without eliciting any systemic effects. The authors concluded: "Local RANKL gene transfer might be a useful tool not only for shortening orthodontic treatment, but also for moving ankylosed teeth where teeth fuse to the surrounding bone".[31] In contrast, local OPG gene transfer inhibited tooth movement by about 50% after 21 days of force application.[32] The authors concluded that local gene transfer is more advantageous than pharmacological therapy because gene transfer "can maintain a local effective concentration and prolonged protein expression, regardless of blood circulation. Second, protein expression occurs at a local site, avoiding systemic effects."[32] Within 40 years, similar procedures may be used by orthodontists to reduce treatment time and improve results.

Pharmacogenomics

Pharmacogenomics is the study of how an individual's genetic composition affects the body's response to drugs.[33] Adverse drug reactions are one of the leading causes of hospitalization and death in the United States, accounting for more than 2.2 million hospitalizations and 100,000 deaths per year.[34] Currently, there is no simple way to determine whether an individual will respond well, poorly, or not at all to a given medication. Therefore, while pharmaceutical companies develop drugs for the "average" person, many patients experience deleterious effects.

Pharmacogenomics holds out the promise that drugs might one day be tailored to each person's particular genetic makeup. At present, however, the benefit of potentially accelerating orthodontic treatment by six to 12 months is outweighed by the risk of death or hospitalization due to adverse drug reactions.

Human clinical trials were conducted in the early 1980s to determine the effect of prostaglandins on the rate of tooth movement. Although local injections of prostaglandins accelerated tooth movement,[35] the main side effect of patient hyperalgesia has limited the use of this technique.[36] Prostaglandins bind to specific receptors on the cell surface; nine such receptors have recently been identified, along with their specific agonists and antagonists, which are now being produced by pharmaceutical companies. The use of prostaglandin receptor-type agonists and antagonists could maximize the benefits of prostaglandins in orthodontic treatment while minimizing adverse side effects.

Various pharmacological therapies for orthodontic anchorage control have been explored in animal studies. Most of these trials have involved the inhibition of osteoclast function (arginine-glycine-aspartic acid peptides[37] and bisphosphonates[38]), under the hypothesis that inhibition of bone resorption would reduce the rate of tooth movement. If pharmacogenomics can help identify the small population of patients susceptible to the associated side effect of osteonecrosis, the clinician would be able to administer bisphosphonates to help maintain anchorage control during orthodontic treatment.

Mechanogenomics

Mechanogenomics is the understanding of the

molecular connections between mechanical forces and gene expression in mammalian cells. In both human and animal orthodontic studies, equal forces have been found to produce substantially different rates of tooth movement in different individuals; conversely, different forces have produced nearly the same rate of movement in different subjects.[39] Similar findings have been noted in the orthopedic literature: for example, the anabolic response to mechanical loading and the catabolic response to unloading in bone are highly variable from patient to patient.[40,41]

Individual variations in the skeletal response to mechanical loading in both humans and mice may largely be determined by genetic factors. The genetic loci for these variations are now being identified in mice, and are likely to be pinpointed in humans within the next 40 years.

Orthodontic practice will be altered by the ability to predict individual responses to the anabolic effects of mechanical loading and the catabolic effects of unloading in bone. For example, a patient's individual genetic code could indicate that person's likelihood of losing alveolar bone after extraction of an unsalvageable tooth. This knowledge would affect orthodontic treatment planning, particularly the role and timing of implant placement.

Stem Cells and Tissue Engineering

Stem cells have several characteristics that other cells in the developing embryo or adult do not have: they can divide for long periods of time, leading to long-term self-renewal; they remain undifferentiated, without assuming the phenotypic characteristics of any differentiated cell type; and they can give rise to multiple (or all) cell types found in an adult. While the debate continues regarding the ethical and political aspects of stem cell science, the science itself continues to progress. Whether we will be using embryonic stem cells from a central cell "bank" containing human leukocyte antigen (HLA) haplotype matches appropriate for all individuals or adult stem cells from differentiated tissues such as fat or teeth will depend largely on the evidence provided by scientific studies over the next few decades.

Biological scientists will need to work with experts in other fields such as chemistry, mechanical engineering, and materials science to reconstitute clinically relevant, functional three-dimensional organs. Tremendous strides have been made in the field of "tissue engineering" with respect to regeneration of craniofacial structures. As Mao and colleagues have recently suggested, "Craniofacial tissue engineering is an opportunity that dentistry cannot afford to miss."[42]

In the case of craniosynostosis, surgical implantation of a functioning, tissue-engineered suture could replace the multiple surgeries often required today. Selective seeding of HLA-haplotype-matched, embryonic-stem-cell-derived osteoprogenitor cells on an appropriate matrix could produce a functional suture lacking the underlying mutation that leads to the recurrence of bony fusion. Such cranial sutures have already been engineered in rabbits.[43]

Within 40 years, it seems likely that stem cell biology and tissue engineering will produce viable biological alternatives for the treatment of missing teeth. Although progenitor or stem cells have been found in a variety of dental tissues,[42] attempts to reconstitute dental tissues from various cellular sources have had limited success. A recent study, however, reported the reconstitution and transplantation of a bioengineered tooth from single cells.[44] Dissociated epithelial and mesenchymal cells from a cap-stage mouse tooth were seeded onto a scaffold, grown in vitro, and transplanted into an extraction site. The bioengineered tooth contained normal hard tissues and supportive periodontal ligament, and viable blood vessels and nerve were found in the dental pulp.

In the future, a patient diagnosed with congenitally missing teeth in the early mixed dentition could be referred for biological tooth replacement. Tissue from the developing third molars could be harvested, and the cells could be expanded in vitro and seeded onto appropriate scaffolds for implantation into the desired sites. Future advances at the interface of the biological, materials, and engineering sciences are likely to bring about this clinically relevant treatment option.

Conclusion

Forty years from now, the underlying biology of an individual may be just as important as the malocclusion in the development of a treatment plan. All the predictions made in this article depend, however, on one highly uncertain variable: the availability of a critical mass of clinician-scientists to translate the inevitable scientific findings into clinically relevant therapies. The recruitment and retention of full-time academic faculty are critical to the continued growth of the orthodontic profession. Many academic programs do not have enough faculty members to fulfill their mission of teaching, much less advancing biomedical research. If orthodontics is to realize its full potential in the next 40 years, time and resources must be committed to developing the clinician-scientists of the future.

One thing is certain: science will progress. The question is whether the new scientific discoveries will be translated into therapies that result in safer, more efficient patient care.

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