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How orthodontic research can be enriched and advanced by the novel and promising evolutions in biomedicine

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Abstract

Recent advances in developmental, molecular and cellular biology as well as biomedical technologies show a promising future for crossing the gap between biomedical basic sciences and clinical orthodontics. Orthodontic research shall utilise the advances and technologies in biomedical fields including genomics, molecular biology, bioinformatics and developmental biology. This review provides an update on the novel and promising evolutions in biomedicine and highlights their current and likely future implementation to orthodontic practice. Biotechnological opportunities in orthodontics and dentofacial orthopaedics are presented with regards to CRISPR technology, multi-omics sequencing, gene therapy, stem cells and regenerative medicine. Future orthodontic advances in terms of translational research are also discussed. Given the breadth of applications and the great number of questions that the presently available novel biomedical tools and techniques raise, their use may provide orthodontic research in the future with a great potential in understanding the aetiology of dentofacial deformities and malocclusions as well as in improving the practice of this clinical specialty.

Keywords

orthodontic research, CRISPR, gene therapy, multi-omics sequencing, stem cells

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Introduction

The unprecedented growth of the biomedical innovations during the last few years has enabled state-of-the-art evaluation of many dental and craniofacial conditions. Nowadays, orthodontic research is attracting many healthcare professionals of various scientific fields. Orthodontics and dentofacial orthopaedics undergo an extensive reshaping by its collaboration and often integration with many scientific fields and clinical disciplines including the other dental specialties as well as surgery, anatomy, anthropology, biochemistry, developmental biology, embryology, engineering, genetics, imaging/radiology, microbiology, pharmacology and physiology. Among the various aims, synergetic research in these fields searches for answers with regard to the aetiology and manifestations of a wide spectrum of dentofacial deformities and malocclusions as well as their treatment (Castroflorio et al., 2017; Crutcher, 1997; English et al., 2002; Goodwin et al., 2014; Young et al., 2016).

It is promising that in the present era orthodontic diagnosis, therapeutics and outcomes will continue to herald future advances and innovations so that orthodontics and dentofacial orthopaedics will significantly contribute to oral healthcare and humans' wellbeing. The sophisticated molecular nature of oro- and dentofacial anomalies with

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their consequences have limited the value of the findings of many major well-done clinical trials to provide precise answers toward questions related to the aetiology, diagnosis and the management of different types of skeletal and dental conditions. It is apparent that here is a rising demand for effective novel strategies for the management of craniofacial anomalies and a great need for accurate, safe and non-invasive biomarkers, which will be capable of diagnosing and staging craniofacial growth as well as the different spectrums of malocclusion. These biomarkers may serve as crucial tools for tracking the progress and predictive potential of orthodontic treatments across different types of malocclusions. The implementation of these novel biomedical advances may introduce new alternatives in dealing with serious challenges in clinical orthodontics, such as the genetic and environmental contribution in the aetiology of malocclusion, and the biological mechanisms of orthodontic tooth movement, growth modification and post-treatment stability.

With the growing awareness of the contribution of genetics and environment in the aetiology of different forms of malocclusions, in addition to the response to the orthodontic interventions, it is crucial for clinicians to implement individualised treatment protocols in clinical practice and apply the best diagnostic and therapeutic tools for patient care. In this perspective, the future of orthodontic research needs to focus more on translational research and personalised orthodontics. The yields from these efforts will benefit from the emergence of novel technologies like CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), gene, cell and protein therapies, digital imaging, personalised medicine and regenerative dental therapies. Therefore, it is critical that orthodontists understand the basics of these novel technologies that are currently used in other medical fields, and that they become familiar with the fundamental concepts of providing individualised approaches to assess patients with skeletal deformities and/or malocclusions. The aim of the present review was to provide an update on the novel and promising evolutions in biomedicine and highlight their current and likely future clinical applications in orthodontics.

Current and future biotechnological opportunities in orthodontic diagnosis and treatment

Although the clinical practice of orthodontics is gradually supported by an increasing evidence-based component, still significant part of the subjects of aetiology of dentofacial deformities and malocclusions, facial growth modification, biology of tooth movement and post-treatment stability are characterised by incomplete understanding, limited knowledge or controversial concepts. In recent years, an increase of biomedical innovations that have been used in a variety of disciplines, including cancer, cardiovascular disease and craniofacial medicine, has taken place (Morris et al., 2020; Wilmes et al., 2013). These innovations include remarkable discoveries and advancements in computational and systems biology, bioinformatics, CRISPR, multi-omics sequencing, the mining of databases of clinical phenotypes, animal models, gene therapy and protein therapy, which are not widely used in orthodontic research (Jheon et al., 2017). At the same time in the field of craniofacial biology, several novel molecular approaches for clinical diagnosis have already been integrated into treatment protocols, especially in the rapidly growing trend of personalised treatment (Lalani, 2017). Many of these technologies produce large datasets that may extend from prions and microbes to humans' genomes, transcriptomes, proteomes and metabolomes with complementary three-dimensional (3D) imaging data. Moreover, the implementation of 3D tools and applications introduces novel solutions for many challenges, such as accurate diagnosis, personalised treatment, including precise appliance manufacturing, and placement. The following research tools and technologies have a great potential in the future of orthodontic research and each of them may open numerous novel pathways in progressing orthodontics by integrating different biomedical scientific fields with clinical applications.

Multi-omics sequencing

Advances in DNA sequencing, computational biology and bioinformatics have facilitated the understanding of the aetiology of many craniofacial anomalies (Dai et al., 2014; Moreno Uribe and Miller, 2015) as well as dental pathological conditions (Nascimento et al., 2017). Next-generation sequencing (NGS) has revealed at remarkable levels the high complexity of the oral microbiome (Belda-Ferre et al., 2012; Simón-Soro and Mira, 2015) metatranscriptome, (Benítez-Páez et al., 2014; Duran-Pinedo and Frias-Lopez, 2015) and metaproteome (Belstrøm et al., 2016). Starting with genomics, the recent development of novel sequencing technologies proved to be highly effective in understanding the aetiology of many craniofacial conditions. The term 'genomics' highlights the cost-effective comprehensive analysis and rapid interpretation of a whole-genome, as well as the study of all genes of interest at once, instead of the analysis of gene by gene (Yadav, 2007). A whole range of further omics technologies has been evolved, with 'omics' referring to the extensive analysis of the capacities, relationships and actions of various types of molecules in the living cells. This includes fields such as epigenomics (the study of the epigenetic regulation of the entire genome) (Stricker et al., 2017), transcriptomics (the study of all genes expression in a cell or organism) (Mutz et al., 2013), proteomics (the analysis of all proteins) (Aebersold and Mann, 2016) and metabolomics (the extensive analysis of all small molecules) (Li et al., 2017).

Moving into orthodontic research, the dynamic and sophisticated nature of skeletal deformities and malocclusions has often been overlooked due to the lack of quantitative

and systems approaches. The implementation of multi-omics technologies in orthodontics can supply clinicians with a substantial understanding of the flow of many clinical conditions, from the aetiology of malocclusions or dental abnormalities (genetic, environmental or developmental) to the functional repercussions or relevant interactions (Civelek and Lusis, 2014). Morris et al. (2020) investigated whether or not variation in craniofacial enhancers contributes to non-syndromic cleft lip and palate (NSCLP) (Morris et al., 2020). Using NGS, they sequenced 20 craniofacial enhancers in NSCLP probands. They identified both common and rare noncoding enhancer variants, individually and in concert, as likely contributors to NSCLP susceptibility.

Multi-omics studies can be used to identify possible cross-talking between different gene-expression spectrums and phenotypic variations like in Class II and/or Class III malocclusions of skeletal aetiology, which in turn will practically apply the concept of personalised orthodontic care. Some of the craniofacial phenotypes arise from mutations in a single gene, while others (like delayed eruption, ankylosis, relapse, etc.) involve many etiological factors, which are usually deterministic in the aetiology of the condition, although the progression and/or severity of many orthodontic clinical problems are affected by environmental factors (Hasin et al., 2017).

One of the biggest advantages of the multi-omics approach is that data can be efficiently and non-invasively generated from patient samples (e.g. extracted teeth, crevicular fluid, blood, periodontal ligament), at various stages of treatment (before, during and after the orthodontic intervention). In this way, a precise mechanistic insight of complex phenotypes can be developed over time and involving both environmental and genetic factors, and clinical tracking for the relevant molecular changes with the progression of malocclusion and/or orthodontic intervention (Cisek et al., 2016; Hasin et al., 2017).

Overall, the use of multi-omics technologies to achieve precision medicine in clinical practice is exceptionally valuable but is still in its beginning. It is anticipated that multi-omics technology will be an important tool in the era of precision orthodontics. In the near future, genetic and epigenetic variants information will be used to answer many clinically controversial questions such as the variability of the rate of orthodontic tooth movement, tissue response to growth modification and post-treatment stability. These advances will not be limited only to precision orthodontics but will be also expanded in a wide range of craniofacial research. It should be the beginning for generating comprehensive and unbiased data that are truly multi-omic.

CRISPR technology

One of the methods that researchers are exploring to combat genetic disorders is gene editing or CRISPR. CRISPR technology provides a precise and highly structured cleavage of a desired target DNA sequence giving the relative ease and simplicity of designing single guide RNAs (sgRNAs) (Miano et al., 2016; Rodrigues and Yokota, 2018). Moreover, this technology has the potential for multiplexibility through the use of different sgRNAs (Lian et al., 2018). Theoretically, gene editing could be used in the area of the genome that causes a certain genetic disorder. It could be used to develop better testing kits and could even be used to edit the human genome to prevent people from being infected by the virus. Recently, the U.S. Food and Drug Administration has granted an emergency use authorisation for a CRISPR-based COVID-19 diagnostic assay. The aim is to use the powerful gene-editing technology in order to figure out who is infected with the novel coronavirus (Kellner et al., 2019).

Dentofacial deformities and malocclusions resulting from genetic perturbations represent a real challenge for clinicians since the genetic problem cannot be modified. However, provision of various treatment plans that deal with the resulting phenotype as a way to alleviate the problem may be proved extremely helpful. In the future, the genetic structure to produce a normal genome sequence using CRISPR technology may be feasible to be manipulated. By identifying more causative genes (and mutations) for specific craniofacial malformations, a CRISPR-driven tool that can detect and fix the genetic structure of the genome could be discovered. For example, CRISPR technology has been used to reveal the importance of C-terminal domain of Msx1 gene in tooth and palate development. Mitsui et al. (2016) targeted Msx1 in mice with CRISPR system in homozygous mice exhibited agenesis of lower incisors with or without cleft palate (Mitsui et al., 2016). In the same context, MSX1 homeodomain also has been identified in non-syndromic tooth agenesis predominantly affecting premolars and third molars (Vastardis et al., 1996). Furthermore, CRISPR could be one of the alternatives that may be used in the fields of temporomandibular disorders and its overlapping pain conditions by targeting genome modification. Recent findings proposed the possibility of using CRISPR to engineer RNA-guided transcriptional activators and repressors targeting genes already linked to pain pathways (Munzenmaier et al., 2014).

Although therapeutic gene editing has started only at the basic research level, its use is still associated with a range of ethical issues, such as safety, equal access and consent. Future practical application of this tool in clinical orthodontics is promising and it may cause a paradigm shift in face growth modification and early orthodontic intervention. However, gene editing in humans must be proven to be absolutely safe before it can be offered as a treatment option.

Gene therapy

It is basic knowledge that the human genome consists of the genome (which represents the whole genes in every somatic cell nucleus), the epigenome (genes specific coding regions)

and the mitochondrial genome (a few genes located in the maternally inherited mitochondria found within each cell). Humans contain 21,000 genes, 19,000 pseudogenes and 37 mitochondrial genes (Feero et al., 2010). Gene therapy is the introduction of genetic material into cells to compensate for abnormal genes or to make a beneficial protein. For example, if a genetic mutation causes a problem in the expression of a necessary protein, gene therapy may be able to introduce a normal copy of the gene to restore the function of the protein. This technique provides health professionals with the chance to treat a disorder by simply inserting a gene of special interest into a patient's cells, rather than using various drugs or surgical interventions. Furthermore, this technique can be implemented to replace a defective or mutated gene that is the main root of the disease with a healthy or normal copy of the same gene. This technique can also be used to inactivate or 'knock out' a mutated gene that is malfunctioning, in addition to introducing a new gene into the body in order to facilitate therapeutic benefits for the patient against the disease.

The application of gene therapy in orthodontics and dentofacial orthopaedics seems to be promising in accelerating orthodontic tooth movement and in managing congenital anomalies due to well-known genetic mutations. Local RANKL gene transfer in mice resulted in acceleration of the orthodontic tooth movement by approximately 150% after 21 days, without inducing any systemic side effects, thus reducing treatment duration. It was proposed that Local RANKL gene transfer might be an efficacious tool not only for reducing the duration of orthodontic treatment but also for moving ankylosed teeth. Moreover, local OPG gene transfer reduced orthodontic tooth movement by about 50% after 21 days of application of force (Kanzaki et al., 2006). Besides controlling orthodontic tooth movement, gene therapy has shown promising results for controlling the pain associated with this process (Andrade et al., 2014).

Gene therapy could foster a paradigm shift in clinical orthodontics by reducing treatment duration and improving treatment efficiency. Notwithstanding that, it raises many unique ethical concerns. The ethical questions concerning gene therapy may include: How can the 'beneficial' and 'harmful' uses of gene therapy be differentiated? Who can decide that certain dentofacial patterns should be considered as normal, and which of them represent disability or disease? Will the additional cost of gene therapy in orthodontic treatment limit it only to wealthy patients? In more generic applications, should this technique be allowed to modify normal human traits, such as facial appearance?

Stem cells and regenerative medicine

Cell therapy describes the approach of introducing new or replaced cells into a tissue or organ to treat a disorder or disease. It is not a recent discovery since the principles for this technique evolved over the past century, and it includes blood transfusion, organ transplantation and bone marrow transplantation.

Stem cells are self-renewal cells that can differentiate toward various cell types (Chamieh et al., 2016; Safari et al., 2018). There are various sources to obtain stem cells like blood, umbilical cord, bone marrow, adipose tissue, periosteum, synovial membrane and teeth (Hass et al., 2011). Previous studies have reported the differentiation and proliferation potential of mesenchymal stem cells obtained from dental origin (Bakopoulou and About, 2016; Krivanek et al., 2020; Sharpe, 2016). Extracted primary teeth or permanent premolar or wisdom teeth are common interventions in orthodontics that can provide stem cells without extra morbidity (Gay et al., 2007; Khojasteh et al., 2015a).

Nowadays, stem cells and their applications in regenerative dentistry are introducing novel therapeutic modalities that may help to overcome some of the challenges encountered in their orthodontic daily practice. Some involve direct implantation of stem cells into the defect site while others use proper scaffolds to support the cells. Recently, stem cells have been used with composite scaffolds to regenerate bone besides restoring alveolar bone integrity in cleft patients (Behnia et al., 2009; Khojasteh et al., 2015b). Furthermore, there are ongoing efforts to use stem cells derived from adipose tissue to develop a tissue-engineered temporomandibular joint disk (Acri et al., 2019; Mäenpää et al., 2010; Melville et al., 2019). These efforts could be revolutionary in the management of internal temporomandibular joint derangements caused by injuries, tumours, inflammation and congenital anomalies (LeResche, 1997; Ta et al., 2002).

Investigators have also proposed the use of stem cells in conjunction with rapid maxillary expansion to minimise post-expansion relapse and increase treatment stability (Ekizer et al., 2015). The local injection of stem cells into the expanded maxilla helped to accelerate the process of bone formation, most probably due to their ability to differentiate into bone cells. Furthermore, the transfer of mesenchymal stem cells to periodontal ligament during maxillary expansion has been found to be effective in minimising orthodontically induced bone resorption and in facilitating enhancement of root repair (Gul Amuk et al., 2020).

To date, there is limited knowledge on the mechanism stem cells use to provide nutrients and particular growth factors as well as immune modulators during development as well as regeneration. Furthermore, there are rising questions about the predictability and the maintenance of stem cells during clinical use. It seems promising that in the coming years mesenchymal stem cells may be able regenerate human teeth in cases with congenital missing ones.

Conceptual change in the future of orthodontic research

In the previous section of the article, explanations have been presented on how orthodontic research shall utilise the advances in biomedical fields including genomics, molecular biology, bioinformatics and developmental biology. This includes uncovering the molecular mechanisms orchestrating craniofacial and dental development, orthodontic tooth movement and the possibilities to biologically intervene in these pathways in a practical and safe approach.

Change in orthodontic research interests should start from within dental academic institutions. Contemporary guidelines on postgraduate orthodontic specialty programmes provide a broad-based higher level of education in orthodontics and its allied biomedical sciences and clinical disciplines (Athanasiou and Eliades, 2015). However, orthodontic research is a few steps behind other medical fields and the number of journals and publications continues to grow exponentially, overwhelmingly with topics related to the development and testing of appliances and procedures used for treatment (Melsen, 2017). During a two- or three-year duration of most of the postgraduate orthodontic specialty programmes, the residents tend to focus on their clinical training, and if a research project should be undertaken, as a requirement for graduation, the selection of the topic depends on various factors (e.g. expertness or research interest of supervisor; availability of research facilities; funding; programme leading to a certificate or a Master's; duration of studies; research background, motivation and interest of the resident). It is a sign of optimism that a significant number of original research papers published in refereed journals are derived from the theses/dissertations of orthodontic residents. However, most of these residents are not engaged in basic research projects, which usually require interdisciplinary collaboration and significant research funding thus limiting the resources for such type of investigations (Al-Moghrabi et al., 2018). Furthermore, the existing number of well-designed prospective randomised controlled trials is not sufficient enough to address important clinical interventions or treatment approaches and, on the other hand, there is a growing trend towards publishing more systematic reviews and meta-analyses even though most of them do not lead to meaningful conclusions (Bastian et al., 2010; Papageorgiou and Eliades, 2019; Richards, 2018). It should be positively noted that many of these research reports are of interdisciplinary character and are published in non-orthodontic journals with impact factors (Alqaydi et al., 2018).

Conclusions

With the increasing prevalence and incidence of dentofacial deformities and/or malocclusions worldwide, novel biomarkers and therapeutics are important to ease the burden of these conditions. Given the breadth of applications and the great number of questions that the presently available novel biomedical tools and techniques raise, their use may provide orthodontic research in the future with a great potential in understanding the aetiology of dentofacial deformities and malocclusions as well as in improving the practice of our clinical specialty. In particular, orthodontic research will be greatly benefitted by performing in the future genetic and gene-environment studies, which can be performed on precisely defined phenotypes using transgenic mice as animal models. Moreover, postgraduate orthodontic specialty programmes can play an important role in promoting the novel evolutions in biomedicine by integrating these biological advances and their orthodontic applications in their educational curriculum and research activities.

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