Current Status of Low Intensity Pulsed Ultrasound for Dental Purposes

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Abstract: Over the past few years, tissue engineering applied to the dental field has achieved relevant results. Tissue engineering can be described by actions taken to improve biological functions. Several methods have been described to enhance cellular performance and low intensity pulsed ultrasound (LIPUS) has shown to play an important role in cell metabolism. The present article provides an overview about the current status of LIPUS as a tissue engineering tool to be used to enhance tooth and periodontal regeneration.

INTRODUCTION

Dental diseases affect millions of people worldwide. Tooth decay, periodontal disease, dental pulp infection and inflammatory root resorption can result in tooth loss and seriously compromise human health and quality of life. In this context, great efforts have been done to prevent oral diseases and restore teeth and periodontal integrity. Over the past few years, tissue engineering applied to the dental field has achieved relevant results. Tissue engineering can be described by actions taken to improve biological functions. Several methods have been described to enhance cellular performance and low intensity pulsed ultrasound (LIPUS) has shown to play an important role in cell metabolism. LIPUS stimulation is a classical therapeutic modality for bone regeneration and its efficiency has been widely reported over the years. Interestingly, recent studies have provided evidence that LIPUS plays an important role in the metabolism of periodontal cells and tissues as well. The present article provides an overview about the status of LIPUS stimulation as tool to be used to enhance tooth and periodontal regeneration.

1. LIPUS AND BIOLOGIC MECHANISMS

It is well known that mechanical stimulation plays a crucial role in regulating bone physiology. During bone remodeling, bone resorption and new bone formation are induced along the dominant local loading direction, suggesting that local regulation of bone remodeling is mediated by mechanical stimuli [1]. Osteoclasts initiate bone resorption in response to determined signals that may relate to local damage [2] and in response to a coupling signal, osteoblasts are recruited to deposit bone matrix, which is later mineralized [3]. The exact nature of the coupling signal is still unclear, but likely relates to local strain (deformation) levels. Osteoclast and osteoblast activity could thus be related to opposite strain modalities [4, 5].

It is well accepted that different patterns of mechanical stimulation can induce distinct cell response. During orthodontic tooth movement, for example, the networked reactions that occur in and around periodontal ligament and alveolar bone cells change compressive and tensile stresses into molecular events, resulting in bone resorption and formation, respectively [6]. Based on these evidences, various studies have been carried out to clarify the biological phenomena underlying orthodontic tooth movement. It has been shown experimentally that cells, located in the compression area, respond differently from those on the tension side in terms of gene expression and enzymatic activity of bone metabolism regulators [7]. Other types of mechanical stimulation could thus modify cell metabolism, including ultrasound.

LIPUS (intensity ranging from 30 - 100 mW/cm^2) is an acoustic radiation that can be transmitted into the living tissues as pressure waves resulting in biochemical events at the cellular level [8]. LIPUS has been shown to stimulate bone and cartilage cells *in vitro*, indicating that ultrasound exerts direct anabolic effects such as production of growth factors and other signaling molecules, osteogenic differentiation and extra cellular matrix production [9]. The mechanisms involved in LIPUS-stimulated tissue repair have not been elucidated yet, however, it is recognized that the anabolic bio-

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physical effects caused by LIPUS are most likely to be caused by mechanical stress and/or fluid micro-streaming impacting on the cellular plasma membrane, focal adhesion and cytoskeletal structures to trigger intracellular signal transduction and subsequent gene transcription [10, 11].

2. LIPUS AND BONE REGENERATION

Mechanical stimulus to bone is of a great importance for maintaining the bone mass and structural stability of the skeleton. When bone is mechanically loaded, movement of fluid within the spaces surrounding bone cells generates fluid shear stress that stimulates osteoclasts and osteoblasts, re-sulting in enhanced anabolic activity for bone remodeling with appropriate bone resorption and the subsequent new bone formation. The mechanisms such as mechanotransduc-tion process, by which osteoclasts or osteoblasts convert the external stimulation from fluid shear stress into biochemical changes, remain unclear [12].

In vivo studies have demonstrated that therapeutic LIPUS can promote bone repair and regeneration, accelerate bone fracture healing, and enhance osteogenesis at the distraction site [13-17]. It has also been shown in *in vitro* studies that LIPUS stimulation can enhance expression of bone forma-tion-related genes such as collagen type I and X, aggrecan, transforming growth factor beta [18], runt related gene-2, osteocalcin [19], insulin-like growth factor-I, bone sialopro-tein [20] and alkaline phosphatase [21]. In addition, LIPUS has been reported to promote protein synthesis and calcium uptake in various osteoblastic cell lines [22]. Moreover, LIPUS stimulation has been reported to enhance COX-2 gene expression and subsequently enhance endogenous prostaglan-din E_2 (PGE_2) synthesis in various osteoblastic cell lineages, playing an important role in bone remodeling [23-25].

3. LIPUS AND PERIODONTAL LIGAMENT REGEN-ERATION

Physiologically, the periodontal ligament is continuously subjected to mechanical stress caused by occlusal forces. Furthermore, remodeling of the ligament and alveolar bone occurs in response to orthodontic forces. Taken together, these clearly indicate that responses of the ligament to me-chanical stress are involved in its cell proliferation and differ-entiation. Periodontal ligament includes precursor cells of ce-mentoblasts at the perivascular area in the middle portion and shows greater differentiation toward the surface of the root [26]. In a previous study, cyclic stretch stimulation mediated periodontal ligament cells differentiation, thus regulating the function of the periodontal ligament as a source of cemen-toblasts and osteoblasts through the EGF/EGF-R system [27].

Regarding LIPUS effects, it has been reported to be ef-fective in releasing fibroblast growth factors from a macro-phage-like cell line [28]. We have reported previously that LIPUS induced early cementoblastic differentiation of hu-man immature cementoblasts from the periodontal ligament by promoting the formation of substrate and increasing alka-line phosphatase (ALP) activity, enabling the regeneration of periodontal tissue destroyed by periodontal disease and the acceleration of the repair of root resorption [29]. Mostafa et al. (2009) demonstrated that ALP and OPN expressions were also induced in human gingival fibroblasts treated with

LIPUS, confirming that after 3 weeks of 5min/day exposure the osteogenic differentiation potential was enhanced [30].

4. LIPUS AND CEMENTUM REGENERATION

Cementum is a thin mineralized tissue covering the tooth root surface and assists in anchoring teeth to surrounding alveolar bone, maintaining the structural stability and physiological function of the dentition [31]. Resorption of the dental root surface can be a relevant adverse outcome of orthodontic treatment. A certain degree of root resorption occurs in most treatment cases, ranging from just a slight apical resorption to a complete tooth root loss [32-36]. It is well accepted that the cementum layer covering the root sur-face plays a crucial role in preventing resorption during tooth movement. In addition, the damaged areas are also repaired in part by cementoblasts lining the root surface. Root resorp-tion during orthodontic treatment is thus confirmed as a mul-tifactorial event and several biological and mechanical fac-tors have been identified to increase its susceptibility, how-ever, the cause still remains unclear [37].

Cementoblasts share many characteristics with os-teoblasts, including similar molecular properties and the abil-ity to promote mineralization [38, 39]. Previous studies have shown that, as in bone, cementum metabolism is also con-trolled by mechanical stimulus. It has been reported that me-chanical loading enhances the expression of phenotypic makers such as OCN and BSP in cementoblast *in vivo*; how-ever, the expression was just moderately stimulated com-pared to osteoblasts [40]. Regarding ultrasound stimulation, a pioneer study published by El-Bially *et al.* (2004) showed that LIPUS prevented root resorption during experimental tooth movement in humans [41]. Studies performed by our group showed that LIPUS up-regulated the expression of several genes related to mineral metabolism in mouse ce-mentoblasts [42, 43]. We also reported in other article that LIPUS simulation significantly up-regulated COX-2 mRNA expression and enhanced PGE_2 production inducing cemen-toblastic differentiation and matrix mineralization through EP2/EP4 prostaglandin receptors pathway [44].

Furthermore, we have also accessed the inhibitory effect of a 21-day LIPUS application on root resorption using an experimental model of tooth replantation involving luxation and immediate replacement of maxillary first molars in rats [45]. The results evidenced that the area of root resorption lacunae was statistically decreased in LIPUS treated sample. The imunne-expression of tumor necrosis factor alpha (TNF-α) was not observed in LIPUS treated sample as was evident as in the control sample. In addition, it was shown *in vitro* that LIPUS may contribute to the reduction of the trauma-induced inflammatory reaction through impairment of the TNF-α signaling pathway, suggesting that LIPUS shows potential as a therapeutic tool to optimize the regenerative potential of periodontal tissues on replanted teeth [45].

5. LIPUS AND GINGIVAL REGENERATION

Clinical trials applying LIPUS in implant dentistry have reported accelerated soft-tissue healing as well as osseointe-gration. In addition, it was suggested that the ultrasound-treated wounds were at a more advanced stage in the repair process [28]. Despite, details of the clinical effects of LIPUS

have not been well characterized, great efforts have been done to clarify the cellular mechanisms underlying LIPUS-induced tissue regeneration. Ikai *et al.* (2008) reported that a daily LIPUS treatment protocol of 20 minutes for a period of 4 weeks has a benefic effect on gingival epithelium cells, accelerating periodontal wound healing after flap surgery [46]. In other study using gingival epithelial cells, Shiraishi *et al.* (2011) reported that LIPUS accelerates soft-tissue healing by increasing the expression of connective tissue growth factor (CCN2/CTGF), an important gene for wound healing and angiogenesis in periodontal tissues [47].

6. LIPUS AND IMPLANT OSSEOINTEGRATION

The use of endosseous dental implants for replacing missing teeth increased considerable over the last few years and may be considered as the current most popular treatment option for edentulous patients. This fact might be related to the great effort on improving the osseointegration process. Attention has been given to the surface and shape of the implant [48, 49]. Other approaches focused on acid etching of titanium surface [50], engineering of dental pulp cells on various implant surfaces [51] and biomimetic implant coatings containing bone morphogenetic protein-2 [52]. Other methods targeted on enhancement of endogenous bone healing around biomaterials through different forms of biophysical stimulations such as pulsed electromagnetic fields [53] or LIPUS [54].

A study of Tanzer *et al.* (1996) showed that LIPUS enhanced the rate and extent of bone growth into fully porous-coated implants inserted into dog femora [55]. Hsu *et al.* (2011) demonstrated *in vivo* that blood flow and mature type I collagen fibers were more prevalent around titanium implants, and bone formation was accelerated by ultrasound stimulation. In addition, tissue culture of MG63 osteoblast-like cells indicated that pulsed ultrasound effectively promoted cell migration and new bone regeneration [56]. Ustun *et al.* (2008) reported that the area, bone volume and bone-implant contact ratio values, which are significant parameters of histomorphometry assessments, have increased by LIPUS stimulation in tibial bone, suggesting that LIPUS application may accelerate and promote bone healing around dental implants leading to a higher quality and faster osseointegration [57].

7. LIPUS AND PULP CELLS DIFFERENTIATION AND TOOTH ERUPTION

Pulp tissue contains precursor cells which have the potential to differentiate into odontoblasts. It is well understood that after physiologic or pathologic stimulation pulp stem cells may be recruited to proliferate and differentiate into odontoblasts, which can produce dentin as a protection mechanism [58]. It is also well documented that LIPUS has the potential to induce bone and periodontal cells differentiation [13-17, 28-30]. In this context, the effect of LIPUS on pulp cells differentiation has risen as a subject of investigation. A previous study showed that LIPUS (30 mW/cm^2) promoted odontoblast-like cells differentiation through stimulation of vascular endothelial growth factor (VEGF) secretion [58]. It has also been shown that ultrasound stimulation (0.5 W/cm^2)

induced pulp stem cells to form reparative dentin formation *in vivo* by optimizing gene transfer of growth/differentiation factor 11 plasmid DNA and subsequently up-regulation of dentin sialoprotein gene expression [59].

An interesting study of El-Bialy *et al.* (2003) examined the effect of therapeutic ultrasound on mandibular incisor development and eruption in mature rabbits undergoing mandibular osteodistraction. The results evidenced that ultrasound application combined with distraction enhanced mandibular incisor growth and eruption. Histologic examination showed that the newly formed tissues at the distraction site and at the cut ends of the incisor segments consisted of osteodentin-like tissue and cementum [60].

8. LIPUS AND GENE DELIVERY

Periodontal disease or inflammatory root resorption is relevant pathologic condition that can lead to tooth loss. In this regard, several tissue-engineering techniques have been proposed to restore periodontal integrity [61]. Administration of growth factors, for example, has proved to exert positive effect; however, some clinical limitations such as proteolytic degradation, rapid diffusion, and solubility of the delivery vehicle have been still problems to be solved [62]. In order to optimize the results, gene transfer methods were introduced; however, progress in experimental and clinical periodontal gene therapy is limited by the immunogenicity and cytotoxicity of viral vectors and low transfection efficiency with regard to non-viral vectors [61, 63]. It has been reported that therapeutic ultrasound associated to echo contrast agents such as nano/microbubbles can optimize gene transfection *in vitro* and *in vivo* [63-67]. Recently, it has been shown that therapeutic ultrasound also provides an effective gene delivery system for bone and periodontal regeneration [68-70]. Because ultrasound-mediated osteogenic gene delivery has been just recently described, no consensus about optimal ultrasound intensity and its exposure protocol have been obtained yet. The use of LIPUS as a tool to optimize gene transfection was described by Watanuki et al. (2009). The study demonstrated that mouse calf muscles injected with BMP-4 plasmids and transcutaneous electroporated showed increased ectopic calcium and total collagen content and bone area when exposed to LIPUS [71].

9. DISCUSSION

Among the causes of teeth loss, inflammatory root resorption has received a great concern due to its unpredictability, difficult control and lack of biological understanding. the resorption of hard tissue in primary teeth is a normal physiologic phenomenon that leads to their exfoliation and ultimately the transition to permanent dentition. On the other hand, the hard tissues of permanent teeth are not resorbed under healthy conditions and the resorption is thus considered a pathologic process [72]. Different causes have been attributed to the root resorption process including pressure, inflammation, neoplastic process and systemic conditions [73]; however, the biological phenomena underlying root resorption are still not completely understood. Tooth root resorption is regarded as a major problem encountered after tooth replantation and orthodontic tooth movement. Eventually, the progression and severity of root resorption cannot

be controlled and culminate with the complete tooth loss. Approaches aiming to attenuate or inhibit root resorption and restore periodontal integrity during the dental practice are still the subject of debate and investigation. Some therapeutic approaches have been proposed to inhibit root resorption after tooth replantation and can induce periodontal regeneration [74-77]. However, with respect to root resorption induced by orthodontic tooth movement, less-accepted clinical protocols have been established, probably due to the difficult application and/or possibly side effects that medicamentous treatment can generate. In this context, non-invasive modality such as LIPUS therapy has been given increased attention and risen as promising therapeutic tool for the regeneration of periodontium.

The effectiveness of LIPUS for bone regeneration is already universally accepted and some recent papers have provided evidence that it can exert benefic effects in other kinds of tissues, including teeth. In addition, LIPUS presents low toxicity, low immunogenicity, non-invasiveness, highly targeted selectivity, and repeated applicability. However, the diversity of techniques, application protocols and ultrasound specifications found in the literature may cause confusion for the clinician. Bains *et al.* (2008) have pointed out that ultrasound application in both diagnosis and periodontal therapy seems to present promising results; however, long-term evidence-based studies are required to use ultrasound in routine periodontal practice [78].

10. CONCLUSIONS

Despite LIPUS therapy has been widely used in the fields of orthopedic surgery and rehabilitation, its availability by dental professionals is still incipient. The effects of LIPUS in bony tissue seem to be well understood, but the literature has still lacked for available information about its effects on periodontal tissues. The present review brings out current evidence that LIPUS has a positive effect on tooth and periodontal cells metabolism, suggesting that LIPUS can be a promising therapeutic tool for the regeneration of tooth support tissues.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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