Chemical and physical influences in bone and cartilage regeneration: a review of literature

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Key words: bone, cartilage, chemical stimulation, physical stimulation

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Introduction

Nowadays several studies demonstrate that influences given by chemical and physical stimulation to bone and cartilage exist. The first studies date back to the 50s and for a long time did not have a strong impact on clinical practice. In recent times, however, the findings arising from these studies are increasingly used to address clinical problems such as osteoarthritis or non-unions. The aim of this article is to make a review of the literature of the state of the art about physical and chemical influences on bone and cartilage.

Physical influences on bone

Updated data show that in the United States of America every year about 6 million fractures occur and that a percentage ranging from 5 to 10% of these fractures evolve in non-union(1). It is shown how the patient affected by non-union has a poor quality of life, even worse than dialysis or ischemic heart disease(2). Some non-unions are surgically treated, with varying success rates based on the type of non-union (atrophic vs. hypertrophic)(3). Delayed bone healing increases the use of resources and increases the costs of health care for both patients and health care suppliers(4).

The aim of this report is to analyze and compare devices known as bone stimulators, which use several technologies that promote bone healing via applying energy fields.

The first use of electric current in the treatment of bone diseases involves the electro-stimulation to induce or increase bone healing. Some reports confirm that Birch in 1827 experimentally used electric currents to heal a tibial non-union.

More recently, Fukada developed the theory of bone healing mediated by electric current, that led to the application of piezoelectric fields in bone healing (5). Yasuda first described the concept of piezoelectric crystals and their action on bone when a mechanical force or stress is applied(6). The bone, similar to the piezoelectric crystals, tends to have an equal distribution of positive and negative electrical charges, symmetrically distributed, that create a neutral equilibrium until a mechanical or electrical stress is applied. These electrical charges are typically associated with the abundant calcium phosphate crystals and transmembrane potential present in the extracellular matrix and cells of this tissue.

When a mechanical stress is exerted on bone, the negative potentials tend to align on the compression side, whereas the positive potentials tend to align on the tension side. Due to the increased presence of negative potentials on the compression side of bone, further research has found that bone production is enhanced at the regions of negative polarity, and bone resorption is stimulated in the areas of positive polarity and tension(1)(7). Moreover, regions with an increased

cellular activity tend to have negative potentials for example in the physis, and electronegative potentials promote cell proliferations in the fracture (8). In addition, of the electric fields stimulate the expression of Bone morphogenetic proteins, transforming growth factor-beta and insulin-line growth factor II in the extracellular matrix of bone and cartilage(9). The positive effect of electromagnetic fields and electric current in bone regeneration has led to the development of new technologies to improve healing. Many of the efforts have focused on the long bones fractures (especially the tibia); but recently, studies have been also focusing on foot, ankle and wrist delayed unions, non-union and arthrodesis(10)(11)(12).

Bone stimulation techniques are mainly used for delayed unions and non-unions. Delayed union is defined as a delay in bone healing following a bone fracture, which shows no signs of healing within six months of the fracture event. Non-union is defined is defined as a fracture that has not healed within 6 months.

These definitions do not take into consideration the physiological differences present in the healing process among different bones. According to Wiss and Stetson The designation of a delayed union or non-union is currently made when the surgeon believes the fracture has little or no potential to heal. (13). This definition, although realistic in clinical practice, does not consider the standardization of treatments but it allows the surgeon to decide on the subsequent therapies. However, it essential to remember that bone stimulation systems are only adjuvants to bone healing, and not the main actors of the process: modern methods of fixation, both internal and external, remain fundamental. Moreover, bone stimulation devices do not correct any deformities (varus/valgus or torsional) that may derive from the fracture.

Over the years, several devices have been developed that aim to stimulate bone healing growth.

The first and most important division is between devices that perform an electrical or an ultrasound stimulation. The subcategories include direct current (DC), capacitive coupling (CC), and pulsed electromagnetic fields (PEMFs, inductive coupling).

Direct current

DC stimulating devices are created to convey a continuous electric current to the healing bone. This technique requires a surgical approach, where a cathode is placed closed to the affected area or in direct contact via a transducer. This device allows to concentrate the maximum amount of energy in the target area and to provide a constant electric current. The obvious disadvantage is that surgery, albeit minimal, is necessary to position the device, with possible complications: infections, wound dehiscence, bleeding, continuous medication and discomfort for the patient. To date, there is no randomized controlled trial that demonstrates the real effectiveness of this type of device, but

clinical studies demonstrate efficacy up to 80%(14). These studies are mainly conducted on delayed unions and corrective osteotomies (15).

Capacitive coupling

The concept underlying the CC is similar: an energy source has more effect if it is concentrated in the area of interest. Unlike DC, the CC is non-invasive but it requires the application of skin electrodes close to the affected area. The DC needs an external transducer, a frequent battery change and can often lead to skin irritation. Several controlled studies have shown the efficacy of this type of treatment: the success rate varies from 60% to 77%(16); the best results have been obtained at the tibia level, probably due to thin layer of skin of this anatomical region(13)(17).

Pulsed electromagnetic fields

PEMF devices are non-invasive and do not require a surgical procedure. They are based on the possibility of conveying this type of energy through soft tissues. This type of device produces a low-intensity electromagnetic field, which mimics the physiological conditions of bone healing.(18) The advantages of this type of stimulation reside in the non-invasiveness and in the possibility of use even on cast devices. The application time is crucial: the manufactures suggest 3 to 10 hours per day as the literature data demonstrate a strong reduction in the reliability for short stimulations (19). When applied correctly, PEMF has shown good results in the treatment of delayed unions and non-unions. Higher-level studies have included a randomized double-blind trial for 31 femoral intertrochanteric osteotomies (20), a multicenter double-blind trial in 45 tibial shaft non-unions (21), and several other small comparison studies focused primarily in long bone delayed or non-unions (11). A meta-analysis of 2011 attempted to summarize four randomized controlled trials, but due to the high heterogeneity of the studies, this was not possible (22). Beherns et al, in 2013, assert that large, randomized, placebo-controlled trials are lacking, and much of the data reflect larger case series and comparative studies. Nevertheless, basic science and clinical evidence support the efficacy of bone growth stimulation as a fracture healing modality in the appropriate clinical situation (23)

The most recent meta-analysis of RCTs on PEMF found that these devices may have significant benefit in healing time of acute fractures (11)(24).

Considering their result, Ehnert et al. suggest that a treatment with gradually increasing frequency might be of interest, as the lower frequency (16 Hz) could enhance bone formation, while the higher frequency (26 Hz) could enhance bone remodeling (25).

Kang et al. carried out a study where they evaluated the effectiveness of electromagnetic fields as pretreatment on mesenchymal stem cells to stimulate osteogenic differentiation. The results were encouraging, as they showed induced osteogenic marker expression via bone morphogenetic

protein, transforming growth factor b, and Wnt signaling pathways based on microarray analyses(26).

Low intensity pulsed ultrasound

The function of Low Intensity Pulsed Ultrasound (LIPUS) is to transmit mechanical forces through soft tissues, to enhance micro-movements of the affected area; micro-movement generates a cast of second messengers that stimulate bone healing process (27). The frequencies range between 1.5 and 3 hertz, with an application of about 20 minutes a day for up to six months. Despite its non-invasiveness, the treatment efficacy decreases in absence of contact between the hand-piece and the skin: this problem can be solved by using a plaster or bandages. LIPUS must be performed on a daily basis and this can decrease patient compliance. The first efficacy studies were conducted on animals (28), while the first studies on human beings were produced starting from the 50s of the last century(29). The results found in the literature are discordant, most of the studies demonstrate only a partial efficacy(30)(31)(32). The effect mostly described in the literature is the decrease fracture healing time (30), that may be due to an enhancement in endochondral ossification as Katano suggests (33). In any case, stable fixation and a modest inter-fragmentation gap are crucial for a proper healing process as emphasized by Roussignol et al (34).

Physical influences on cartilage

Physical stimulations, such as pulsed electromagnetic fields, have been used for a long time in the context of bone regeneration and healing. Lately, several studies have been conducted also on osteochondral lesions.

Cheng et al. have shown that PEMFs cause an increase in DNA and collagen synthesis in chondroblasts (35).

According to Iwasa, PEMFs stimulate chondrocyte proliferation, differentiation and extracellular matrix synthesis due to the release of anabolic morphogenic proteins such as BMPs and anti-inflammatory cytokines by adenosine receptors A2A and A3 in both in vitro and in vivo investigations. It is noteworthy that in clinical translational investigations a beneficial effect was observed on improving function in knee osteoarthritis (36).

Results show that BMC and PEMFs might have a separate effect on osteochondral regeneration, but it seems that they have a greater effect when used together. Biophysical stimulation is a non-invasive therapy, free from side effects and should be started soon after BMC transplantation to increase the quality of the regenerated tissue (37).

Hilz et al. have shown that seeded chondrocytes on 3D scaffold express higher levels of chondrogenic markers when stimulated with electromagnetic fields(38).

Chemical influences on bone

Bone repair can also be influenced by chemical mediators. These chemical mediators can be conveyed in situ directly or by synthetic bone grafts enriched by these molecules. Therefore, these materials are both osteoconductive and osteoinductive (i.e. allogeneic or autologous bone graft).

The properties of a bone substitute; such as granule size, macroporosity, microporosity and shape, have been shown to influence the cellular inflammatory response. Ghanaati et al. analyzed the in vivo tissue reaction to three bone substitute materials (granules) with different chemical compositions (hydroxyapatite (HA), beta-tricalcium phosphate (TCP) and a mixture of both with a HA/TCP ratio of 60/40 wt%. Results showed that the chemical composition of bone substitutes significantly influenced the cellular response. When compared to HA, TCP significantly attracted greater number of multinucleated giant cells within the implantation bed (39). Hydroxyapatite has always been considered a material with excellent osteoinductive capabilities, excellent biocompatibility and controlled biodegradability. This material is composed of a calcium and phosphate reticulum, with a hexagonal bipyrididal structure (40). The Ca/P ratio of calcium phosphate is a a crucial factor that should be considered when selecting nano-to-micron particulate calcium phosphates for various orthopedic applications(41).

Frasnelli et al. have shown that the replacement of carbon with strontium in the hydroxyapatite promotes cell growth without affecting the morphological characteristics of the cells (42).

In recent years the attention of biologists and researchers focused on the use of mesenchymal stem cells (MSCs). A study by Castano-Izquierdo tried to define the ideal culture conditions to promote osteogenic differentiation of MSCs: the medium was enriched with a-MEM supplemented with 10% fetal bovine serum (FBS), ascorbic acid, b-glycerophosphate, and 10 nM dexamethasone. This study showed that chemical mediators present in the medium are essential for differentiation; moreover, culture time is also crucial: longer pre-culture periods lead to a progressive decrease in osteogenic potential (43).

Chemical influences on cartilage

To date very few studies deal with chemical influences on articular cartilage on humans: most of the researches are focused on animal models, mainly due to the difficulty in human samples' harvesting.

Osteoarthritis (OA) is a degenerative condition caused by an alteration of multiple molecular signaling pathways, that leads to a subsequent self-sustaining degradation of the articular cartilage.

Matrix metalloproteinases (MMPs), especially MMP-13, are key enzymes in the cleavage of type II collagen, which is a vital component for cartilage integrity. Transforming growth factor beta (TGFβ) can protect against pro-inflammatory cytokine-mediated MMP expression. With age there is a change in the ratio of two TGFβ type I receptors (Alk1/Alk5), a shift that results in TGFβ losing its protective role in cartilage homeostasis. In this case, TGFβ promotes cartilage degradation which correlates with the spontaneous development of OA in murine models. However, the mechanism underlying changes in TGFβ action with age has not been extensively studied (44).

In 2018 Kosik-Bogacka and colleagues demonstrated the correlation between calcium (Ca), magnesium (Mg), zinc (Zn), and lead (Pb) in degenerated cartilage obtained during hip replacement procedures. They found significantly higher concentrations of Ca, Mg, and Zn in men than in women. They also demonstrated a higher concentration of Pb in cartilage of patients over 65 years old. On the other hand, no relationship was found between Ca, Mg, Zn, and Pb levels and BMI. (45)

Lately, the use of mesenchymal stem cells represents a valid treatment for osteoarthritis, due to their very well-known anti-inflammatory and regenerative properties (46). There are numerous preclinical studies that demonstrate the efficacy of MSCs in osteoarthritis (OA), but still few randomized controlled clinical trials. One of the few recent studies refers to the preliminary results on the application of autologous stem cells in the treatment of knee osteoarthritis (47). However, clinical evidences on the efficacy of MSCs in OA are still missing; thus, further clinical trials are required.

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	Chemical influences	**Physical influences**
Bone	hydroxyapatite (HA), beta-tricalcium phosphate Ca/P ratio Strontium MSCs	*Direct current* *Capacitive coupling* *Pulsed electromagnetic fields* *Low intensity pulsed ultrasound*
Cartilage	Matrix metalloproteinases Transforming growth factor beta Calcium (Ca), Magnesium (Mg), Zinc (Zn), and Lead (Pb) MSCs	*Pulsed electromagnetic fields*

Table 1 Summary table on chemical and physical influences on cartilage and bone