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Review Article

Hylonome

Biomaterial osseointegration Enhancement with biophysical stimulation

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Abstract

Despite the ongoing improvement in implant characteristics, bone intrinsic potential for regeneration may be stimulated with adjuvant therapies to standard surgical procedures, as it is important to achieve the best possible implant osseointegration into the adjacent bone and to ensure therefore long-term implant stability. For this purpose various pharmacological, biological or biophysical modalities have been developed, such as bone grafting materials, pharmacological agents, growth factors and bone morphogenetic proteins. Biophysical stimulation of osseointegration includes two non-invasive and safe methods that have been initially developed to enhance fracture healing: pulsed electromagnetic fields (PEMFs) and low-intensity ultrasounds (LIPUS), for which most studies confirm their beneficial effects. The present paper is an overview of bone-implant osseointegration and the current trends on its enhancement, focusing mainly on the two methods of biophysical stimulation.

Keywords: Osseointegration, PEMFs, LIPUS, Biophysical Stimulation

Introduction

One of the main goals in orthopaedics, as well as dental and maxillofacial surgery and a pre-requisite for clinical success is to achieve good and fast bone implant osseointegration[1]. It is of great clinical importance, as it would provide early fixation with long-term implant stability, and it would minimise the risk of aseptic loosening; a serious complication in reconstructive surgery and joint replacement, thus reducing patient morbidity and health care cost[2].

I. Osseointegration

Discovery and definition of osseointegration

The initial observations of osseointegration were made in the 1950s by Branemark in a study on the circulation in bone marrow in rabbits[3]. After implantation of a titanium implant with a central canal and a transverse opening at one level to allow bone and vessel growth into bone, he observed permanent incorporation of titanium screws with bone which could be useful for supporting dental prostheses on a long-term basis, thus initiating the ongoing research on titanium implants and their various clinical applications. He first introduced the term osseointegration to describe the direct histological bone-implant contact and stated that the successful outcome of implant placement depends on direct structural and functional contact between living bone and the surface of a load-carrying implant[4].

Since Branemark initial observations, osseointegration has been intensively studied and the research is ongoing. Currently, an implant is considered as osseointegrated when there is no progressive relative movement between the implant and the bone with which it has direct contact[5,6] (Figure 1). Essentially, the process of osseointegration reflects an anchorage mechanism whereby nonvital components can be reliably incorporated into living bone and which persist under all normal conditions of loading.

Biology of osseointegration

Bone healing around implants involves the activation of a sequence of osteogenetic, vascular and immunological

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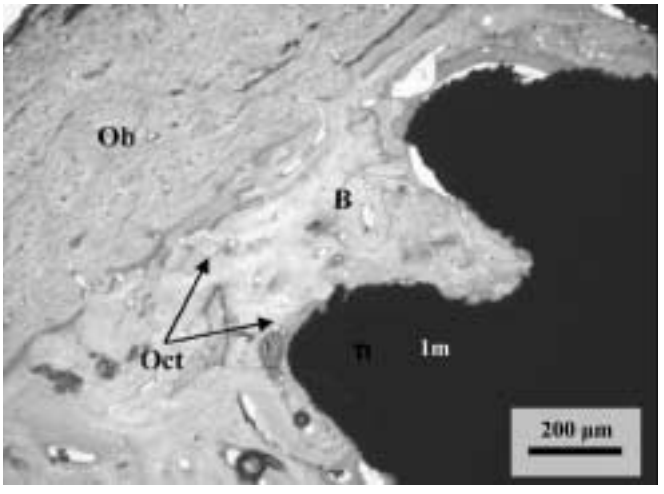

Figure 1. Photomicrograph taken by a light microscope. The newly formed bone (B) is in direct contact with the implant (Im). (Oct: osteocytes, Ob: old bone). (*Reprinted from Publication: Materials Science and Engineering C, 24, ECS Rigo, AO Boschi, M Yoshimoto, S Allegrini Jr, B Konig Jr, MJ Carbonari, 'Evaluation in vitro and in vivo of biomimetic hydroxyapatite coated on titanium dental implants', 647-651, Copyright (2004), with permission from Elsevier).*

events that are similar to those occurring during bone healing[7]. Various cell types, growth factors and cytokines are involved and interact throughout the stages of osseointegration, including inflammation, vascularisation and bone formation and ultimately bone remodelling[8].

The primary host response after implantation is an inflammatory reaction elicited by the surgical trauma and modified by the presence of the implant. Initially, a haematoma is formed at the bone-implant interface and may play a role as a scaffold for peri-implant bone healing[9]. The host response consists of platelet activation, migration and activation of inflammatory cells, vascularization, mesenchymal cells and osteoblast adhesion, proliferation, protein synthesis, and local factor composition[9-12]. From the implant side, an oxidation of metallic implants has been observed[13]. Osteoblasts also attach on the implant surface from day one of implant insertion[14]. Furthermore, the deposition from osteogenic cells on the implant surface of a layer of non-collagenous proteins that regulate cell adhesion and binding of minerals has been described during the early stages of host response[15].

A few days after implantation, osteoblasts begin to deposit collagen matrix either in direct contact with the implant surface[14] or directly on the early afibrillar interfacial zone comparable to cement lines, which is rich in non-collagenous proteins such as osteopontin and bone sialoprotein[16]. The early deposition of new calcified matrix is followed by woven bone formation to ensure tissue anchorage and ultimately is substituted by lamellar bone, thus completing the biological fixation of the implant[17]. Peri-implant osteogenesis progresses either from the host bone towards the implant surface (distance osteogenesis) or from the implant towards to the healing bone (contact osteogenesis or *de novo* bone formation)[11].

Vascularisation is essential during osseointegration, as it influences tissue differentiation and ossification[18]. Bone remodelling ultimately occurs for reshaping or consolidation of bone at the implant site, providing a mechanism for self repair and adaptation to stress. Overall, osseointegration of implants in humans is a slow process and can take up to several months[19,20].

However, a better understanding of the cascade of events that occur at the cellular and molecular level at the bone-implant interface is needed, in order to optimise osseointegration of implants.

Factors enhancing osseointegration

Bone implant osseointegration depends on several factors which can be separated into different groups: implant-related factors, the status of the host bone bed, the mechanical stability, the use of adjuvant therapies and the remodelling of periprosthetic bone at the interface (Table 1).

i) Implant-related factors

These include implant design and chemical composition and topography of the implant surface[18]. The different materials, shape, length, diameter, implant surface treatment and coatings have been proposed to enhance clinical performance.

The biocompatibility of the material is of great importance and a predictor of osseointegration, as it is essential to establish stable fixation with direct bone-implant contact and no fibrous tissue at the interface[21].

Pure Titanium (Ti) is widely used as an orthopaedic metallic implant material as it is highly biocompatible, it has good resistance to corrosion, and no toxicity on macrophages or fibroblasts, lack of inflammatory response in peri-implant tissues and its surface is composed of an oxide layer and has the ability to repair itself by reoxidation when damaged[22-26]. Other materials have also been proposed either as alternative to Ti or as alloy systems, including tantalum, aluminium, nionium, nickel, zirconium, and hafnium[27-31].

The most frequent implant surfaces and types can be subdivided into implants with roughened surfaces with a coating (e.g., titanium plasma-sprayed or hydroxyapatite–coated), implants with machine-processed (e.g., machined or polished) titanium without a coating, and implants with roughened surfaces without a coating (e.g., sand-blasted, acid-etched or anodically roughened)[32-35]. Rough surfaces enlarge the implant area in contact with the host bone favouring primary stability, and enhancing peri-implant bone formation compared to smooth surfaces[34]. Roughness positively affects osseointegration[36] and, in particular, it seems to affect directly osteoblast attachment and subsequent proliferation and differentiation[37]. In general, moderately rough surfaces

Factors	Enhancement of osseointegration	Inhibition of osseointegration
Implant-related factors	- design - chemical composition - surface topography - coatings	- inappropriate porosity (wide or narrow porous)
The status of host bone bed	- minimal surgical trauma - vascularity and cellularity of implantation site	- bone defect - osteoporosis - rheumatoid arthritis - smoking - advanced age
Mechanical stability	- primary implant stability no micromotion	- excessive implant mobility (interface motion)
Adjuvant therapies	- bone grafting (autogenous or allograft) - osteogenic coatings (BMPs, TGF-β) - biophysical stimulation (PEMFs and LIPUS) - systemic administration of ibandronate and human parathyroid hormone 1-34	- irradiation - pharmacological agents: (cyclosporin A, methotrexate, cis-platinum, warfarin, indomethacin)

Table 1. A summary of the main factors that influence positively or negatively biomaterial osseointegration.

favour peri-implant bone growth better than smoother or rougher surfaces[38].

The pore size of a porous coated implant seems to be a major determinant of osseointegration[39]. Among different pore sizes, a pore size above 80 μm improves bone ingrowth in both hydroxyapatite and tricalcium phosphate materials[40].

ii) The status of host bone bed and its intrinsic healing potential of bone[41]

A healthy bone bed and with minimal surgical trauma is important as it is the source of almost all cells, local regulatory factors, nutrients, and vessels that contribute to the bone healing response. The implantation site influences the osseointegration process through different levels of bone cellularity and vascularity[42]. A high-quality bone also seems to be important for the initial implant stability[43]. Other factors, such as the geometry and size in the case of recipient defect sites, have also been described to influence bone implant osseointegration[44].

iii) The mechanical stability and loading conditions applied on the implant[7]

Primary implant stability consists in rigid fixation between the implant and the host bone cavity with no micro-motion of the implant or minimal distorsional strains[4,45]. Primary stability depends on the surgical technique, the implant design, and the implantation site. In cortical bone a higher mechanical anchorage to the implant is observed compared to cancellous bone[46].

iv) The use of adjuvant therapies

These adjuncts to optimise implant osseointegration include bone grafting, the use of osteogenic coatings and biophysical stimulation to enhance the amount of bone ingrowth.

After primary implantation of a biomaterial and especially after revision surgery, gaps of different size remain between the implant surface and bone. To close larger gaps or bone defects, autologous bone graft is regarded as the gold standard for bone regeneration, due to its intrinsic osteoinduction, osteoconduction and osteogenic potential[47]. However, harvesting-related complications in combination with its limited available quantity dictated need for the development of alternative methods of biological stimulation, including the use of allogenic bone graft or other bone graft substitutes[48-49] and the use of "biological" coatings to induce osseointegration.

Several growth and differentiation factors have been used either alone or combined[50-53] as biocoatings of conventional implants to accelerate and enhance the bone ingrowth and to strengthen the osseous fixation. Bone morphogenetic proteins (BMPs), and in particular BMP-2[54-57] and BMP-7 or osteogenic protein-1 (OP-1)[58,59], have been extensively studied and have been shown to augment bone formation and osseointegration of implants. Other growth factors used to improve implant osseointegration are platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF)[50], transforming growth factor-beta 1 (TGFβ-1)[60] alone or with IGF-1[51], TGFβ-2[60,61]. However, further research is required to ascertain the beneficial effects from the use of

such osteoinductive factors, since there are contradictory results[62] and to solve issues regarding their optimal dose and safety for clinical use.

Additionally, other coatings that have been used include collagen and other extracellular matrix proteins, like fibronectin and vitronectin, to improve the osseointegration of titanium implants by enhancing bone formation, or even coating of the implant with autologous osteoblasts, which represents a more "physiological" approach to improve and accelerate bone regeneration[63-66].

Finally, an interesting adjuvant therapy to improve implant osseointegration includes the systemic administration of pharmacological agents such as ibandronate[67] and human parathyroid hormone 1–34[68], with positive preliminary results.

Biophysical stimulation methods of osseointegration will be more thoroughly described in the following sections of this review article, as they represent its main topic.

Factors negatively affecting osseointegration

An important factor that negatively affects osseointegration is excessive implant mobility which results in tensile and shear motions, stimulating a fibrous membrane formation around the implant and inhibiting osseointegration[45,69].

Additionally, an inappropriate porosity of the porous coating of an implant can inhibit bone ingrowth. Narrow pore throats were found to inhibit tissue differentiation in pores, possibly because of inadequate vascularization[70]. In areas of a very close contact between implant and host bone no early bone formation has been described[71], whereas excessively wide gaps of more than 500 μm reduced the quality or quantity of the newly-formed bone and delayed the rate of gap filling[72].

Although irradiation seems to delay bone remodelling pre- and post-implantation[73,74], a recent study on the effects of radiotherapy pre- and post- implantation around mandibular implants in dogs showed that although resorption was more pronounced in the -irradiated after implantation- group, osteon formation appeared unvarying and osseointegration was shown to be compatible with bone irradiation. However, the authors underline that the timing and length of delay between irradiation and implantation appear decisive in order to ensure the correct osteogenic reaction and osseointegration[75]. Additionally, after evaluation of the tissue response to bone-anchored implants retrieved from irradiated sites in patients, Bolind et al. found that it is possible to achieve bone anchorage of implants in irradiated tissue, but they did not conclude on radiation dose and bone tissue response[76].

Various pharmacological agents were found to impair implant osseointegration, including cyclosporin A, methotrexate, and cis-platinum[77-79]. The administration of warfarin was found to significantly impair both the attachment strength and the ingrowth of bone uncoated porous implants made of cobalt-chromium-molybdenum alloy; however, no such inhibitory effect was observed in hydroxyapatite-coated implants[80]. Also, it has been suggested that perioperative administration of indomethacin causes a transient decrease in attachment strength at early periods, but it does not seem to significantly affect long-term osseointegration of porous-coated implants[81].

There are various patient-related conditions that compromise the status of the host bone bed and impair fracture healing, which can potentially impair osseointegration. These are local and systemic diseases and therapies, of which the most frequently encountered in orthopaedic-implanted patients are osteoporosis and rheumatoid arthritis, advanced age, nutritional deficiency, smoking, renal insufficiency and drugs often used by orthopaedic patients because of chronic pain and inflammation or tumours[82-85].

In particular, osteoporosis, one of the most frequent diseases at least in the Western world, seems to affect cell proliferation, protein synthesis, cell reactivity to local factors, and mesenchymal cells numbers[85,86]. Although, the research on osteoporosis is ongoing including fracture prevention and numerous treatment modalities, the knowledge on biomaterial osseointegration after implantation in osteoporotic bone remains limited.

Studies have demonstrated that bone alterations in osteoporosis may not compromise only fracture healing[87,88], but also the osseointegration of implants[89]. The increased risk of implant failure in osteoporotic bone is secondary to various factors that are present and alter its structural, biological and mechanical properties. In osteoporosis, the number of and activity of cells of the osteogenic lineage (mesenchymal cells and osteoblasts) is decreased, the number and activity of osteoclasts is increased, and vascularisation is impaired[90,91]. The balance between anabolic and catabolic local cytokines and growth factors that affect bone formation and remodelling is disturbed with a spontaneous increase in pro-inflammatory cytokines (TNF-α, IL-6, IL-1, PGE2) and a decrease of bone-forming factors (IGF-1, TGF-β)[18,91].

Researchers have reported slower biomaterial osseointegration and higher rate of prosthetic device failures in the presence of osteoporosis, *in vitro* as well as in animal studies[83,92-95]. A possible explanation for these negative findings could be that osteoporotic tissue may not provide a firm initial stabilisation of the implant; which requires a high-quality bone and is crucial for long-term clinical success[43].

In conclusion, osteoporosis seems to compromise the biological and mechanical fixation of biomaterials used for fracture fixation and joint replacement, but further research is needed in order to elucidate the effect of osteoporosis and of other metabolic and degenerative pathologies, in the process of osseointegration of the implanted biomaterials.

II. Biophysical stimulation

As mentioned, it is essential for clinical success to achieve good and fast bone implant osseointegration, and for that purpose various strategies have been developed. Among the

aforementioned methods that mainly include optimisation of the implant characteristics, biophysical stimulation represents a non-invasive and locally applied strategy to enhance endogenous bone regeneration around an implant. Therefore, the two methods of biophysical stimulation have been used: pulsed electromagnetic fields (PEMFs) and low intensity pulsed ultrasounds (LIPUS). Although initially developed and currently used to stimulate bone regeneration during fracture healing, their positive clinical outcomes and safety demonstrated their potential use as an adjunct to enhance implant osseointegration.

1. Pulsed electromagnetic fields

Bassett et al. first demonstrated the use of electromagnetic fields as a noninvasive method to stimulate fracture healing[96]. Since then, numerous animal and human studies have shown the effect of pulsed electromagnetic fields in different clinical situations to enhance bone regeneration.

Greenough, in 1992, observed that pulsed electromagnetic fields may affect tissue healing through a primary effect on vascular growth[97]. Roland et al. also supported the angiogenic effect of PEMFs *in vivo*, and suggested its possible use to increase the quality of revascularized tissue[98]. Finally, Smith et al. showed that local application of PEMF waveforms elicited significant arteriolar vasodilation in the rat muscle, supporting this potential mechanism for stimulation of the healing process[99].

In animal studies, PEMF exposure enhanced maturation and consolidation of the regenerate bone in a distraction model[100,101] and accelerated fracture healing[102,103].

In humans, PEMF treatment has been applied to stimulate osteogenesis in patients with fracture non-union[104,105], as well as an adjunct for delayed healing of foot and ankle arthrodesis, but with a relatively low success rate[106]. Moreover, in spinal surgery, the adjunctive use of PEMFs increased the success of radiological spinal fusion and accelerated the regeneration process[107,108]. They have also been used in a case of anterior cervical fusion non-union with success[109]. During limb lengthening procedures including the humerus, femur and tibia, PEMFs were found to encourage callus formation and maturation at the distraction site, allowing earlier removal of the external fixation devices[110]. Electromagnetic stimulation has even been applied in two cases for the treatment of femoral head osteonecrosis[111].

The molecular mechanisms of PEMF stimulation have not yet been determined. It was found that, *in vitro*, PEMFs significantly alter the expression and functionality of adenosine A2A receptors in human neutrophils in an intensity, time and temperature-dependent way, resulting in a reduction of superoxide anion production. This may limit the inflammatory response, but further research is required to clarify the role of this mechanism on osseointegration and aseptic loosening[112]. It has also been shown *in vitro* that PEMF stimulation of osteoprogenitor cells and osteoblasts increased their proliferation and differentiation[113,114], as well as the production of extracellular matrix and growth and differentiation factors including TGF-β1, BMP-2 and BMP-4[115-117]. Studies also suggest that the positive effect of PEMFs on bone ingrowth may be the result of a primary effect on vascular growth and increased angiogenesis, secondary to the release of angiogenic factors such as IL-8, bFGF and VEGF[118]. In a recent study, Chang et al. showed that PEMF accelerated the apoptosis of osteoclasts derived from primary osteoblasts and bone marrow cell cultures, and suggested the potential for PEMF applications on some osteoclast-associated bone diseases such as osteoporosis[119]. Also, a statistically significant increase and decrease of osteoclastogenesis and bone resorption areas were found when bone marrow cultures (mice) were exposed to PEMF with different intensities. These data demonstrated that PEMFs with different intensities could regulate osteoclastogenesis, bone resorption, OPG, RANKL, and M-CSF concentrations in marrow culture system[120].

Researchers, in a study comparing the mechanisms of ultrasound (US) on osteoblast proliferation with those of pulsed electromagnetic field (PEMF) by different signal transduction pathway inhibitors, showed that there are different transduction pathways for US and PEMF stimulation leading to osteoblast proliferation, although their pathways all lead to an increase in cytocolic Ca^{2+} and activation of calmodulin. These findings offer a biochemical mechanism to support the process of ultrasound and PEMF-induced enhanced bone regeneration[121].

There are several animal studies on the effect of PEMFs on bone implant osseointegration. Shimizu et al.[122], while studying the effect of PEMF on bone ingrowth into porous hydroxyapatite (HA) and porous tricalcium phosphate (TCP) after implantation in rabbit tibiae, found accelerated bone formation and bone maturation. A significantly greater amount of bone and thicker bone trabeculae was noted, mostly at 3 to 4 weeks post-implantation, but no effect in the TCP pores. They also observed that the greater the diameter of the pore of the material, the greater the effectiveness of PEMF stimulation.

Spadaro et al.[123] reported that, after intramedullary implantation of stainless steel implants in rabbits and in cases of movable implants, PEMFs enhanced bone formation around the implant, and that this effect was more obvious in the femur than in the tibia. These findings demonstrated the potential clinical application of PEMFs for the treatment of aseptic loosened orthopaedic implants.

In another animal study, Ijiri et al. examined the effect of PEMFs on bone ingrowth into a porous coated implant. Using a titanium implant coated with beads of 250-300 microns in diameter around a stem after implantation into the humerus of Japanese albino rabbits, the researchers observed a significant improvement in bone ingrowth into the porous coated implant in the PEMF-treated animals compared with the controls[123]. An improved bone contact ratio and bone area ratio of rough-surfaced implants was also observed after insertion into the femur of Japanese

white rabbits, and this improvement was found to be dependent on dosage and exposure time[125].

Fini et al. investigated the effects of PEMFs on osseointegration of hydroxyapatite (HA) cylindrical nails in the femur of rabbits, and noticed significantly improved bone-HA contact ratio, bone mineralization and mechanical fixation in the PEMF-treated group, thus suggesting a positive therapeutic effect of PEMFs in accelerating HA osteointegration in trabecular and cortical bone. They also observed that the surgically damaged bone, which was the bone directly adjacent to the implant, was the only one responding to treatment[126,127].

In a study of the events at the hydroxyapatite implant material/tissue interface after implantation of porous ceramics (natural and synthetic) in bone defects created at the proximal tibiae in rabbits, Ottani et al. concluded that synthetic HA possess more osteoconductivity than the natural HA, and that electromagnetic stimulation resulted in accelerated bone formation at early time periods in both forms of apatite[128].

However, Buzza et al.[129], whilst studying bone regeneration around pure titanium implants after implantation in the metaphyses of rabbit tibiae and after stimulation with PEMFs, did not find significant differences in the osseointegration process between the PEMF-treated and non-treated animals. They explained their controversial findings by the different biomaterial used, the duration of the electromagnetic stimulation as well as by the different intensity of the electromagnetic power.

PEMF stimulation has been applied in humans as well to improve implant osseointegration, and particularly hip prostheses. In 1995, Steinberg reported a case of a 44-year-old patient with osteolytic changes around the distal end of the femoral prosthesis, which were reversed with the use of anti-inflammatory medication and pulsed electromagnetic field stimulation[130].

Konrad et al. used PEMF treatment in 24 patients with aseptic loosening of total hip arthroplasties and observed that at the end of the treatment, at 6 months and 1 year later, pain and abduction-adduction hip movements were significantly improved[131]. Although radiological findings did not significantly change, ultrasonography as well as Tc^{99m} scintigraphy findings improved, and this effect was maintained for a year. However, the cases with severe loosening and pain at rest did not respond to PEMF treatment. They concluded, therefore that PEMF treatment was effective, but not in severe cases.

Kennedy et al.[132] in a double-blind clinical trial studied 37 patients with loosened cemented hip prostheses. After completion of the 6-month stimulation for the group of PEMF treatment, they reported a success rate of 53%, statistically significant compared to 11% reported for the placebo group. However, they observed a 60% relapse rate among the active successes at 14 months post-stimulation, which increased to 90% at three years, despite maintaining the therapy for one hour per day. Hence they concluded that the use of PEMFs for loosened cemented hip prostheses is an option only to delay revision hip surgery.

In general, the clinical use of PEMFs remains controversial. Well-designed studies are required to verify its beneficial effects as a biophysical method to enhance implant osseointegration and to determine the optimal magnetic intensity and the most effective duration per day application and length of treatment.

2. Low intensity pulsed ultrasound

LIPUS is a form of mechanical energy that is transmitted through and into living tissue as acoustic pressure waves above the human audible range. It is absorbed at a rate proportional to the density of the tissues it passes through[133]. It has been hypothesised that the micromechanical strains produced in biological tissues may result in biochemical events that could stimulate fracture healing.

In vitro studies have shown that LIPUS application to human osteoblasts, as well as fibroblasts and monocytes, induced cell proliferation, differentiation, and bone formation (collagen/non-collagenous protein production), and angiogenesis. Anabolic and angiogenic factors such as TGF-β1, b-FGF, IL-8, PGE2 and VEGF were released, while pro-inflammatory cytokine levels, such as IL-6 and TNF-α were decreased[134-137]. More recent studies have shown that LIPUS stimulation directly affects osteogenic cells, leading to mineralised nodule formation[138] and enhances chondrogenesis of bone marrow-derived mesenchymal stem cells[139].

In early animal studies, high intensity ultrasound was used to stimulate osteogenesis, but non-consistent results regarding new bone formation were observed. In higher intensities, researchers reported reduced callus formation, delayed bone healing, necrosis and dense fibrous tissue formation, whereas, in lower intensities, they found increased callus formation and acceleration of fracture healing[133]. These conflicting results dictated the need for further research and eventually led to the development and use of LIPUS to stimulate osteogenesis[140].

LIPUS treatment has been shown in various experimental studies to accelerate the restoration of mechanical properties of bone, such as maximum torque and torsional stiffness during fracture healing[133]. It was shown that ultrasound accelerated fracture healing when applied during the inflammatory and early proliferative phases of bone regeneration[141]. However, when applied in the late proliferative phase, it stimulated cartilage growth, suggesting that the time of application is important. Many *in vivo* studies suggest the LIPUS does not affect the remodelling phase of fracture healing, but the earlier inflammatory or callus formation phases of healing and mainly the endochondral ossification[142], as well as the angiogenesis phase[143]. Positive effects of LIPUS treatment on the maturation process of the bone regenerate during distraction osteogenesis have also been found in animal models, with significantly more callus formation, higher mineral content, and a higher bone regenerate stiffness[144,145]. Recently, it has been shown that the effects of LIPUS are even more pronounced when applied during the lengthening phase compared to the maturation phase

and that possibly its effect is mediated via endochondral pathways[146]. In an animal study, it was shown that LIPUS enhanced bone regeneration under rapid distraction, and this effect was dose-dependent[147].

Numerous studies also showed the important role of the intensity of the ultrasound and possibly its frequency, but this needs to be clarified[148].

LIPUS effects have been studied in various clinical studies for the acceleration of fracture healing and for the treatment of fracture non-unions in humans[149]. As an adjunct for the treatment of closed or grade 1 open tibial shaft fractures, Heckman et al.[150] observed a reduced time to achieve clinical and radiological union. LIPUS has also been used for the treatment of distal radius fractures[151], and of scaphoid fractures[152]. However, contradictory results have also been reported in a clinical study on fresh tibial shaft factures fixed with a reamed and statically locked intramedullary rod with or without stimulation with LIPUS[153]. The researchers observed that LIPUS treatment did not reduce the healing time of the fracture. For the treatment of fracture non-unions[154-157], LIPUS gave comparable healing rates to those obtained with electrical stimulation or extra-corporeal shock wave therapy[158,159]. It was also used as an adjunct to cast immobilisation for the treatment of a congenital pseudoarthrosis of the tibia with success[160].

The LIPUS effect is mainly produced at tissue borders of soft and hard tissue, like connective tissue and bone, which implies a potential clinical application on implant osseointegration. An animal study in rabbits demonstrated that LIPUS enhanced osteogenesis at the healing interface of the bone-tendon junction[161]. It does seem to stimulate osteogenesis in intact bone, which suggests that it does not affect bone remodelling[133].

The mechanisms by which ultrasound may accelerate bone regeneration are still unclear and under research. Different mechanisms have been proposed. It has been hypothesised that LIPUS may stimulate changes in cells and tissues by increasing the temperature with energy absorption, as it has been shown that even a small increase in temperature ($<1^{o}C$) may affect some enzymes, such as matrix metalloproteinase 1 (or collagenase 1)[162]. However, more recent studies support the view that LIPUS may be associated with non-thermal effects on cells and tissues, such as acoustic streaming and cavitation, which result in an increase collagen synthesis and overall cellular activity with enhanced gene expression, and that it seems to affect diffusion rates and membrane permeability. Also, LIPUS may produce increased blood flow and mechanical stimulation by inducing micromotion[133,148].

To date, there are no clinical studies in humans on bone-material osseointegration and LIPUS stimulation. There are only animal studies indicating increased bone ingrowth and bone apposition rate[163-165].

Tanzer et al.[165] showed that LIPUS enhanced bone ingrowth into porous intramedullary rods (tantalum) after implantation into the ulnae of dogs. An average of 119% more bone growth into the ultrasound-treated implants compared with the controls was observed. In another study, Tanzer et al.[164] showed an 18% increase in bone ingrowth into fully porous transcortical implants (titanium) in the LIPUS-treated group compared to the non-treated controls. LIPUS treatment had its greatest effect in the first 2-3 weeks of stimulation. These findings suggest the potential use of LIPUS to improve osseointegration of non-cemented porous-coated total joint replacements in humans.

As the potential for external application of ultrasound to augment implant osseointegration in humans is implied, further research is needed to elucidate its mechanisms of stimulation. Well-designed clinical studies are required not only to confirm its beneficial effects, but also to optimise the ultrasound parameters and establish a protocol for clinical use.

In conclusion, LIPUS has a positive effect on bone regeneration, but the biophysical processes of this effect remain unknown. It could represent a non-invasive method to augment bone ingrowth into porous implants and, therefore, a safe and inexpensive adjuvant in the armamentarium of arthroplasty surgeons to maximise and accelerate cementless arthroplasty osseointegration.

Conclusion

Despite the ongoing improvement in biomaterials (design, properties such as porosity and roughness, etc.), bone intrinsic potential for regeneration may be stimulated with adjuvant therapies to standard surgical procedures, in order to accelerate and maximise bone ingrowth. To achieve the best possible implant osseointegration into the adjacent bone is of a major clinical significance and for this purpose various pharmacological or biophysical modalities have been tested. Such adjuvant therapies that improve the bone-prosthetic device interface by locally enhancing bone ingrowth, include bone grafting materials, pharmacological agents, growth factors and bone morphogenetic proteins.

Regarding the biophysical stimulation of the osseointegration process, both LIPUS and PEMFs constitute two non-invasive, exogenous and locally applied methods, for which most studies confirm their beneficial effects. No systemic or local side effects from their application have been encountered thus far.

The controversial observations on their effects can be attributed to various factors. For example, different animal species have been used in the different studies, various implantation sites were used (trabecular or cortical bone, intramedullary), with a variety of biomaterials being inserted (ceramic or metallic), and with different intensity and duration of the stimulation.

Further research is therefore needed to establish their beneficial effects in experimental studies, to elucidate the mechanisms of stimulation at the molecular level, and to determine the optimal parameters for both of these biophysical stimulation methods. The parameters that need to be determined include the minimal intensity required for stimulation, the most effective duration of daily exposure and of

the overall period of treatment.

Finally, so far there are only a few clinical studies, case series and case reports on the efficacy of PEMFs and no human studies of LIPUS stimulation on implant osseointegration. It is therefore essential to design well-controlled randomized clinical studies to assess and confirm the efficacy from their clinical use that would result in increased implant longevity when applied initially and in the delay and even the inhibition of the process of aseptic loosening, which represents a serious complication in joint replacement and reconstructive surgery.

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