

Immunomodulation of Osseointegration Through Extracorporeal Shock Wave Therapy

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Abstract

Introduction: Dental implants are a routine procedure within the therapeutic range of dentists. Many loading protocols are based on techniques and biological times that consider the biology of the host bed. However, early or late complications may occur, such as lack of osseointegration, peri-implantitis, and marginal bone loss. Nowadays, treatments for total or partial failure in osseointegration are often complex and unpredictable. It has recently been postulated that osseointegration is rather an immunomodulated event, which is the result of an equilibrium response to a foreign body reaction. Given this new evidence, there is a need to develop new therapeutic protocols and approaches to improve osseointegration and the prognosis of implant treatments. **The Hypothesis:** Human bone marrow-derived mesenchymal stem cells (HBM MSC), resident in the maxillary and mandibular bones, immunomodulate osseointegration through the bioactivating effect of extracorporeal shock waves therapy (ESWT). **Evaluation of the Hypothesis:** Local immunomodulation is currently considered one of the main functions of mesenchymal stem cells to maintain tissue homeostasis, and it has been demonstrated that ESWT manages to stimulate the activity of HBM MSC. Clinical and experimental reports demonstrate the therapeutic potential of ESWT in medicine and dentistry. **Conclusion:** ESWT medical devices could become a new therapeutic strategy to immunomodulate osseointegration. The bioactivating effect of ESWT on resident HBM MSC can have the potential of guiding the tissue response to a more favorable outcome, with the objective of improving clinical success and decreasing the complications of dental implant treatments.

Keywords: ESWT, HBM MSC, immunomodulation, osseointegration

INTRODUCTION

Brånemark defined osseointegration as a direct, structural, and functional connection between ordered living bone and the surface of an implant subjected to functional loading.^[1] Since the formulation of this definition, it has been established that dental implant osseointegration is a biological process similar to a fracture repair. Therefore, loading protocols have been established based on techniques and biological stages that consider not only the biology of the host bed, but also the systemic characteristics of the patient.^[2] Recent studies have shown the complexity of the bone tissue dynamics;^[3] in fact, the balance between osteoblasts and osteoclasts is important not only for bone homeostasis but also for the cells themselves as a part of the immune system.^[4] Both, osteoblasts and osteoclasts respond to cytokines produced by innate and adaptive immune cells, and it has been shown that osteoclasts can act as antigen presenting cells.^[5]

After the surgical insertion of a dental implant, a predictable healing process is expected to end with osseointegration, but this is not always the case. Treatments with titanium dental implants can present early complications such as lack of osseointegration,^[6] which can be observed histologically as an interface of nonmineralized connective tissue between the bone and the implant,^[7] leading to implant failure.^[8] Late complications of dental implants include marginal bone resorption, observed mainly around the 1st year,^[9] and peri-implantitis, which affects between 28 and 56% of the patients,^[10] having a complex and unpredictable treatment.^[10,11]

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Different theories explain the failures and complications of dental implants, including excessive surgical trauma, bacterial contamination, premature overload, and impaired healing response of the host.^[8] The compromised healing/adaptation theory, states that the failure of an implant depends on the combined action of several subfactors that can alter the bone cells and their vascular supply.^[12] Koka and Zarb proposed two terms, “Osseosufficiency,” defined as the ability of the host site to correctly heal and allow osseointegration, and “Osseoseparation,” defined as the inability of the host site to maintain osseointegration.^[13]

The host healing capability seems to be a crucial factor, even more, if we consider that osseointegration would be an immunomodulated healing process, as proposed by, Trindade and Albrektsson. These authors introduced the concept of foreign body equilibrium (FBE) for dental implants, and described osseointegration as a local host response that leads to the isolation of the implant by the gradual apposition of cortical bone. Osseointegration is considered a mild chronic inflammatory response that allows implant function, with a bone-implant interface that remains in

a state of equilibrium, susceptible to changes in its local environment.^[3,4,9,14]

Paradigm Shift?

It has been shown that dental implants, when in contact with host tissues, present immediate adsorption of proteins onto their surface. The physicochemical interaction between host proteins and the implant surface leads to a change in the molecular conformation of one or more of these host proteins exposing sequences of previously hidden amino acids that act as antigenic epitopes.^[3,14] Hu *et al.* showed that adsorbed fibrinogen is the main protein responsible for the accumulation of macrophages on the surfaces of implanted biomaterials.^[15] The presence of antigens on the surface of dental implants could trigger immune and inflammatory responses that initiate a foreign body reaction (FBR), which finally reaches a FBE allowing the normal function of the implant [Figure 1].^[14] This phenomenon was indirectly detected in studies of the 1980s, which describe a heterogeneous interface.^[16] Connective soft tissue, blood vessels, bone marrow, and a layer of nonmineralized

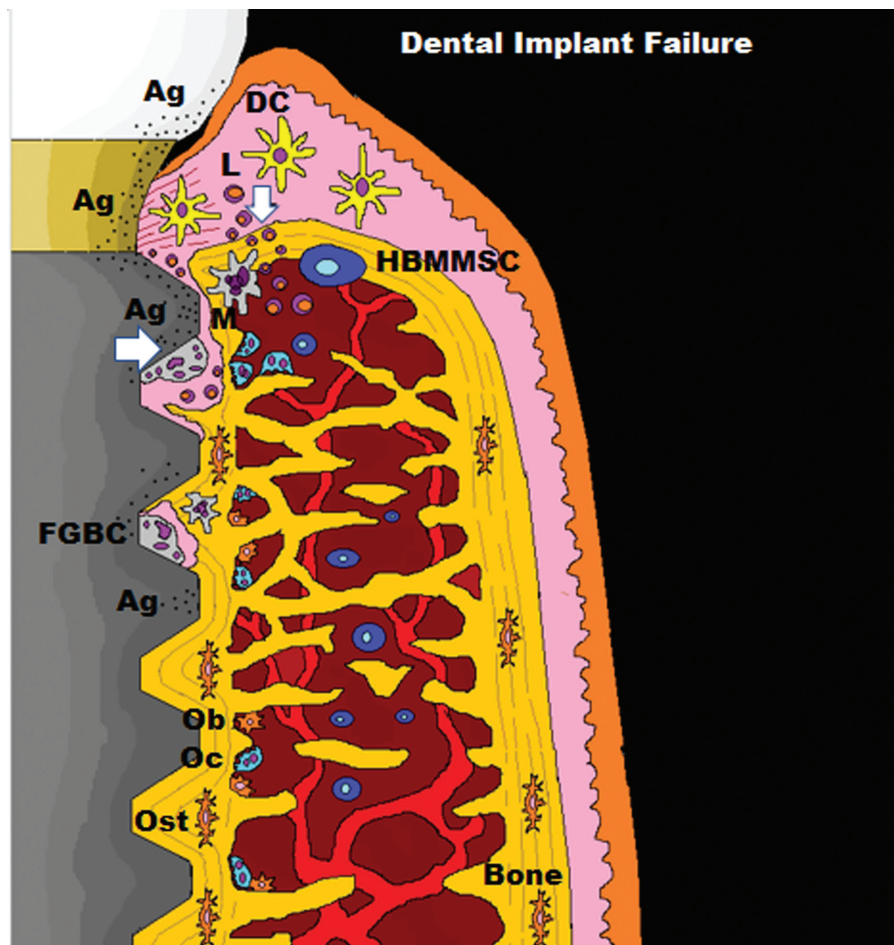


Figure 1: The presence of antigens on the surface of dental implants could trigger immune and inflammatory responses that initiate a foreign body reaction (FBR), which finally reaches a foreign body equilibrium (FBE) allowing the normal function of the implant. The loss of the foreign body equilibrium (FBE) could be the main cause of peri-implant bone loss. Ag = antigen; DC = dendritic cell; L = lymphocyte; HBMMS = mesenchymal stem cells derived from the bone marrow; FGBC = giant foreign body cell; M = macrophage; Ob = osteoblasts; Oc = osteoclast; Ost = osteocyte

amorphous tissue, of 100 to 400 nm in thickness, between the bone and implant surface was observed, with between 56 and 85% of contact between bone and titanium.^[16]

Some studies have shown an absence of bone implant-contact (BIC) in 100% of the implant surface, which reinforces the idea that osseointegration is not equivalent to the repair process of a fracture. Up to date, it is not known which is the ideal BIC that allows an adequate clinical function.^[15] Osstell developed an implant stability quotient to evaluate the rigidity of the bone-implant interface. This method is currently widely being used among clinicians; however, it does not provide conclusive histological information regarding the bone-implant interface.^[17]

The factor that initiates FBR on the surface of dental implants has not yet been determined; however, the complement system seems to play a key role.^[14] Arvidsson *et al.* showed that the interaction between titanium and plasma coagulation factors, such as factor XII, could lead to complement activation through the alternate pathway, producing C3b.^[18] Since many innate and immune cells express receptors for C3b, this could explain immune cell infiltration on the surrounding bone tissue.^[3,15]

Macrophages are recruited in response to the presence of a foreign entity in the body, fusing and forming foreign giant body multinucleated cells (FGBC).^[3] Donath *et al.* described through histological studies, the presence of FGBC on the surface of titanium implants, which were present in multiple cases of FBRs.^[19]

The loss of the FBE could be the main cause of peri-implant bone loss.^[3] This concept is reinforced by the fact that osteoclasts can be formed by the fusion of multiple macrophages, and some authors even suggest that macrophages can perform bone resorption functions,^[15] and this point highlights the importance of continued clinical care for patients treated with osseointegrated implants and reveals how dynamic and fragile osseointegration can be.^[20]

The Hypothesis

The human bone marrow-derived mesenchymal stem cells (HBMMSCs), resident in the maxillary and mandibular bone, immunomodulate osseointegration, through the bioactivating effect of extracorporeal shock waves therapy (ESWT) [Figure 2].

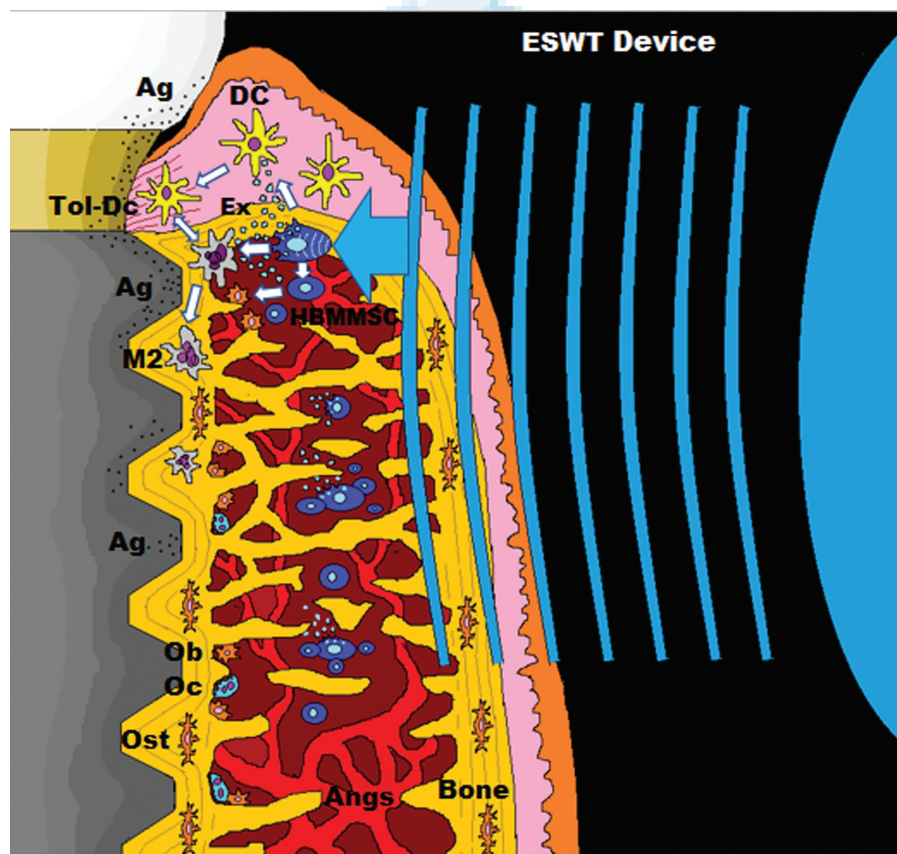


Figure 2: HBMMSCs residing around the peri-implant bone tissue immunomodulate the osseointegration process, through the ESWT bio activation effect. The mechanical stimuli generated by ESWT trigger the release of exosomes by HBMMSCs, generating tolerogenic dendritic cells (Tol-Dcs) and increasing the presence of the M2 phenotype of the macrophage. Also ESWT increases the angiogenesis what have a fundamental role in the maintenance of the HBMMSCs. Ag = antigen; DC = dendritic cell; Tol-Dcs = tolerogenic dendritic cells; Ex = exosomes; HBMMSC = mesenchymal stem cells derived from the bone marrow; FGBC = giant foreign body cell; M2 = M2 phenotype of macrophage; Ob = osteoblasts; Oc = osteoclast; Ost = osteocyte; Angs = angiogenesis

Evaluation of the hypothesis

Human bone marrow-derived mesenchymal stem cell and immunomodulation of osseointegration

Mesenchymal stem cells (MSCs) represent one of the most promising tools in regenerative medicine, thanks to their potential for proliferation, differentiation, and immunomodulatory functions.^[21] More than 400 studies have explored the immunomodulatory effect of MSCs for the treatment of various autoimmune conditions, including graft-versus-host disease, diabetes, multiple sclerosis, Crohn's disease, and organ transplantation.^[22] The finding that cultured MSCs have immunomodulatory properties comes from experiments that show direct inhibition of T cell proliferation by MSCs. Currently, it is known whether MSCs affect not only T cells, but also other cells of the immune system, such as dendritic cells (DCs) and macrophages.^[23]

Langerhans DCs are present at the peri-implant mucosa, and constitute a part of the first line of defense against infection. In addition, it has been observed that Langerhans cells are more effective in stimulating T cells than DCs from the skin.^[3] HBMMSC modulate the immune response through a series of mechanisms; among these, the generation of tolerogenic DCs (Tol-DCs). It has been demonstrated that HBMMSC act on DCs, inhibiting the differentiation of precursors, and also by suppressing their maturation and chemotactic activity. In addition, DCs cultured with HBMMSC lose their ability to stimulate CD4+ T cells. The presence of Tol-DCs could not only help to better tolerate grafts in transplant areas,^[24] but also to modulate the local response in favor of osseointegration. It has been suggested that some biomaterials favor DC maturation and influence their phenotype.^[3] This can alter the FBE, especially considering the wide range of "biomaterials" used in implantology, including cemented implant crowns.^[3]

HBMMSCs also modulate B cells, NK cells, and macrophages.^[24] HBMMSCs stimulate IL-10 production by resident macrophages.^[24] IL-10 inhibits the production of other inflammatory mediators such as IL-1,^[25] which is the most prevalent cytokine detected in peri-implant bone defects. Moreover, it has been shown that IL-1 receptor blockage changes the healing response, by modulating proinflammatory cytokine production and increasing the number of M2 macrophages.^[14] In animal models, the administration of HBMMSC has been able to reduce IL-4, IL-5, and IL-13.^[22] This is particularly interesting because it is known that IL-4 promotes the formation of FGBC *in vivo*.^[14]

Macrophages seem to play a fundamental role in the maintenance of FBE in implants, so the ability of these cells to present different phenotypes depending on changes in environmental conditions becomes relevant. The M2 phenotype of anti-inflammatory activity is involved in the healing and repair of tissues, instead of the purely phagocytic activity of the proinflammatory M1 phenotype.^[3,9]

Extracorporeal shock waves therapy and stimulation of human bone marrow-derived mesenchymal stem cells

Extracorporeal shock waves are supersonic waves, generated by different types of devices, such as electrohydraulic, piezoelectric, electromechanical or pneumatic, which generate transient pressure changes that propagate through the tissues where they are applied. This wave is characterized by high energy, rapid ascent and slow descent with a negative energy phase known as cavitation. These waves spread through tissues of different densities where they are applied, generating a cellular and extracellular biological response. At present, these waves are widely used in the context of therapeutic mechanotransduction.^[26] Mechanotransduction is the mechanism, by which a mechanical disturbance influences gene expression and cellular behavior. The cells are sensitive to shear, tension, and compression forces, and can respond to cell proliferation, migration and tissue repair.^[27]

Biophysical stimuli, particularly the treatment with extracorporeal shock waves (ESWT) and pulsed electromagnetic fields, can induce the proliferation and differentiation of MSCs from different origins. It has been proven that mechanical stimuli of low amplitude stimulate the activity not only of the bone cells but also of MSCs of the HBMMSC.^[28]

It has recently been demonstrated *in vitro* that ESWT acts as an effective bioactivator over HBMMSC, increasing their rate of growth, proliferation, migration, and healing responses and in contrast reduces the apoptosis of these cells. Therefore, it is suggested that ESWT could be an adequate tool for the preconditioning HBMMSC to express all its therapeutic potential, and, thus, be able to counteract the complications that arise from graft rejection by the host in transplant environments.^[29] It has also been shown that ESWT promotes the growth and differentiation of HBMMSC towards osteogenic cells.^[30]

Extracorporeal shock wave therapy in medicine and dentistry

Multiple experimental and clinical studies show the efficacy of ESWT in accelerating tissue repair and regeneration in various wounds.^[26] Potential mechanisms include the ability to induce neoangiogenesis, recruitment of MSCs, stimulation of cell proliferation and differentiation, anti-inflammatory and antimicrobial effects, as well as nociception suppression.^[31]

Currently, ESWT is applied to treat various medical pathologies. In orthopedics, it is used mainly in the treatment of tendinopathies, treatment of nonunion in fractures of long bones, avascular necrosis of the femoral head, chronic diabetics, nondiabetic ulcers and ischemic heart disease.^[32]

In dentistry, ESWT has been used in extracorporeal lithotripsy of salivary stones^[33] and painful mielogelosis of

the masseter.^[34] Recently, Falkensammer *et al.* used ESWT as a supplement in Orthodontics,^[35] finding an absence of deleterious effects in the maxillofacial tissues or for pulpal vitality.^[36] Li *et al.* hypothesized that ESWT could be a complementary treatment for the management of peri-implantitis, due to its multiple biological effects at the bone level.^[37]

Mechanism of action of extracorporeal shock wave therapy

Recently Holfeld *et al.* suggested that the mechanical stimuli generated by ESWT cause an increase in the permeability of the cell membrane, triggering the release of cytoplasmic ribonucleic acid (RNA) through an active process dependent on exosomes. This RNA can stimulate the Toll-like receptor 3 (TLR3) in healthy adjacent cells. TLR3 is part of the innate immune system and modulates inflammation by the stimulation of the production of several cytokines. However, the signal transduction mechanism of TLR3 receptors has not yet been elucidated.

It is important to consider that bone microvascular circulation plays a fundamental role in maintaining the function of resident MSCs and perivascular pericytes, which are currently considered indistinguishable from MSCs. Therefore, an adequate vascular supply is essential.^[21,38] It has been observed that ESWT stimulates angiogenesis and vasculogenesis, increasing the number of capillaries and increasing blood perfusion in the treated tissues. **Therefore, ESWT has been proposed today as an “endogenous cell therapy.”**^[39,40]

CONCLUSION

ESWT medical devices could become a new therapeutic strategy to immunomodulate osseointegration. The biostimulator effect on resident HBMMSC can have the potential to guide the tissue response to a more favorable outcome, with the final goal of improving clinical success and reducing the number of complications in dental implant treatment.

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Conflicts of interest

There are no conflicts of interest.

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