

COST Action: BM1309 - European network for innovative uses of EMFs in biomedical applications (EMF-MED)

Working Module proposal for WG2 – Alessia Ongaro

Working Module topic

Pulsed Electromagnetic Fields as an innovative approach for functional tissue engineering of connective tissues.

Working Module description

This Working Module proposal aims to study the role of Pulsed Electromagnetic Fields (PEMFs) in functional tissue engineering of connective tissues with particular interest for joint tissues such as articular cartilage and bone.

The tissue engineering is a multidisciplinary field with the aim to repair the tissue combining the use of biomaterials and cells, whose activity can be controlled by the addition of signal molecules, such as growth factors or modulated by physical stimuli. Tissue engineering require two phases: an *in vitro* phase to develop the construct in culture and an *in vivo* phase including the surgical implantation of the construct to repair the tissue defect and the outcome evaluation.

It is well known that pulsed electromagnetic fields (PEMFs) (1.5 mT, 75Hz) plays several positive effects on cartilage and bone tissues and they are used in orthopedics with benefit for patients. More recently, PEMFs have been studied in combination with tissue engineering to improve cartilage and bone repair.

The rationale for using PEMFs in tissue-engineering techniques for cartilage repair is based on two important findings: (1) the increase in anabolic activity of chondrocytes and cartilage explants exposed to PEMFs and (2) preventing the catabolic effects of inflammation due to osteoarthritis or surgical trauma and the lesion itself, thanks to the agonistic activity for the A2A adenosine receptor.

Since PEMFs are efficacious in facilitating ununited fracture repair in animals and humans, the osteogenic effect of PEMFs on human mesenchymal stem cells (hMSCs) has also received considerable attention as new strategies for bone tissue engineering optimization.

On the basis of these considerations, PEMFs may be useful in promoting the formation of the new cartilage in the engineered construct during its preparation *in vitro*, to protect the construct when surgically implanted in an inflammatory environment and to favor its integration with the surrounding host tissue. On the other hand, PEMFs favor the osteogenic differentiation of hMSCs cultured in osteoinductive medium, increasing the production of osteogenic markers.

However, several gaps have been highlighted from the clinical outcome evaluation and they deserve attention to improve the use of PEMFs as an useful tool for cell-based regeneration of cartilage and bone defects in orthopedics.

This Working Module aims to encourage discussion on the specific issues described above, hence facilitating the translation from cell biology, biophysical stimulation and technical progresses into clinical and therapeutic applications.

Comprehensive review (state-of-the-art)

Tissue engineering is an interdisciplinary field that use the combination of cells, biomaterials, and suitable biochemical factors to improve or replace biological functions and it is addressed to the repair of tissues. Specialized connective tissues, such as cartilage and bone, require certain mechanical and structural properties for proper functioning. Powerful developments in tissue engineering have yielded a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors,

and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. Among the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in laboratory-grown tissues destined for transplantation.

Considering that cartilage has very limited regeneration capability, the improvement of cartilage repair procedures is required and it represents the first topic of this Working Module.

To treat articular cartilage damage effectively, it is necessary to fill the defect with a repair tissue with the same mechanical and functional characteristics of hyaline cartilage and to promote the integration of the repair tissue with the host cartilage and the subchondral bone. To fulfill these aims, several procedures have been proposed also by using human mesenchymal stem cells (hMSCs). So far, clinical research has failed to identify surgical procedures that can reproduce the biological composition and biomechanical properties of native cartilage. Although no definitive treatment for cartilage repair is available, significant effort has been made to optimize treatment modalities, in particular those based on engineered cartilage tissue to speed up and improve chondral and osteochondral regeneration, thus allowing patients a swift return to previous activity levels and preventing or, at least, delaying osteoarthritis.

Tissue-engineering procedures aim at overcoming the current limitations of traditional surgical treatment by offering functional regeneration in the defect region. They require an initial step of *in vitro* culturing of chondrocytes or hMSCs alone or in the presence of natural or synthetic extracellular matrix (ECM)-based scaffolds followed by a second step consisting in the surgical implantation of the engineering-construct into the cartilage defect. By taking into consideration and controlling all the scaffold- and cell-related critical factors, the final laboratory phase requires the evaluation of the tissue substitute's biomechanical and biological properties before it is implanted; the *in vivo* result depends on the capability of functional remodeling and integration with host tissue (Fini et al., 2013). However, the lesion site scenario completely differs from the strictly controlled *in vitro* tissue conditions. In fact, in the joint environment, potent catabolic mediators could severely impact chondrocyte metabolism and ECM maintenance (Lima et al., 2008). Most cartilage tissue engineering is being done for trauma, which alters the physiological joint environment, leading to an increase of proinflammatory cytokines such as interleukin (IL)-1 and IL-6 in the acute phase of inflammation. In the long term, the chronic presence of elevated levels of IL-1 and IL-6 in the synovial fluid could be responsible for cartilage catabolism, leading to development of osteoarthritis. In addition, surgical procedures themselves trigger an inflammatory response that is detrimental to the engineered cartilage. Increased IL-1 concentration has been documented after joint surgery, and its levels have been found to correlate with the severity of cartilage damage, showing the importance of controlling the joint environment for clinical success following tissue engineering procedures. In the presence of IL-1, hMSCs differentiation is directed toward the fibroblast rather than chondrocyte phenotype and transplanted chondrocytes synthesize fibrocartilage instead of hyaline cartilage, with a loss of functional properties of articular cartilage. In an inflammatory environment, prostaglandin E2 (PGE2) is released and it promotes chondrocyte apoptosis. This implies that even transitory exposure to chemical factors, which may arise from inflammation, might have long-lasting effects on the development of immature tissue within the joint.

The second topic of interest in this Working Module is focused on bone repair. Differently from cartilage, bone is a vascularized tissue with known regeneration capability. However, bone tissue engineering is considered a promising treatment for bone injuries and damage caused by trauma, infections, tumors, and abnormal skeletal developments (Cancedda et al., 2007). *In vivo*, bone formation is a complex, tightly regulated process, influenced by multiple biochemical and physical factors. To develop a vital bone tissue engineering construct, all of these components have to be considered and integrated to gain an *in vivo*-like stimulation of target cells. In particular, for what concerns bone tissue engineering, the identification of stimuli able to address hMSCs toward the specific osteogenic differentiation is widely required.

Several *in vitro*, *in vivo*, and clinical studies have shown that biophysical stimulation with pulsed electromagnetic fields (PEMFs) plays a regulatory role of connective tissue, particularly cartilage, bone and synovia with a global positive effect on the joint tissues (De Mattei et al., 2004, 2007, 2009; Ongaro et al., 2011, 2012a). Most of these studies have shown that PEMFs affect

chondrocytes in several experimental models (monolayers, cartilage explants, and 3D scaffolds), by significantly increasing cell proliferation and synthesis of specific cartilage ECM components, including proteoglycans (PGs) and collagen type II. Further, PEMFs counteract the IL-1 β -triggered cartilage ECM degradation in healthy and osteoarthritic-joint-derived cells. Moreover, using human cartilage explants, we showed that PEMFs increase PGs synthesis of the same magnitude as that induced by the IGF-1, the main cartilage anabolic growth factor (Ongaro et al., 2011). Together, these *in vitro* data support the active role of PEMFs in the phase of development and manipulation of the construct in culture, suggesting that the combination of tissue engineering and low-frequency PEMFs might improve cartilage repair techniques.

The effect of PEMFs can be important also in the surgical phase to control and limit the inflammatory microenvironment. It has been shown that PEMFs inhibit the negative effect of the cytokine IL-1 β on the production of ECM components in cartilage explants. These results suggest that PEMFs stimulation during the implantation phase may prevent the catabolic effects induced by inflammatory molecules on both implanted and host cells, thus protecting the construct in the long term. Recent data suggest that the IL-1 β inhibitory activity on the expression of specific chondrocyte markers, including aggrecan and type II collagen and accumulation of PGs during TGF β 3-induced chondrogenic differentiation of bovine and human MSCs, can be counteracted by PEMF exposure (Ongaro et al., 2012b; Fini et al., 2013). PEMFs may act also on cell membrane receptors and may affect membrane protein distribution. In particular, *in vitro* PEMFs mediate the up-regulation of A2A adenosine receptors on chondrocytes and synoviocytes enhancing their anti-inflammatory effects as they reduce cyclooxygenase 2 (COX2) expression and prostaglandin E (PGE)₂ production in bovine and human synoviocytes (De Mattei et al., 2009; Ongaro et al., 2012a). These effects could be attributed to the capability of PEMFs to potentiate the density and functionality of A2A adenosine receptors, which, in turn, inhibit the NF- κ B signaling pathway, resulting in decreased synthesis of inflammatory molecules.

For what concerns the role of PEMFs on osteoblasts, we have reported in previous studies that PEMFs significantly increase their proliferation dependently on the presence of serum in culture, suggesting that growth factors can mediate the cellular proliferative response to PEMFs [De Mattei et al., 1999]. Other *in vitro* studies have indicated that PEMFs may stimulate both matrix organic component production and mineralization. For many years, PEMFs have been applied with beneficial effects to promote bone ununited fracture healing in clinics (Assiotis et al., 2012). All these effects can be related to the activity of PEMFs on osteoblasts, but also to the ability of PEMFs to favor, in adequate conditions, the osteogenesis of hMSCs.

The rationale for using PEMFs in bone tissue-engineering is based on the evidences showing that PEMFs can influence the behaviour of hMSCs, particularly their differentiation into the osteogenic lineage (Schwartz et al., 2008; Saino et al., 2011). Further, we have recently reported that PEMF exposure may stimulate an early osteogenic induction in hMSCs grown *in vitro* in an osteogenic microenvironment, by modulating osteogenic markers such as alkaline phosphatase activity, osteocalcin levels and matrix mineralization (Ongaro et al., 2014). Furthermore, our results indicate that in hMSCs PEMFs can exert an osteogenic role also independently by the presence of the osteoinductive bone morphogenetic protein (BMP)-2.

Gaps, challenges and objectives

Despite recent progress in the study of the role of biophysical stimulation in tissue engineering of cartilage and bone, the challenges remain significant. The main gaps are the following:

1. Clinical validation for cartilage repair. After construct implantation, degeneration of the new tissue and surrounding host cartilage has been reported. Cartilage repair often results in fibrocartilaginous tissue without the stratified organization of normal hyaline cartilage. Where hyaline cartilage is produced, it is typically immature and does not show a true articular surface. Functionally, the repair tissue is not mechanically competent and fails to withstand the mechanical stress applied to articular cartilage. Furthermore, successful lateral integration between the host cartilage and repair tissue is largely lacking; so, future degeneration is almost inevitable.

OBJECTIVE: To confirm and extend the previous experimental data and preliminary clinical applications and to perform larger clinical studies to evaluate the long-term effects of biophysical stimulation with PEMFs on tissue engineered constructs.

2. Bone regeneration and action mechanisms. Although several studies have reported effects of PEMFs to favor osteogenic differentiation of hMSC both in early and in the later phases of the process, however the action mechanisms by which PEMFs play a role in osteogenic differentiation remain to be elucidated.

OBJECTIVE: To study the mechanisms of action by which PEMFs enhance osteogenic differentiation in order to identify the cellular pathways and molecular targets involved in PEMF activities during osteogenesis.

3. PEMF parameters. It has been previously evaluated the dose-response effect of PEMF (amplitude, frequency, and exposure time) on cells and cartilage explants to identify the optimal exposure conditions to be used subsequently *in vivo* and in clinical studies. Other research results supported the finding of a strict relationship between the physical characteristics of PEMFs and the response of the exposed biological system, thus explaining the lack of effects reported in some studies.

OBJECTIVE: To finalize a common protocol of PEMF exposure and to optimize how to apply them in daily practice. In addition, with the contribution of engineers and physicists new signals PEMFs with shorter exposure time could be investigated with appreciable results for the clinical applications.

4. Tendon. It is known that PEMF treatment is able to give good results in human tendon cell culture increasing tendon cells proliferation and the expression of extracellular matrix components (De Girolamo et al., 2014), but there are no study investigating the role of PEMF in tendon tissue engineering.

OBJECTIVE: To study the effects of biophysical stimulation with PEMFs on tendon cells focusing on the potential applicability of this cell source for regenerative medicine purpose, both in surgical and in conservative treatment for tendon disorders.

Proposed research activities

Research activities are based on the list of gaps and objectives discussed above. The continued success of tissue engineering, and the eventual development of true human replacement parts, will grow from the convergence of engineering and basic research advances in tissue, matrix, growth factor, stem cell, and developmental biology, as well as materials science and bioinformatics. On this basis, this Working Module promotes the integration of different competence and the sharing of different abilities to study, in a multidisciplinary approach, the role of PEMFs in tissue engineering of connective tissue.

Moreover, this Working Module could be a platform for coordinating common activities among different groups participating to this Cost Action in order to enlarge the knowledge and the use of the biophysical stimulation in the field of tissue engineering with application in regenerative medicine.

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