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The Role of Pulsed Electromagnetic Fields (PEMFs) in the Regenerative Medicine of the Musculoskeletal System

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WM topic and description

The present proposal focuses on exploring new approaches and potential future translational perspectives of application of Pulsed Electromagnetic Fields (PEMFs) in the Regenerative Medicine of the musculoskeletal system. Specifically, here we propose innovative ways of applying PEMF stimulation *in vitro,* both in static and dynamic conditions and in in *vivo* models, aiming to devise protocols enhancing commitment and differentiation of selected mesenchymal stem cell models, specifically Synovial Fibroblast-like cells (SFs). Moreover these protocols will be finalised to induce a maximised biological response of human resident Tendon Cells (hTCs), a population located in tendon tissue, mainly composed by tenocytes and Tendon Stem Progenitor Cells (TSPCs). The protocols will aim to favour the biological activity and differentiation, of SFs and TCs, even in the presence of a pro-inflammatory environment. A deeper understanding of the biological responses and pathways present in inflamed *in vitro* cell cultures, and their modulation through specific protocols of biophysical stimulation will inform the generation of more complex models. In particular we propose the analysis of PEMF effects on several levels, from micro to macro systems, to include:

- modelling of stem cell niches and the role of biophysical stimulation within them,

- *in vitro* modelling of inflamed tendon and evaluation of anti-inflammatory, PEMF-related mechanisms,

- application of multiple stimulation (biochemical, biophysical and tribological) in bioreactors, which may closely represent the complexity of the joint microenvironment,

- analysis of PEMF activity in enhancing *in vivo* regeneration of tendon in a collagenase-induced Achilles tendinitis rat model.

Comprehensive review (state-of-the-art)

In the last few years, clinical evidence suggests the efficacy of PEMFs in favouring the healing of bone delayed union or non-union fractures and the management of articular cartilage disease, including early osteoarthritis (Ganesan et al. 2009; Shi HF et al. 2013).

PEMFs have been shown to modulate the detrimental activity of a pro-inflammatory microenvironment *in vitro* and *in viv*o (Veronesi *et al*, 2014; De Mattei *et al*, 2009; Benazzo *et al*, 2008), together with the ability to counteract the catabolic effect of selected pro-inflammatory cytokines, also during chondrogenic differentiation *in vitro* (Ongaro *et al*, 2012 b).

Studies conducted in the last few years on different cell types, including human neutrophils, chondrocytes and synovial fibroblasts, elucidated one of the potential mechanisms by which PEMFs can exert their functions, with an emerging primary role of selected adenosine receptors, mainly A_{2A} and A_3. The bioavailability and biological activity of such receptors was enhanced by specific biophysical stimulation (Vincenzi *et al*, 2013, Ongaro *et al,* 2012 a; De Mattei *et al*, 2009). Remarkably, PEMFs had a positive effect in modulating cell proliferation, tissue-specific gene expression and cytokine release in hTCs (de Girolamo et al. 2014), suggesting a role of PEMFs in the modulation of key aspects of hTCs biology.

Moreover, literature evidence suggests a role for PEMFs in promoting differentiation of mesenchymal stem cells (MSCs) or their mesengenic precursors towards selected differentiation lineages, with specific reference to chondrogenic (Esposito *et al.* 2013; Mayer-Wagner *et al*, 2011) and osteogenic lineages (Ongaro *et al*, 2014; Wang *et al*, 2014).
The summarised evidence underlines how the biophysical stimulation with selected PEMFs represents a useful tool to counteract inflammatory statuses and, interestingly, there is some evidence supporting their role in stem cell commitment and/or differentiation.
The various levels of experimental evidence obtained were recently reviewed by Fini *et al*. (2013), where a roadmap for the application of PEMF stimulation has been considered with a holistic approach. The authors hypothesised the application of biophysical stimulation in tissue engineering, with specific reference to:

- the optimisation of scaffolds/constructs seeded with cells (specifically: to facilitate cell seeding, homing and proliferation of cells within the construct),
- the utilisation post-surgical intervention (to facilitate grafting/integration of the construct into the receiving tissue and to minimise the detrimental activity of an inflamed tissue microenvironment).

Gaps and challenges

The emerging role of biophysical stimulation as a tool to promote and enhance events of cell differentiation and/or tissue repair within the musculoskeletal system still needs to be fully understood and explored.

The mere observation of *in vitro* and/or *in vivo* effects does not necessarily correlates with a full understanding of the mechanisms by which the biophysical stimulus exerts its action. Moreover the analysed studies display a heterogeneous range of ways to administer the biophysical stimulus, with or without the combination of selected biochemical stimuli.

The majority of *in vitro* studies though agrees on the utilisation of selected biophysical parameters characterising the PEMFs (75Hz, 1.5mT), as per optimised previously by De Mattei *et al* (2007).

Moving on, it can be argued that cell responses to a biophysical stimulus may not be unique, especially considering the cell type and, in the case of a stem cells, its stage of commitment and differentiation. The lack of a unique protocol of application of biophysical stimulation may already empirically reflect that a specific optimisation of biophysical and biochemical stimuli is paramount in the differentiation of diverse type of stem cells towards different lineages. Even though there is evidence of the role of biophysical stimulation on stem cells, a great debate still remains to suggest the preferential stem cell type to be used in combination with EMF stimulation, especially in promoting repair of cartilage and bone.

Mesenchymal stem cells of various derivations, mainly from bone marrow and adipose tissue, seem to be a preferential model, due to their relatively easy accessibility, low-immunogenicity and plasticity demonstrated towards chondrogenic and osteogenic lineages (Ongaro *et al*, 2014; Fu *et al*, 2014).

However, other models of mesenchymal-like cells have emerged from the literature, displaying equally interesting characteristics: specifically Synovial Fibroblast-like cells (SFs) and Tendon Cells (TCs). Both cell models display ability to functionally respond to biophysical stimulation (de Girolamo *et al*, 2014; Ongaro *et al*, 2012 a; De Mattei *et al*, 2009) and differentiate towards typical mesenchymal lineages (Stanco *et al*, 2015), even in the presence of a pro-inflammatory environment, counteracted by PEMFs (Ongaro *et al*, 2012 b). These cell models emerge as particularly attractive because of their location within the joint, and because of their documented role in the molecular cross-talk existing between different cell types at the articular level, in the inflamed tendon (Mobasheri and Shakibaei, 2013) and the osteoarthritic joint (Scanzello and Goldring, 2012). Although some of their differentiation potential has been unveiled, together with a combined suggested role of PEMF for the SFs, their full biological functions in response to biophysical stimulations remain partly understood.

The application of biophysical stimulations within bone and cartilage, as a tool to promote cell differentiation, grafting and tissue regeneration may be complicated by the presence of an inflamed microenvironment, due to pre-existing pathologies or to the surgical intervention itself (Fini *et al*, 2013). The gap of knowledge in relation to the intracellular inflammatory pathways, which may be modulated by PEMFs in inflamed cell models, needs to be addressed more deeply to create tailored protocols of application for bone and cartilage and tendon regeneration.

In particular, for what concerns the complex pathophysiology of tendons, the role of inflammation is still under great debate, as well as the mechanisms of tendon regeneration. Despite the advance in the last three decades in the surgical treatments aiming to solve tendon disorders, the recovery is often long and not always successful (Sharma and Maffulli, 2006). For these reasons the investigation of PEMF effects in an *in vitro* model of tendon inflammation, could give precious insights into the development of selected preclinical models, such as the collagenase-induced Achilles tendinitis in rat. Taken together, these studies could inform us on several aspects of tendon pathophysiology, to include the potential modulation of molecular pathways through biophysical stimulation, and the resulting possible effects on tendon regeneration.

Another important aspect is to understand how the above suggested cell models (SFs and TCs) behave within the joint in response to biophysical stimuli, and more in detail, how they behave at the interface between different tissues. The stem cell niche is defined as an anatomical compartment where populations of adult stem cells receive mechanical support from other resident cells. Another important role of the niche is to integrate humoral and nervous signals coming from the surrounding, providing the stem cells with the correct cues for quiescence, proliferation, and differentiation (Moore and Lemischka, 2006). In bone, and at the interface between bone and cartilage, bone and tendon and bone and synovium, cells are susceptible to a plethora of biophysical signals; to include hydrostatic pressure, shear forces, compression, tension, ultrasounds and EMFs. However, so far, there is a minimal understanding of how such signals can be integrated within the niche; therefore an understanding of such mechanisms emerges as a scientific need (Govey *et al*, 2013).

Finally, bioreactor technologies have been shown as a valid application to study complex cell-to-cell interactions, such as the ones present at the stem cell niche (Papadimitropoulos *et al*., 2014). Bioreactors are tools capable of mimicking *quasi vivo* environments, thanks to the combination of complex levels of biochemical, biomechanical and biophysical cell stimulation. Interestingly, it has been shown how the combination of selected regimens of biophysical and tribological stimulation in cartilage cell constructs, grown in bioreactors, enhances the quality of the scaffold architecture itself, compared to a biomechanical stimulation alone (Hilz *et al*, 2014).

Understanding whether and how it is possible to apply such level of complex modelling may indeed help to elucidate mechanisms of cell proliferation versus differentiation at the niche level, in response to specifically combined biophysical stimuli, enhancing the overall awareness of EMF mechanisms of action at the cellular level.

Objectives to be achieved

With the present WM proposal, we aim to achieve the following objectives:

a) to set *in vitro* models of TCs and SFs differentiation towards selected musculoskeletal lineages (osteogenic and chondrogenic), in the absence and in the presence of optimised biophysical stimulation regimens (exposure time, frequency and intensity of the EMF)

b) to set *in vitro* models of TCs and SFs inflammation, in the presence of optimised biophysical stimulation, to deepen the understanding of intracellular pathways and mechanisms of actions of EMFs and to inform future applications into a selected preclinical model

c) to combine the evidence obtained at points a) and b) to further:

1- elucidate functional role and mechanisms of actions of biophysical stimulation in counteracting the detrimental activity of inflammation, during processes of stem cell differentiation

2- deepen the level of analysis of cell-to-cell interaction and cell-to-environment interaction, during the differentiation sustained/favoured by biophysical stimulation, via the modelling of stem cell niches and approaches of cell engineering in bioreactors. This last point will focus mainly on SFs and TSPCs, but other mesenchymal cell models could be analysed (bone marrow and adipose tissue-derived mesenchymal stem cells).

Proposed research activities

The research activities will spring from the potential level of interest triggered by the proposed topic, namely the application of PEMFs in the regenerative medicine of the musculoskeletal system, with main reference to bone and cartilage tissue. However, the emerging role of biophysical stimulation in the broad field of stem cell biology and regenerative medicine can well go beyond the points suggested here.

As a result of a successfully started collaboration between University Campus Suffolk (UK) and IRCCS Orthopaedic Galeazzi Institute (Milano), a project aiming to establish a model of *in vitro* tendon inflammation and its modulation through selected PEMF exposure has been funded and initiated recently (1st call for STSM COST BM 1309 EMF-MED). This study would be potentially informative for the development of a preclinical model of tendinopathy as well as to increase the knowledge about the tendon cells response to inflammation and the pathological process underlined in tendinopathies.

Finally, this project represents the stepping-stone to a more comprehensive approach in the utilisation of TSPCs in translational medicine and preventative medicine.

However, thanks to the common views and shared needs between the researchers involved, the project has been the elected milieu to start investigating the modelling of the putative interaction at the niche level, between TSPCs and SFs *in vitro*, and its potential modulation with biophysical stimulation.

Other objectives such as the setting of *in vitro* models of chondrogenic/osteogenic differentiation, in the presence and in the absence of biophysical stimulation and/or a pro-inflammatory microenvironment will continue upon realisation of the objectives of the above mentioned project and upon securement of additional funds, without excluding a joint application through Arthritis Research UK. Moreover, thanks to the exciting and diverse environment we will be surrounded by in this meeting, we aim to maximise the networking with other colleagues potentially interested into PEMF applications within regenerative medicine and, more broadly, tissue and cell engineering. The possibility to establish other STSMs to favour exchange of ideas and expertise will be further explored during the meeting in Madrid.

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