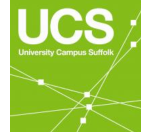

COST EMF - MED (Action BM1309):
European network for innovative uses of EMFs in biomedical applications

STSM Report:

Evaluation of PEMF anti-inflammatory effect on *in vitro* models of tendinopathy

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Abstract:

Tendon injuries are accompanied by inflammation, and up-regulation of Interleukin-1β (IL-1 β), a pro-inflammatory cytokine. The role of cytokines and pro-inflammatory pathways in tendon pathology is still poorly understood. Pulsed electromagnetic field (PEMFs) had remarkable effects on tendon cells *in vitro*, inducing proliferation, anti-inflammatory cytokines' release, and ECM component synthesis. The aim of this study was to obtain an *in vitro* model of tendon cell inflammation and to evaluate whether selected low-energy and low-frequency PEMFs (1.5mT; 75Hz) may exert an anti-inflammatory activity on the validated cell model. This study will help to better understanding pro-inflammatory pathways in tendon cells *in vitro*, aiming to mimic, at least in part, the inflammed microenvironment typical of tendinopathies.

A. Purpose of the STSM

The purposes of this STSM project were:

1- To obtain, a validated *in vitro* model of tendon cell inflammation, by using two different protocols,
2- To evaluate whether the exposure to selected PEMFs (1.5mT;75Hz) could modulate specific markers of inflammation and impact on the metabolic activity of the validated *in vitro* model of tendon cell inflammation.

B. Work Description

Human tendon cell (TCs) samples were isolated at IRCCS Galeazzi Hospital from 4 healthy donors, expanded *in vitro* up to the 3rd passage, frozen and then sent to UCS.

Subsequently, at UCS, the cells were thawed, further expanded *in vitro* and plated at the 4th passage for conducting the experiments. Prior to the experimental tests, the cells were starved in a medium with a low concentration (2%) of foetal bovine serum (FBS). One of the aims of the study was to produce a reliable model on inflamed tendon cell *in vitro*; as such two regimens of treatment were optimised:

1-the utilisation of the pro-inflammatory cytokine IL-1β, at different doses (0.1 to 10ng/ml) for 24h and the monitoring of selected outcomes at different times (24 and 48h after IL-1β treatment),

2-the application for 24h of conditioned medium (CM) derived from previously cultured SW982 synovial cell line, grown in the presence (CM+) or in the absence (CM-) of a high dose of IL1-β (10ng/ml), and the measurement of selected outcomes at different times (24 and 48h after CM treatment).

The aim was to achieve a moderate state of cell inflammation, without being too detrimental to the cell metabolism. As such, cell morphology was monitored and Alexa Fluor-568 phalloidin was used to fluorescently label actin filaments and nuclei were counterstained with DAPI. Cell metabolic activity was measured with MTT test. A multi-parameter ELISA was conducted to evaluate the release of selected cytokine in cell culture supernatants, as well as colorimetric Griess test was performed to measure the release of nitric oxide in the medium. One of the tests still under current optimisation is the immunofluorescence for COX-2, a typical mediator of inflammation and pain, which is a suggested target in tendinopathies and tendon injuries.

The optimised models of cell inflammation were selected on the basis of preliminary results: in particular, we selected TCs treated for 24h with 1ng/ml of IL-1β, and TCs treated with CM+ for 24h, as they were displaying increased metabolic activity and release of selected cytokines up to 24-48h after the removal of the pro-inflammatory stimuli. The hypothesis was that the subsequent biophysical stimulation with PEMFs could modulate and/or counteract such state of moderate inflammation.

Following the optimisation of the *in vitro* models of inflamed TCs, biophysical stimulation was applied. During the 24h of treatment in the presence of the two pro-inflammatory regimens (1ng/ml of IL-1β, and CM+, respectively) the two inflamed models of TCs were exposed for 8h to PEMF (1.5mT; 75 Hz). All tests were conducted in triplicate. Cells were processed for analysis immediately after the treatment (0d) and at 1 day and 5 days after the application of treatments. Cell metabolic activity, cytokine release, morphological analysis and ECM gene expression profiling are still in progress.

C. Results

At all time points (24 and 48h after treatment), TCs treated with different concentrations of IL1-β (0.1, 1 and 10 ng/ml) appeared viable and displayed a similar morphology. Analogously, TCs treated with CM+ and CM- from SW982 medium had healthy metabolic rates and displayed no significant changes in morphology.

TCs were removed the pro-inflammatory stimuli after 24h; the cell metabolic activity measured after additional 24h of culture was increased in comparison to untreated control cells, in particular in the presence of IL1-β 10ng/ml and in CM+ samples (+64% and +69%, respectively). On the other hand, only cells treated with CM+ for 24h still displayed a significant increase in cell metabolic activity (+25%) 48h hours after treatment removal.

Interestingly, there was no release of nitric oxide in the medium in both control and stimulated cells.

The analysis of cytokines with multi parameter ELISA, displayed an IL1-β-dose- dependent increase in the release of IL-6 , which is known to be a powerful pro-inflammatory cytokine. Indeed, the highest increases were observed in the samples treated with IL1-β and 10 ng/ml and CM+.

Given these findings, we deemed optimised two protocols to our subsequent scope of PEMF application:

1-TC cultures treated for 24h with IL1-β at 1 ng/ml (IL1-β+)

2- TC cultures treated with pre-activated SW982 cell-derived medium (CM+).

Both IL1-β+ and CM+ TCs were then exposed for 8 hours to PEMF (1,5mT, 75Hz), during the 24h of inflammatory stimulation, with the aim of inhibiting/counteracting inflammatory events during the stimulation phase.

The preliminary results suggest that PEMF exposure does not alter significantly the cell morphology in all samples and at all time points At 1 and 120h from PEMF exposure, cell viability of IL1-β+ cells showed an increase at day 1 of +19%, and at day 5 + 37%, respectively ($p<0.05$), in comparison to untreated cells. This may suggest that the biophysical stimulation can possibly counteract the detrimental activity IL1-β *in vitro*, by promoting the metabolic activity of TCs, even on a long term-basis, further to the exposure to an inflamed microenvironment.

At present, evaluations of cytokine release, COX-2 expression and gene expression of tendon-related markers are in progress.

D. Future collaboration with host institution

The scope of the STSM will indeed go above and beyond the three months of visit. Considering that part of the analysis is still in progress, we aim to extend the collaboration for at least additional three months. A research agreement between the two Institutions involved (IRCCS Galeazzi and University Campus Suffolk) has been produced and it details terms and conditions of the collaboration, in a frame of shared research outputs and expanded collaborative projects. Plans for mutual financial effort in the completion and expansion of the current project have also been discussed, testifying how both parts truly believe in the value associated to this STSM experience. Moreover, the applicant and the contact referent at the receiving Institution are in the process of consolidating ideas and networks to initiate a multicentre study to evaluate markers of inflammation/joint damage in elite football players. The project had the endorsement of the Scientific Director of IRCCS Galeazzi and of other relevant parties at UCS. Recently, the applicant and the referent contact at the receiving Institution were accepted as members of a new COST working module on PEMF and Immunomodulation. The current work being done in TCs treated in the presence of synovial cell line-derived medium, aiming to represent a model of pro-inflammatory niche, could be a useful tool to expand the understanding of immunomodulatory mechanisms mediated by EMFs.

E. Expected Publications

In the frame of collaboration and collective effort initiated and consolidated throughout the STSM, there is the aim of producing at least 2 peer-reviewed manuscripts by the end of 2016.

1- A manuscript discussing the modulation of inflammation with PEMFs in TCs treated in the presence of IL1-β

2- A manuscript detailing the behaviour of TCs in response to synovial cell-derived CM, as a model of cell niche and its modulation by PEMFs

F. Other Comments

N.A.

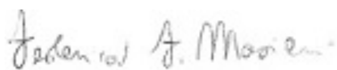
Confirmation by the host institution of the successful execution of the STSM:

We confirm that Ms. Deborah Stanco has performed the research work as described above and we are pleased by the positive outcomes of the STSM.

Dr Federica F. Masieri

Signature

Signature



Deborah Stanco