The method of optimization includes: i) cell type; ii) source of dexamethasone; and iii) percentage of oxygen. In the experiment three different cell culture types were used: chondrocytes, human mesenchymal stem cells from bone marrow (hMSC) and combination of chondrocytes and hMSC in 2:1 ratio. Cells were grown in 3D culture, incorporated in a peptide hydrogel RADA (BD PuraMatrix Peptide Hydrogel). In order to induce chondrogenesis, cells were put in a differentiation medium containing ascorbic acid-2-phosphate, L-proline, ITS, TGFβ-1 and dexamethasone. Dexamethasone was added in a differentiation medium or it was incorporated with cells within a peptide hydrogel. In order to demonstrate the effect of oxygen level on the efficiency of chondrogenic differentiation, cells were grown in normoxic (20% O2) and hypoxic conditions (5%O2). Expression levels of two important cartilage marker genes, SOX9 and agreccan, were evaluated by quantitative PCR in samples on day10 and day21 after induction of chondrogenesis.

Our results provide important insights into effects of how different cell type, dexamethasone source and oxygen level affect efficiency of in vitro chondrogenesis.

PLATELET RICH PLASMA IN THE TREATMENT OF SPORTS INJURIES: INDICATATIONS AND TECHNIQUE

Trpimir Vrdoljak, Tomislav Šušnjar, Saša Janković, Damir Hudetz, Alan Ivković

KB Sveti Duh, Zagreb, Croatia

Platelet-rich plasma (PRP) due to its role in wound healing, has spanned various fields of orthopaedics and sports medicine . Among athletes muscle injuries are common and may be associated with impaired functional capacity. The vulnerability of athletes to strains and contusions represents a substantial problem for professional players and their clubs. Such injuries involve significant time lost from training and competition. Presently there are no drugs available to hasten restoration of muscle function after injury and the results of healing with conventional therapy including rest, ice, compression, and elevation (RICE) are often inadequate. Therefore platelet-rich plasma (PRP) therapies may help athletes by promoting muscle regeneration , enhancing the process of soft-tissue healing and to decrease time to recovery.PRP is generally considered an elective treatment for subacute and chronic conditions. In orthopaedics and sports medicine is used to treat tendinopathies, ligament sprains, muscle strains, degenerative joint conditions. PRP is obtained from a sample

of patients blood drawn at the time of treatment,and it is prepared in a process of differential centrifugation,where acceleration force is adjusted to sediment certain cellular constituents based on different specific gravity. Suspensions have different concentration of platelets and leucocytes depending on the method and time of its centrifugation.PRP injections may be performed unaided or under ultrasound guidance. Multiple injection techniques have been employed including intramuscular delivery of a singular bolus, multiple depots at the site of maximal injury, or a single injection into the muscle insertion site.

A NEURAL CREST ORIGIN OF TENDON CELLS?

Herbert Tempfer, Renate Gehwolf, Christine Lehner, Ludwig Aigner, Andreas Traweger

Institute of Tendon and Bone Regeneration,
Spinal Cord Injury and Tissue Regeneration Center Salzburg,
Austrian Cluster for Tissue Regeneration, Salzburg, Austria

We have previously shown that tendon perivascular cells (TPCs) express markers associated with tendon cells and neural stem cells, such as Nestin and Musashi1. Findings that tendon cells express a variety of neuron associated markers such as acetylcholine, M2 acetylcholine receptors or substance P now lead us to hypothesize that tendons harbour a cell population of neural crest origin.

By immunohistochemistry using antibodies specific for tendon, neural crest and neuron associated markers on control- and Rosa26-YFP-Sox10-Cre mice as well as on Scleraxis-GFP mice and by in vitro differentiation assays on cultured murine tendon cells we characterized tendon cell in vitro and in vivo.

Murine Achilles tendon cells coexpress p75NTR, Tenomodulin, Doublecortin, Neurofilament and TUJ1.

Tendon cells from sox10cre mice express YFP together with tenomodulin the tail tendon anlagen of of E13.5 embryos. YFP is detectable in the Achilles tendons of adult animals. Tendon cells in tails of Scleraxis-GFP e13.5 embryos partly co-express p75 and GFP.

In vitro murine tendon cells differentiate into functional neurons with the capacity to generate spontaneous action potentials, detected by multi electrode array.

With this work we show that murine tendon cells express a variety of neuron associated markers. Analysis of Rosa26-YFP-Sox10-Cre and Scleraxis- GFP mouse tendons reveals a potential neural crest origin of a tendon cell population.