Synergy of growth factors and mineralizing agents in bone remodelling: assessment on cultured osteoblasts for tissue engineering

M. Leigheb[1,3], M. Bosetti[3], M. Cannas[3], C. Cisari[2], M. Cherubino[4], F.A. Grassi[1]

[1]S.C. Orthopaedics and Traumatology; [2]S.C. Functional Rehabilitation, University Hospital "Maggiore d.c." (Novara, ITALY);
[3]Laboratory of Biocompatibility and Tissue Engineering, Department of Clinical and Experimental Medicine, University of Eastern Piedmont (Novara, ITALY);
[4]S.C. Plastic and Reconstructive Surgery, University of Insubria (Varese, ITALY)

Objective We have investigated and compared the influence of three isoforms of transforming growth factor beta (TGF-ß1, TGF-ß2 and TGF-ß3), three fibroblast growth factors (FGF-2, FGF-4 and FGF-6) and the active metabolite of Vitamin D [1,25-(OH)2D3] on proliferation, alkaline phosphatase activity and mineralization of human primary osteoblasts (hOB).
Material and methods HOB were cultured for a period of 24 days and divided into the following groups relating to the GF and/or mineralizing agents added: untreated cells (negative control), FGF-2, 4 and 6 respectively, TGF ß1, ß2 and ß3 respectively, Vitamin D. Proliferation was assessed via ATP + Luciferin + O2 α Oxyluciferin + AMP + PPi + CO2 + light, differentiation via P-nitrophenilphosphate + dietanolamine α P-nitrophenol, mineralization via Calceine staining and densitometric quantification.
Results TGF-ß isoforms and three FGFs examined have been proved to be inducers of osteoblasts proliferation (higher extent for TGF-ß and FGF-2) and inhibitors of alkaline phosphatase activity and osteoblasts mineralization. Combination of these growth factors with the active form of Vitamin D induced osteodifferentiation. In fact Vitamin D showed an additive effect on alkaline phosphatase activity and calcium content, induced by FGF-2 and TGF-ß in human osteoblast.
Conclusions These results highlight the potential of proliferating cytokines combination with mineralizing agents for in vitro bone growth induction in bone tissue engineering.

NGF and TrkA receptor expression in chronic tendon ruptures: potential role in the pathogenesis of degenerative tendon lesions

M. Surace, M. Ronga, L. Murena, A. Fagetti, D. Marcolli

Dipartimento di Scienze Chirurgiche Ricostruttive e Tecnologie Avanzate (Varese, ITALY)

Aim of our study was to investigate the expression of nerve growth factor (NGF) and its tyrosine kinase receptor (TrkA) on tendinosic tissue harvested from Achilles and rotator cuff tendons. The presence of NGF and TrkA receptor was proven through immunofluorescence. We randomly recruited 6 patients that underwent rotator cuff arthroscopic repair and 10 patients that were treated for an acute Achilles tendon lesion. During surgery we harvested samples of the rotator cuff and of Achilles tendon. Furthermore we took a sample of macroscopically healthy tissue from each Achilles tendon.
From each specimen 4 slides were obtained. Two slides were employed for the search of NGF, one was treated with specific antibodies and marked with FITC (Fluorescein Isothiocyanate Conjugated), the second slide was for control purposes and was exposed to FITC, but without prior exposition to the specific antibody. The same procedure was repeated to obtain on two more slides in order to repeat the search for Trka, with specific antibodies. All the slides were studied on a fluoromicroscope.
The analysis of these specimens revealed the presence of the NGF and of the TrkA in all the rotator cuff specimens: the immunoistochemical reaction between the specimens and the specific antibodies marked with FITC was seen under fluoromicroscopy, but in none of the control cases treated with only FITC.
The samples of Achilles tendon revealed the presence of NGF in 9 of 10 cases. In one case the sample was negative for NGF and TrkA receptor and no inflammatory reaction was spotted.
The specimens harvested from the macroscopically healthy Achilles tendon revealed no inflammatory reaction and immunofluorescence revealed no NGF or TrkA receptor expression.
There is considerable evidence that shows that the system constituted by the NGF and his high-affinity receptor TrkA plays a fundamental role in the molecular processes underlying the main forms of persistent pain.This indicates a possible therapeutic area for the antibodies that could block the NGF/TrkA system, in order to modulate the frequency and the duration of the action potential of nociceptive neurons during chronic inflammation.
NGF and TrkA were absent on normal tissue and increased on degenerative tendon specimens. These findings could suggest a role of NGF in the pathophysiology of degenerative tendon rupture.

Bioactive micro-structured scaffold for annulus fibrosus repair and regeneration

G. Vadalà[1], A. Rainer[2], M. Loppini[1], M. Centola[2], M. Trombetta[2], V. Denaro[1]

[1]Area of Orthopaedics and Trauma Surgery; [2]Laboratory of Chemistry & Biomaterials, University Campus Bio-Medico of Rome (Rome, ITALY)

Objective Annulus fibrosus (AF) tissue engineering is reaching increasing interest [1] in developing strategies to both reduce recurrent disc herniation (DH) rate and increase effectiveness of intervertebral disc regeneration techniques. This study evaluate the use of a bioactive microfiber scaffold in Poly-L-Lactic Acid (PLLA) releasing the growth factor TGF-β1 and investigate both cell toxicity and the extracellular matrix produced by bovine AF cells (bAFCs) and human mesenchymal stem cells (hMSCs) cultured on these scaffolds in vitro.
Material and methods Scaffolds were fabricated by electrospinning a PLLA solutions loaded with TGF-β1 and characterized in terms of morphology and release rate of TGF-β1. Bare PLLA scaffolds were use as control. bAFCs and hMSCs were cultured on the scaffolds and cell toxicity was evaluated at 4, 6 and 24 h. bAFCs were seeded at the density of 5×105 cell/cm^2 on the scaffold and cultured for 3 weeks. bAFCs were tested to quantitatively assess glycosaminoglycans (DMMB assay) and total collagen production (Sirus Red Assay). Histology was performed and the neo-ECM thickness measured.
Results PLLA and PLLA/TGF-β1 membranes were composed by fibers with diameter of 1.5 ± 0.9 μm and 0.6 ± 0.2 μm respectively. The scaffolds were not toxic for both hMSCs and bAFCs at all time points. PLLA/TGF-β1 released TGF-β1. bAFCs cultured on PLLA/