Establishing procedures for using a goat model for studying autologous tissue engineering strategies for the repair of cartilage and bone defects

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Statement of Purpose: The aim of this study was to establish a goat model for evaluating autologous tissue engineering strategies for the regeneration of bone and cartilage tissue defects, based on biodegradable starch based scaffolds seeded with goat bone marrow cells (GMCs). For this purpose, it was established and optimized cell isolation and culturing methodologies in order to differentiate, *in vitro,* GMCs into osteoblasts and chondrocytes and compare the respective cellular behaviour in autologous and non-autologous conditions. The cell performance before and after being cryo-preserved was also evaluated. Additionally, static and dynamic (bioreactor) cultures were used to determine the best differentiation and proliferation methodology. Furthermore, it is envisioned the assessment of i*n vivo* functionality of the constructs, by the implantation of cell seeded scaffold during the repair process of osteo and chondral-induced defects using goat models.

Methods: Marrow stromal cells (MSCs) were isolated from the iliac crest of adult goats and cultured with DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% of either fetal bovine serum (FBS) or goat serum isolated from goat peripheral blood. Cells were grown until confluence and sub-cultured twice (P2) before being cryopreserved. To induce osteogenic differentiation, cells were cultured under different dynamic conditions and/or in the presence osteogenic supplements (10^{-8} M dexamethasone, 50μg/ml ascorbic acid, and 10mM β-glycerophosphate). Osteogenic differentiation was assessed by von Kossa staining, alkaline phosphatase activity (ALP) and calcium quantification procedures. To induce chondrogenesis, GMCs were cultured with TGF-β in 3D (pellets). The presence of chondrocytes was studied by Alcian Blue, Toluidine Blue and Safranin O staining as well as by GAGs quantification kit and immunocytochemistry for type II collagen. In both, cell viability and proliferation was assessed by DNA quantification and by the MTS test, while cellular morphology was assessed by scanning electron microscopy (SEM). Fiber-mesh based on SPCL, (a blend of corn starch with polycaprolactone, 30/70% wt) with a porosity of about 75%, was used as scaffold materials. These scaffolds have been used with very promising results in previous studies[1,2]. In order to evaluate the scaffolds ability to promote cell attachment, proliferation and differentiation, the materials were seeded with a cell suspension (GMCs primary cultures at Passage 2 or Passage 3) at a concentration of $33.3x10^5$cells/ml and cultured for 14days for osteogenic- and a concentration of $2x10^6$ cells/pellet for 28 days for chondrogenic- differentiation.

Results / Discussion: The proliferation potential from cryopreserved cells is very similar to that of freshly obtained marrow cells. Mineralization node like-structures were firstly seen after 6days of osteogenic medium cultures and chondrogenic pellets (0.9mm diameter) were obtained after 21days in chondrogenic 3D cultures.

Significant differences between autologous and non-autologous approaches viability (MTS) (Figure 1) and proliferation (DNA) tests were not found in *in vitro* studies; although they might be found during the on going *in vivo* experiments as far as our preliminary results seem to indicate.

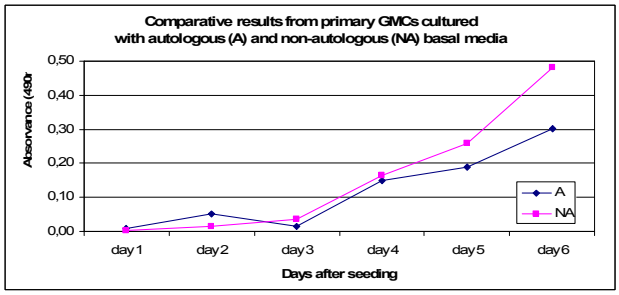

Figure 1 - Comparative results from primary GMCs cultured with autologous (A) and non-autologous media (NA).

Conclusions: Results show that GMCs behaviour as a primary culture demonstrates high cellular density and proliferation rate as well as high 2D culture stability. Cryopreserved cells have a very good recovery process, maintaining the initial proliferation potential.

In addition, GMCs differentiation and proliferation when seeded and cultured onto the starch based scaffolds, indicates that these scaffold-cell constructs may provide adequate tissue-like substitutes.

In summary, it was established an autologous tissue engineering model strategy which allows for obtaining tissue substitutes *in vitro* that will be subsequently implanted to repair bone and cartilage defects.

References:

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