In vivo evaluation of the suitability of starch-based scaffolds for bone tissue engineering constructs using Adipose Derived Adult Stem Cells and transgenic mice

T. C. Santos[1,2,3], K. Tuzlakoglu[1,2], T. Morton[3], K. Lang[3], K. Reise[3], A. P. Marques[1,2], A. G. Castro[4], H. Redl[3], R. L. Reis[1,2] and M. van Griensven[3]

[1]3Bs Research Group-Biomaterials, Biodegradables and Biomimetics, Univ. of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[2]Dept. of Polymer Engineering, University of Minho, Campus de Azurém, 4800-058 Guimarães, Portugal
[3]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Donaueschingenstrasse 13, A-1200 Vienna, Austria
[4]Institute of Health and Life Sciences, School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal

Statement of Purpose: One of the biggest challenges in the Tissue Engineering field is the achievement of the ideal construct. In recent years some research have been done concerning the use of natural polymers, combined with cells and growth factors, in order to obtain the adequate shape and cell organization in the assembly of constructs for bone tissue engineering. The aim of this work was to investigate the suitability of the constructs composed by starch-based fiber meshes, obtained by a wet-spining methodology, embedded in fibrin sealant (Baxter®), seeded with Adipose Derived Adult Stem Cells (ADAS), isolated from human adipose tissue, and complemented with growth factors, for bone tissue engineering.

Methods: A qualitative *in vitro* assay was performed by seeding ADAS cells, labeled with a fluorescent dye (DyeI®), on the starch-based scaffolds. The results were analysed by confocal microscopy after 5 days. For the *in vivo* approach, different tissue engineering constructs were assembled, by combination of starch-based scaffolds, fibrin sealant, ADAS cells and growth factors (bone morphogenetic protein-2 – BPM-2; vascular endothelial growth factor – VEGF or fibroblast growth factor-2 – FGF-2). After 24 hours of incubation in cell culture medium, at 37ºC and 5% CO2, the constructs were subcutaneously implanted in transgenic mice. VEGFR2-luc transgenic mice provide data concerning processes involving the vascular endothelial growth factor receptor-2 (VEGFR-2) while OC-luc transgenic mice reveal osteocalcin (OC) expression patterns. The constructs with VEGF and FGF-2 were implanted in VEGFR2-luc transgenic mice and the constructs with BMP-2 were implanted in OC-luc transgenic mice. Four animals per condition and two implants in each animal (n=8) were used. The results were assessed with a back-illuminated CCD (charged couple device) camera (Xenogen®) and a follow up every 3 days of the implantation was performed. At the end time point of the experiment (13 days) the explants were retrieved from the animals to perform histological analysis and Polymerase Chain Reaction (PCR) analysis for osteo- and chondrogenic differentiation, in the case of the OC-luc mice, and for VEGF and VEGR-R1 in the case of the VEGFR2-luc mice. In addition to those parameters, a basic inflammatory reaction analysis was performed.

Results/Discussion: The *in vitro* results of confocal microscopy showed that the ADAS cells were able to grow along and bridging the fibers of the starch-based scaffolds after 5 days of incubation. The preliminary data obtained by the living image system shows that after 3 days of implantation in the VEGFR2-luc transgenic mice, the constructs containing FGF-2 induce a slight increase of the luminescence signal while the constructs containing VEGF did not induce changes in the luminescence signal. However, after 9 days of implantation, the luminescence signal increased for all the tested conditions, in contrast with the negative control (pockets instead of implanted constructs), that showed a constant luminescence signal, which started to decrease 9 days after implantation. This data was substantiated with the PCR results, since in all tested conditions, except for the negative control, was found the expression of VEGF-R1. After 3 days of implantation in the OC-luc mice, all the constructs showed a slight increase of the luminescence signal, which started to decrease after 9 days of implantation. The exception was observed for the scaffold with fibrin sealant, which showed a considerable increase of the luminescence signal at day 3 and a slight decrease 13 days after implantation. Again, these data are supported by the PCR results that show expression of the CBFA1 (RUNX2) and Osteocalcin genes for the scaffold and the construct with ADAS cells and fibrin sealant. Furthermore, the CBFA1 (RUNX2) gene was expressed in the animals where the construct with BMP-2 was implanted, and the Osteocalcin gene was expressed in the cases of implantation of the scaffold combined with fibrin sealant and ADAS cells. In addition, the histological analysis showed that subcutaneous implantation induced a normal and expected mild inflammatory response to all the implanted constructs.

Conclusions: The results of *in vivo* imaging showed that the introduction of growth factors (VEGF, FGF-2 and BMP-2) and ADAS cells did not have a strong influence in the expression of the Osteocalcin and VEGF-R2 genes. Even though the PCR results suggest that the SPCL scaffold is able to induce the expression of VEGF-R1, CBFA1 (RUNX2) and Osteocalcin genes. The addition of fibrin sealant, ADAS cells and growth factors, such as VEGF, FGF-2 and BMP-2, could be considered to enhance the performance of the scaffolds and to obtain a suitable construct for bone tissue engineering applications Those constructs showed to be promising tools since they were able to induce the expression of osteo- and vascular differentiation and did not induce an unexpected inflammatory response.

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