Polyhydroxybutyrate-co-valerate/La-containing apatite composite for tissue engineering

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(BMP-2) variants onto collagen beads in a site directed manner and test their osteogenic potential *in vitro* and *in vivo*. Because of the problems observed in all the approaches developed to incorporate growth factors into biomaterials, we decided to immobilize the growth factor BMP-2 site-directly to collagen beads by the use of ''click chemistry''. This method potentially overcomes the drawbacks connected with surface absorption and encapsulation. For this purpose, we created two BMP-2 variants through site directed mutagenesis, comprising one unique non-natural amino acid substitution in each chain of the mature polypeptide, allowing a site-specific coupling by a specified coupling chemistry.

Apart from the physical and chemical aspects connected to the immobilization, we focused our attention on improving the growth factor's bioactivity by a caption of the BMP antagonist Noggin. BMPs perform their pro-osteogenic effect by binding to and oligomerizing of membrane receptors. Noggin binds to BMPs and interferes with their ability to induce receptor dimerization. Here we identify peptide sequences, through phage display method and analyze them in terms on their Noggin-binding characteristics and their potential to inhibit BMP-2 mediated biological responses. Both tools, the covalently coupled BMP-2 and the peptides, which might act as Noggin ''deflectors'' might produce an innovative biomaterial with superior bone healing properties.

Polyhydroxybutyrate-co-valerate/La-containing Apatite Composite for Tissue Engineering

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Three-dimensional (3D) structures (scaffolds) have been widely studied for bone regeneration in tissue engineering, mainly polymeric scaffolds. Therefore, this study developed a bioactive-radiopaque polymer based on polyhydroxybutyrate-co-valerate (PHBV) and La-containing apatite ($La_{20}AP$) (PHBV/$La_{20}Ap$) for 3D printing. First, the composite was prepared in a twin screw extruder for producing standardized filaments. A technology similar to ''fused deposition modeling'', 3D printer - Fab@CTI (open source, Fab@Home), was used for printing the 3D scaffolds. The samples were characterized by Scanning Electron Microscope (SEM), Digital Radiography, Differential Scanning Calorimetry (DSC) and Dynamic Mechanical Analysis (DMA). Cytotoxicity was evaluated using CHO-K1 cells. SEM images showed the presence of apatite particles homogeneously distributed throughout of the extruded material. Radiographic images revealed that the composite presents suitable radiopacity for diagnostic imaging. The DSC results showed that thermal properties of polymer are maintained after incorporation of $La_{20}Ap$ into the PHBV; moreover, PHBV/$La_{20}Ap$ composite revealed better mechanical properties than PHBV polymer. *In vitro* assays demonstrated no cytotoxic effects in CHO-K1 cells for PHBV/$La_{20}Ap$ composite. In conclusion, the PHBV/$La_{20}Ap$ composite produced by extrusion process might be a potential material for 3D printing and for application in tissue engineering.

Generation of a Bone Organ by Human Adipose-derived Stromal Cells through Endochondral Ossification

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Among mesenchymal progenitors (MSC), only skeletal MSC from bone marrow form ectopic bone through endochondral ossification (ECO). The goal of this study was to assess whether chondrogenically or hyperthrophically-primed human adipose derived stromal cells (ASC) were capable to form bone organ through ECO. Minimally-expanded ASC were either cultured as micromass-pellets or onto type-I-collagen scaffolds in chondrogenic medium containing TGF-β3 and BMP-6 for 4 weeks (early hypertrophic templates). A medium supplemented with β-glycerophosphate, L-thyroxin and IL1-β was added for 2 additional weeks to further induce hypertrophy (late hypertrophic templates). Constructs were subcutaneously implanted in nude mice and analysed 8 weeks post-surgery.

In vitro, deposition of a cartilaginous matrix, positive for glycosaminoglycans, type II collagen and Indian Hedgehog was shown. Upon induction of hypertrophy, gene expression analysis showed up-regulation of type X collagen, BSP and MMP-13. RT-PCR data were confirmed at protein level. *In vivo*, both early and late hypertrophic constructs underwent cartilage remodelling evidenced by the presence of MMP-13 and TRAP positive cells and further developed into bone tissue, containing osteocyte-like cells embedded in a matrix rich of BSP. Newly-formed bone was vascularized and included a bone marrow cavity. *In situ* hybridization for human-specific sequences and staining with a human specific anti-CD146 antibody demonstrated a direct contribution of ASC not only to bone tissue formation but also to the bone marrow stroma. In conclusion, human ASC can undergo a developmental program of ECO. This specific bone regenerative potential should now be evaluated in an orthotopic model of bone repair.

Effect of Elastic Moduli of Micro-Fiber/Collagen Composites on Ligament Development

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Traditional hydrogels lack the topographical and mechanical cues to guide mesenchymal stem cell (MSC) differentiation into oriented tissues. Toward the development of a ligament-like tissue, we hypothesized that the incorporation of sparse electrospun fibers within a 3D collagen gel could confer both topographic cues and anisotropic mechanical properties to guide MSC alignment and differentiation. Further, we postulated that a low density of fibers would allow MSCs to proliferate and migrate freely, and to remodel the collagen gel without being impeded by the electrospun fiber network.

Toward this goal, sparse aligned fiber networks with elastic moduli of 5.6 (soft), 15.1 (moderate), and 31 (stiff) MPa were electrospun from blends of polycaprolactone and polyurethane. MSCs in collagen were introduced to the fibers to form seeded fiber/collagen composites, and cultured for 14 days. Confocal microscopy revealed that soft fibers induced orientation of cells both attached to the fibers and with the collagen bulk, while cells exposed to stiff fibers were primarily localized to the fibers with remaining cells in the collagen relatively unoriented. Additionally, expression of the ligament transcription factor scleraxis and the contractile fibroblast marker α-smooth muscle actin were enhanced on the soft and moderate fiber composites, while collagen 1 α1 expression was increased in stiff fiber composites. These results indicate that sparse fibers, regardless of stiffness, induce cell alignment, with softer fibers promoting ligament phenotype development. Moving forward, our ongoing effort is to guide MSCs toward a ligament phenotype in thicker composites suitable for implantation.

Tissue Forming Capacity of Nasal and Articular Derived Chondrocytes for Intervertebral Disc Regeneration-Effect of Oxygen and TGFβ Stimulation

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