Starch-Poly(E-Caprolactone) Fiber Based Scaffolds Are Suitable For Cartilage Tissue Engineering Approaches

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Statement of Purpose: Tissue Engineering has emerged in the last decade as an alternative to the currently used therapies within the bone and cartilage reparative/regenerative medicine field.[1] In this particular field it is believed that a biodegradable 3D template- a scaffold- is needed. These scaffolds will eventually allow cells to adhere and proliferate, which lately will lead to ECM elaboration with consequent tissue regeneration. Current gold standard materials within the field are mainly based on PLA, PLGA or their co-polymers. However, these materials release acidic by-products, which can jeopardize the tissue engineered construct integration and consequent tissue regeneration. Natural origin polymers have been put forward in the last years, as an alternative to the referred materials. Among this class of natural polymers, starch seems to be one of the most promising, according to its biocompatibility and biodegradability features, among others.[2] Even so, some of its intrinsic properties limit the range of processing methodologies, and blending this material with a synthetic and more easily processable one appears to be a good option. Being so, in the present work we tested the suitability of starch-poly(ε-caprolactone) scaffolds for pursuing a cartilage tissue engineering approach.
Methods: The methodology used in scaffolds production was fiber extrusion followed by fiber bonding using controlled temperature near the melting point of the synthetic polymer. The produced scaffolds had cylindrical shape and dimensions of 7x3mm. Bovine articular chondrocytes were isolated from cows legs, according to enzymatic based procedures. Briefly, trypsin and collagenase type II were used in combination in order to digest the extracellular matrix (ECM) present in the small minced cartilage pieces. The chondrocytes were then isolated by centrifugation and cultured with medium containing FGF-2.
When the sufficient cell number was obtained, cells were trypsinised and put in suspension in spinner flasks, where the sterilised scaffolds were cell seeded for a 3-day period. Afterwards, they were cultured under rotational agitation in non-tissue culture treated Petri dishes, and the expansion culture medium (with FGF-2), was maintained until day 7. Afterwards, it was exchanged by differentiation medium, containing insulin and L-ascorbic acid. The constructs were cultured for up to 6 weeks, and, in defined time periods, constructs were analysed by the following techniques: Hematoxylin-eosin, Toluidine blue, Alcian blue, Immunohistochemistry for collagen types I and II, Scanning Electron Microscopy (SEM) and Glycosaminoglycans (GAG's) quantification assay. PGA non-woven scaffolds and bovine native articular cartilage were used as controls.

Results / Discussion: The cells were able to proliferate and differentiate within the scaffolds structure for the full-time of the experiments. The performed analysis included general cell observation methods, as well as articular cartilage components identification. The images below show the results obtained for some of the analysis described in the Methods part.

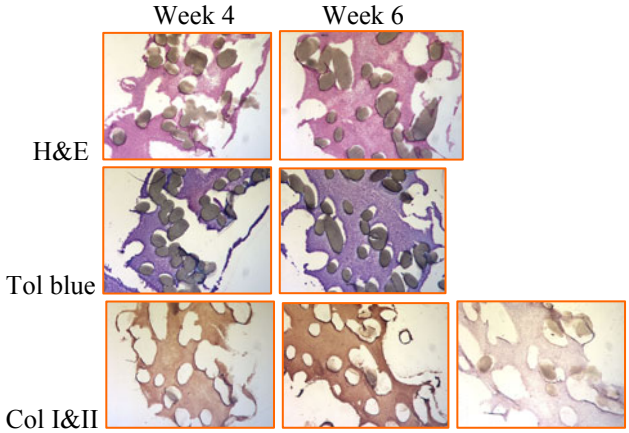

Fig.1. Hematoxylin-eosin, Toluidine blue and Immunohistochemistry results for weeks 4 and 6 (x40).

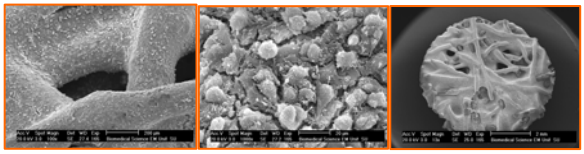
Fig 2. SEM pictures of the tissue engineered constructs at weeks 2, 4 and 6 (left to right, respectively).

The Toluidine blue evidences the presence of GAG's present in the newly synthesized matrix. The immunological results clearly show a predominance of collagen type II, the most prevalent collagen type in articular cartilage, in the produced ECM.
Conclusions: The results herein reported show that the studied starch-poly(ε-caprolactone) fiber-based scaffolds scaffolds support cell adhesion, proliferation and differentiation, for up to 6 weeks.We therefore conclude that these scaffolds should be considered for further studies in the cartilage tissue engineering field.

References

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