In vivo performance of gellan gum-based structures: A comparison study between hydrogels and spongy-like scaffolds for osteochondral tissue regeneration

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Statement of Purpose: The orthopaedic field has been facing challenging difficulties when it comes to regeneration of large defects as found in osteochondral defects (OCD). Autologous osteochondral mosaicplasty has been used as valid option for OCD treatment. But donor site morbidity remains a big issue with most used autografts [1]. Promising strategies for the regeneration of large osteochondral defects (Grade 4) implies the application of biomaterials, growth factors, and cells alone or in combination. But the ideal treatment remains unclear. Our group has been proposed bilayered structures to regenerate osteochondral defects [2]. The underlying hypothesis of our study was to compare the *in vivo* responses of bilayered hydrogels and spongy-like hydrogels applied as 3D implants for OCD regeneration following an acellular strategy. Both structures (hydrogels and spongy-like) present the same backbone material. However different physical properties due to the different processing methods can imply a different response in OCD regeneration. The study was investigated using two different *in vivo models.* The subcutaneous inflammatory response was assessed in mice model and the materials performance such as materials stability, cellular behavior, cells and macrophages infiltration, matrix deposition and new bone and cartilage formation assessed in rabbit critical size OCD model.

Methods: Low Acyl Gellan Gum (LAGG) was purchased from Sigma-Aldrich (USA). Cartilage-like layer was obtained from a 2wt% LAGG solution and bone-like layer consisted of a 2wt% LAGG incorporating different HAp ratios (20-30wt%). Both structures followed different processing methods. While hydrogels were made *in situ*, the spongy-like were previous produced by means of freeze-drying and posterior sterilization. Either hydrogels or spongy-like were subcutaneous and surgical implanted. The subcutaneous implantation (mice models) and critical size OCD surgical implants (rabbit model, diameter 4 mm and 5 mm depth) studies were carried out for 2 and 4 weeks time, respectively. Both approaches were tested for inflammatory responses and cellular behavior by means of histology staining and biochemical function, as matrix deposition by immunohistochemistry. Moreover, the OC structures stability and new bone formation was assessed using x-Ray *Vivo*- and micro-computed tomography (micro-CT).

Results: Subcutaneous implantation was performed in mice model. Observing the histology characterization by Haematoxylin-eosin and Masson's Trichrome stainings showed no acute inflammation, no macrophages invasion nor fibrous capsule were observed at both mice implants. The bone- and cartilage-like layers are clearly seen in the implanted spongy-like hydrogels.

OC regeneration was tested in OCD in rabbit using two different structures: bilayered hydrogels in comparison to bilayered spongy-like hydrogels. Hydrogels crosslinking *in situ*, following an acellular strategy was addressed. OC empty defects were used as controls. From photographic images the defects sites with implanted spongy-like showed the formation of tissue surrounding the defect with a bright and natural coloration. In the case of empty defects a thin membrane of tissue was formed with no tissue invasion below. Comparing empty defects and the defects with implanted spongy-like is evident that there is hard tissue invasion as analyzed by micro-CT. Furthermore, almost in all cases the amount of soft tissue is largely higher than hard tissue. Soft tissue can be considered fibrous tissue and cartilage-like ECM, while hard tissue can be considered bone-like ECM. From histologic studies, it was possible to observe the invasion of collagen and reabsorption of LAGG in the defects with implanted spongy-like structures. Immunohistochemistry and X-ray Vivo-CT have been performed to both strategies and results compared as feasible strategies for OCD regeneration.

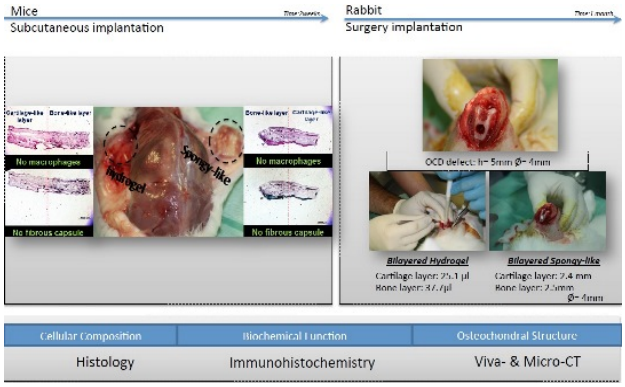

Figure 1. Mice subcutaneous and rabbit surgical implantation of both structures: Bilayered hydrogels and bilayered spongy-like implanted in critical size OCD.

Conclusions: Both strategies had shown promising results concerning regeneration of OCD defects. The combination of both might be interesting for clinical application. The hydrogel reinforcement within spongy-like hydrogels might enhance mechanical properties. As well, a balanced catabolism *vs.* anabolism process while neo-tissue is being formed can lead to proper cellular and physiological behavior.

References:

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