TS021
Mechanical performance and biocompatibility study of methacrylated Gellan gum hydrogels with potential for nucleus pulposus regeneration

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Methacrylated gellan gum hydrogels, obtained either by ionic- (iGG-MA) and photo-crosslinking (phGG-MA), have been investigated as potential biomaterials for supporting *nucleus pulposus* (NP) regeneration and/or repair [1,2]. In previous work, some advantages were attributed to GG-MA hydrogels, such as: (i) the possibility to control endothelial cells infiltration and blood vessel ingrowth's, (ii) tunable and improved mechanical properties, and (iii) *in situ* gelation, within seconds to few minutes. In this study, the mechanical and biological performance of these hydrogels was firstly evaluated *in vitro*. Human intervertebral disc (hIVD) cells obtained from herniated patients were cultured within both hydrogels, for 1 up to 21 days. Dynamic mechanical analysis and biological characterization (calcein-AM staining, ATP and DNA quantification and PCR) were performed after specific times of culturing. A biocompatibility study was also performed *in vivo*, by subcutaneous implantation of acellular iGG-MA and phGG-MA hydrogels in Lewis rats for the period of 10 and 18 days. Tissue response to the hydrogels implantation was determined by histological analysis (haematoxylin-eosin staining). The *in vitro* study showed that both cell loading and culturing time do not have an effect on the mechanical properties of the hydrogels. Regarding their biological performance, the iGG-MA and phGG-MA hydrogels showed to be effective on supporting hIVD cells encapsulation and viability up to 21 days of culturing. Human IVD cells were homogeneously distributed within the hydrogels and maintained its round-shape morphology during culturing time. The *in vivo* biocompatibility study showed that iGG-MA and phGG-MA hydrogels do not elicit any deleterious effect, as denoted by the absence of necrosis and calcification, or acute inflammatory reaction. A thin fibrous capsule was observed around the implanted hydrogels. The results presented in this study indicate that the iGG-MA and phGG-MA hydrogels are stable *in vitro* and *in vivo*, support hIVD cells encapsulation and viability, and were found to be well-tolerated and non-cytotoxic *in vivo*, thus being potential candidates for NP regeneration.

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References:

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TS022
Advanced mimetic materials for meniscus tissue engineering: targeting segmental vascularization

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Meniscus lesions are among the most common orthopaedic injuries which can ultimately lead to degeneration of the knee articular cartilage. The human meniscus has a limited healing potential, partly due to a poor vasculature, and thus meniscus regeneration using tissue engineering strategies has recently been investigated as a promising alternative to total/partial meniscectomy [1]. Advanced scaffolds for tissue engineering of meniscus should be able to mimic and preserve the asymmetric vascular network of this complex tissue, *i.e.* enable controlling the segmental vascularization during the regeneration process. Novel scaffolds were produced combining a silk polymeric matrix (12 wt%) [2] and the methacrylated gellan gum hydrogel (iGG-MA), which has been shown to be able to prevent the ingrowth of endothelial cells and blood vessels into the hydrogels [3,4]. The angiogenic/anti-angiogenic potential of acellular and cell-laden silk-12 scaffolds combined with iGG-MA hydrogel was investigated *in vivo*, using the chick embryo chorioallantoic membrane (CAM) assay. For producing the cell-laden scaffolds, human meniscus cells (HMC's) were isolated from morphologically intact human menisci using an enzymatic-based digestion and expanded using standard culture conditions. The HMC's-laden hydrogel/silk scaffolds were produced by encapsulating the HMC's into a 2 wt% GG-MA hydrogel, followed by impregnation onto the 12 wt% silk scaffold and ionic-crosslinking in a saline solution. A CAM assay was used to investigate the control of segmental vascularization of the acellular and HMC's-laden hydrogel/silk scaffolds by the effect of GG-MA hydrogel, until day 14 of embryonic development. The *in vivo* study allowed investigating the number of macroscopic blood vessels converging to the implants. The evaluation of possible inflammation and endothelial cells ingrowths was performed by histological (haematoxylin and eosin - H&E - staining) and immunohistochemical methods (SNA-lectin staining). When the silk-12 scaffold was combined with the hydrogel, an inhibitory effect was observed as demonstrated by the low number of convergent blood vessels. Results have shown that iGG-MA hydrogel prevented the endothelial cells adhesion and blood vessels infiltration into the HMC's hydrogel/silk scaffolds, after 4 days of implantation. This study showed that the hydrogel/silk scaffolds enabled controlling the segmental vascularization, thus it can possibly mimic the native vasculature architecture during meniscus regeneration.

References:

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