0324 Combinatory approach for developing silk fibroin-based scaffolds seeded with human adipose-derived stem cells for a cartilage tissue engineering applications

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Several processing technologies have been combined to create scaffolds for different tissue engineering (TE) applications. Hydrogels have been extensively used for cartilage TE applications, presenting several structural similarities to the natural extracellular matrix of cartilage tissue environment[1]. From the different biodegradable materials proposed as matrices for cartilage scaffolding[2], silk fibroin (SF) presents high versatility, processability and tailored mechanical properties, which make this protein attractive for the development of innovative matrices for cartilage TE purposes[3]. In a previous study, we proposed fast formed SF hydrogels produced through a horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) crosslinking reaction, taking advantage of the presence of tyrosine groups[4]. In this work, macro-/micro-porous SF scaffolds derived from enzymatically cross-linked SF hydrogels by a HRP/H_2O_2 complex were produced in combination with salt-leaching and freeze-drying methodologies. The scaffolds morphology, mechanical properties and chemical characterization were assessed by mean of different characterization techniques (SEM, micro-CT, Instron, FTIR and XRD). The scaffolds structural integrity was evaluated by swelling ratio and degradation profile studies. The *in vitro* ability to support the adhesion, proliferation and differentiation into the chondrogenic lineage was tested using human adipose-derived stem cells (hASCs) cultured over 28 days in basal and chondrogenic conditions. Cell behaviour in the presence of the SF scaffolds was evaluated through different quantitative (GAGs/DNA and RT-PCR) and qualitative (live/dead, SEM, histology and immunocytochemistry) assays. The *in vivo* biocompatibility of the SF-based scaffolds was also assessed by subcutaneous implantation in mice for 2 and 4 weeks and analysed by means of hematoxylin & eosin (H&E) staining and immunohistochemical analysis of CD31 angiogenic marker. The results showed highly porous and interconnected SF structures that allowed cell adhesion and infiltration into the scaffolds. *In vitro* cell viability and proliferation were also observed over the 28 days of culturing in basal conditions and a significant increase of GAGs content was detected on constructs cultured in chondrogenic differentiation medium. *In vivo* results showed that the implanted scaffolds allowed tissue ingrowth's and blood vessels formation/infiltration. The obtained results demonstrated that the innovative approach of combining enzymatically cross-linked SF hydrogels with the salt-leaching and freeze-drying methodologies allowed to produce more versatile scaffold architectures with appropriate mechanical properties and large swelling ability. The positive influence over *in vitro* chondrogenic differentiation and *in vivo* response, revealed by the new tissue formation and angiogenesis within the porous scaffolds, validates the proposed macro-/micro-porous SF scaffolds for being used in cartilage TE applications. Moreover, the versatility of these combinatory approach can allow for further applications in other musculoskeletal TE strategies.

References

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