

Methacrylated Gellan Gum Hydrogels for Application in Nucleus Pulposus Regeneration: *In Vitro* and *In Vivo* Studies

Joana Silva-Correia^{1,2}, Vera M. Gonçalves^{2,3}, J. Miguel Oliveira^{1,2*}, Rui M. Reis^{2,3} and Rui L. Reis^{1,2}

¹3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, S. Cláudio de Barco, 4806-909 Taipas, Guimarães, Portugal

^{2*}ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal, miguel.oliveira@dep.uminho.pt

³Life and Health Science Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

INTRODUCTION

Natural-based hydrogels have been attracting great deal of attention for tissue engineering of nucleus pulposus (NP). Gellan gum is an extracellular microbial polysaccharide from *Sphingomonas elodea* that forms a firm and transparent gel with interesting features for use as an *in vitro* 3D cell support, or as an *in vivo* injectable system. Recently, gellan gum-based hydrogels (ionic- and photo-crosslinked methacrylated gellan gum) have been proposed as potential candidates for NP regeneration¹. An important feature of these hydrogels will be their capacity to control blood vessel growth, since the NP is naturally avascular. Our aim was to investigate the biological performance of the developed hydrogels, *in vitro*. The angiogenic/anti-angiogenic potential of the GG-based hydrogels was also carried out *in vivo*, using an optimized adaptation of the chorioallantoic membrane (CAM) assay.

EXPERIMENTAL METHODS

Low acyl gellan gum (GG) was reacted to glycidyl methacrylate (GMA) at pH 8.5, to enable incorporation of methacrylate groups in GG structure. Ionic- and photo-crosslinked methacrylated Gellan gum (GG-GMA) hydrogels were obtained respectively by immersion in phosphate buffered saline (PBS, pH 7.4) solution or by UV exposure (366 nm). The materials were physico-chemically characterized. The *in vitro* cytotoxicity screening and cells encapsulation efficiency was also investigated by means of using an immortalized mouse lung fibroblasts cell line (L929 cells). Sterile hydrogel discs ($n=10$) made of GG, ionic- and photo-crosslinked GG-GMA were placed on the CAM at day 10 of embryonic development. Filter paper or gelatin sponge with VEGF, and filter paper or gelatin sponge were used as controls. The assay proceeded until day 14 of embryonic development and images were acquired *in ovo* and *ex ovo* using a stereomicroscope by the end of the assay. The images obtained were image-processed using the ImageJ program for facilitating the counting, which was performed by three independent observers. Haematoxylin & Eosin (H&E) staining was performed in order to investigate possible inflammation and cells ingrowths.

RESULTS AND DISCUSSION

FTIR and ¹H NMR analyses demonstrated that GG was successfully methacrylated and allowed to produce both ionic- and photo-crosslinked GG-GMA hydrogels. The developed GG-GMA hydrogels possess improved mechanical properties as determined under DMA

analysis. The water uptake ability and degradation rate of the developed hydrogels were also investigated. The *in vitro* cell culture studies revealed that both ionic- and photo-crosslinked GG-GMA hydrogels are non-cytotoxic over L929 cells. Moreover, cells encapsulation within the hydrogels was possible and the hydrogels supported cellular functions over a period of 14 days.

The angiogenic potential of the hydrogels was investigated by performing a CAM assay until day 14 of embryonic development (*in ovo* and *ex ovo*). The blood vessels converging toward the implanted hydrogels were quantified (Figure 1).

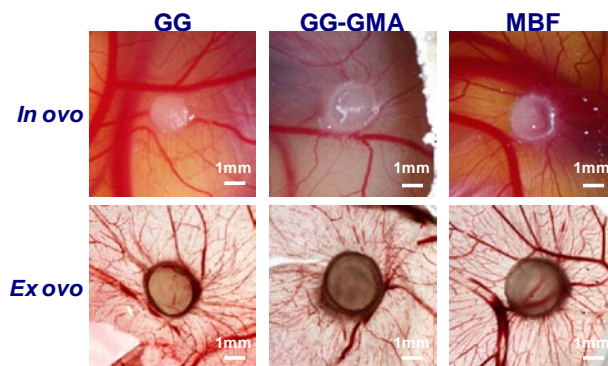


Figure 1. Photographs of the gellan gum (GG), ionic-crosslinked GG-GMA (GG-GMA) and photo-crosslinked GG-GMA (MBF) hydrogels after 14 days (CAM assay).

All the developed hydrogels were partially adsorbed during the assay. However, resorption rate was inferior as compared to controls (data not shown). The results indicate that no significant differences exist between ionic- and photo-crosslinked methacrylated Gellan gum (GG-GMA) hydrogels in respect to its angiogenic potential, *i.e.*, do not induce vascularization. Moreover, H&E staining revealed no inflammation. This work demonstrates the great potential of the GG-based hydrogels for application as NP substitute.

REFERENCES

1. Silva-Correia J. *et al.*, J. Tissue Eng. Regen. Med. 5:e97-e107, 2011

ACKNOWLEDGMENTS

The authors would like to thank the EU FP7 project Disc Regeneration (Grant No. NMP3-LA-2008-213904) for providing financial support to this project.