In vivo study on the angiogenic potential of gellan gum-based hydrogels for application in nucleus pulposus regeneration

Authors

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Abstract

Objectives: Tissue engineering of nucleus pulposus (NP) offers a promising alternative strategy to current ineffective clinical approaches for treating intervertebral disc degeneration. Gellan gum-based hydrogels (ionic- and photo-crosslinked methacrylated gellan gum) have been recently proposed as potential candidates for NP regeneration. 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 *in vivo* the angiogenic/antiangiogenic potential of the developed hydrogels, using an optimized adaptation of the chorioallantoic membrane (CAM) assay.

Methods: Sterile hydrogel discs (n=10) made of gellan gum, ionic- and photo-crosslinked methacrylated gellan gum were placed on the CAM at day 10 of embryonic development. Positive (filter paper or gelatin sponge with VEGF) and negative (filter paper or gelatin sponge) controls were also tested. The assay proceeded until day 14 or 18 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.

Results and Discussion: The evaluation of the angiogenic response was performed by analysing the convergence of the blood vessels toward the implanted discs. Some degree of variability was found between replicates and inflammation occurred frequently, which hindered the analysis of the formation of new blood vessels. The reduction of the assay duration (from 18 to 14 days) resulted in a decrease of inflammation/contamination. All the materials were partially adsorbed during the assay. However, the controls didn't present a regular response and the gelatin sponge was often completely adsorbed.

Conclusions: *In ovo* quantification method was more complex as compared to *ex ovo*. The results indicate that no differences exist between the hydrogels tested in what concerns to their angiogenic potential.

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