Visible light-crosslinked methacrylated gellan gum hydrogels for the embedding of human chondrocytes

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INTRODUCTION

Cartilage has a minimal ability to heal for the lack of a vascular network, making the repair of a defect challenging [1]. The aim of cartilage tissue engineering is to restore the tissue using cells and scaffolds to replace defected cartilage. Hydrogels have emerged as attractive biomaterials due to their biocompatibility and ability to mimic the tissue extracellular matrix. Among them, gellan gum represents an interesting candidate due to its structural similarity with native articular cartilage glycosaminoglycans [2]. This manuscript reports the investigation of visible light-crosslinked methacrylated gellan gum (GGMA) hydrogels to treat cartilage defects. Visible light-mediated crosslinking was investigated under different photocrosslinking conditions (photoinitiator concentration and dose).

MATERIALS AND METHODS

Methacrylation was carried out by following the procedure reported in [3]. Lyophilized GGMA was then autoclaved for 70 min before use. For the formation of a crosslinked hydrogel, GGMA was dissolved at a concentration of 1 and 2 % wt. in PBS, in a bath at 37 ° C for about 1 h. Then, tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate (Ru) and sodium persulfate (SPS) were added to the solution at a concentration of 0.1/1, 0.2/2 and 0.5/5 mM. Hydrogels were prepared by adding 200 µL within a cell crown, which blocked a polystyrene membrane. GGMA hydrogels were crosslinked using a white LED source at an intensity of 18 mW/cm^2 for 30, 60 and 120 s.

To evaluate the sol fraction, all samples were weighed, then lyophilized and weighed again to measure their dry weight (W_{dry1}). Then, PBS was added to all samples, which were left at 37°C for 24 h. Each sample was blotted and lyophilized, then weighed (W_{dry2}). The sol fraction (%) was calculated as:

$$Sol\ fraction\ (\%) = \frac{W_{dry1} - W_{dry2}}{W_{dry1}} \times 100$$

Human chondrocytes (HCs, Cell applications, Inc) were embedded within GGMA hydrogels (density: 400,000 cells/sample). Subsequently, Ru/SPS were added, and the solutions crosslinked with the LED light. Live/Dead tests were performed after 2, 7 and 14 days to evaluate HC viability. Furthermore, the release of lactate dehydrogenase (LDH) and metabolic activity through PrestoBlue assay were assessed.

RESULTS AND DISCUSSION

The first screening of GGMA formulations highlighted that two conditions at 2 % showed the lowest sol content. More in detail, for the case at Ru/SPS 0.1/1 mM with a crosslinking time of 60 s (GGMA-A) the average sol content was 9.3 %, while in the case of 0.2/2 mM with a crosslinking time of 30 s (GGMA-B) the average sol content was 2.6 %. We focused on these cases, with the hypothesis that a lower sol content means higher crosslinking efficiency and, subsequently, higher hydrogel stability over time [4]. On such formulations, we investigated the HC viability (Figure 1b), cytotoxicity (Figure 1c), and metabolic activity (Figure 1d).

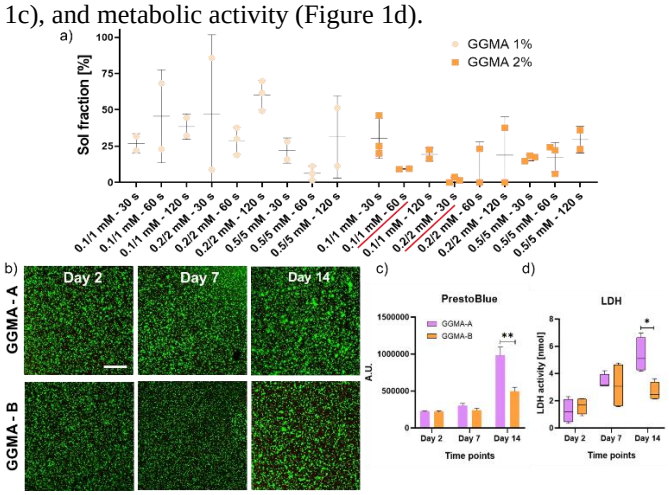

Figure 1. a) Sol content analysis for GGMA. Analysis of b) cell viability, c) metabolic activity and d) cytotoxicity.

Results showed that GGMA safely encapsulated HCs for up to 14 days. Interestingly, a smaller Ru/SPS ratio formulation promoted a higher metabolic activity, despite a higher LDH release after 14 days.

CONCLUSIONS

The production of visible-light-crosslinked GGMA hydrogels, able to constitute a safe environment for chondrocyte encapsulation, is reported. These findings open the way for using different cell sources (i.e., stem cells) to be encapsulated in these gels, for cartilage tissue engineering.

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