Cellular Response and Immunocytochemistry of Hydrogel Chitosan-Glycidyl Methacrylate-Xanthan

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1. Abstract

We have successfully synthesized a novel hybrid natural-synthetic hydrogel Chitosan-Glycidyl Methacrylate-Xanthan (CTS-*g*-GMA-X). (CTS-*g*-GMA)-X hydrogel was synthesized by two different methods: 1) using a neutral aqueous media during a 4-hour reaction, and 2) using an acidic aqueous media following neutralization. These hydrogels show interesting properties for use in the biomedical field, such as scaffolds for tissue engineering. Encouraging preliminary results of *in-vivo* assays prompted us to investigate the potential inflammatory response of these materials *in vitro*. Human epidermal keratinocytes (HEK) and nerve cells (NG108-15) were cultured onto the biomaterial and was preformed to assess their viability over several time intervals. This viability has been quantified by the DNA (proliferation) assay. Additionally for HEK immunocitochemistry (ICC) was carried out through fibronectin, E-cadherin, and Laminin antibodies. The above analyses show satisfactory results of the CTSGMA-X when compared to pure chitosan and positive controls.

2. Introduction

Synthesis of the hydrogel (CTS-*g*-GMA)-X, (Z), was carried out by two different methods: 1) using a neutral aqueous media, and 2) using an acidic aqueous media following neutralization.[1,2] Both synthesis methods yield reproducible materials with similar properties.[3]

Human epidermal keratinocytes (HEK) and nerve cells (NG108-15) were seeded over the synthesized hydrogels and its viability was demonstrated by calcein stain. The viability for keratinocytes and nerve cells cell culture has been quantified by the DNA (proliferation)

assay, over several time intervals. The analysis showed satisfactory results of the Z's when compared to pure chitosan.

Additionally for HEK, fibronectin, laminin and E-cadherin antibodies were detected in the cells by ICC protocol. The results shows an adequate stain for human epidermal keratinocyte identification and can be compared with positive control. These results indicate that the biopolymers do not affect the normal growth and proliferation of this type of cells.

3. Experimental Conditions

The Z hydrogels were synthesized by two different methods: neutral and acid aqueous media. The stoichiometric molar ratios of CTS:GMA are 1:1, 1:2, 1:3 and 1:4 and labelled as Z11, Z12, Z13 and Z14 respectively; and identified with a c, for the neutralized materials, and h for the neutral reaction.[1,2] a film of chitosan (0.5%) in acetic acid 0.4m, was used as background. All materials used were conditioned prior to use. [2]

3.1 Culture of Keratinocytes and DNA Assay for Counting Cells

Human epidermal keratinocytes were used and their proliferation was assayed in T75 flasks, with a DMEM:F12 media supplemented with 50 μl of hydrocortisone per litre of media, and maintained at 37º C in a humidified incubator with 5% CO_2. DNA Assay: cells were seeded at density of 4 x 10^4 cells/ml in DMEM:F12 onto each disc of polymer, additionally glasses were used as a positive control. The times for this assay were 1, 3, and 7 days, after which, media was removed and samples rinsed with PBS. Each sample was transferred to a separate replicate multiwell plate. Distilled water was added to each new well and samples were freeze-thawed three times. Aliquots of 100 μl of the samples, standards and blank (distilled water) were placed into a 96 well plate and 100 μl of Hoechst Stain was added to each well. The plate was shaken for 10 seconds and fluorescence measurements at 355 nm excitation and 460 nm emission were taken using a fluorescence plate reader (Fluostar Optima).[3]

3.2 Nerve cells line NG108-15

Nerve cells line NG108-15 were used, their proliferation was assayed in T75 flasks with

surface treatment for ensuring optimal cell attachment and growth, with DMEM high glucose (5 % FBS, 1 % HAT and 1 % of antibiotic) media without antibiotic and maintained at 37 °C in a humidified incubator with 5 % CO2.

3.3 Immunocytochemistry (ICC)

This test has been made only for the HEK. Fibronectin, laminin and E-cadherin antibodies were detected in the cells by ICC protocol. Secondary antibody for fibronectin and laminin was rabbit IgG; and for E-cadherin, mousse IgG and rabbit IgG.

4. Results and Discussion.

Figure 1 shows the growth onto biomaterials, the results indicate that most of samples show a better behaviour to positive control, increasing the cell number across the time.

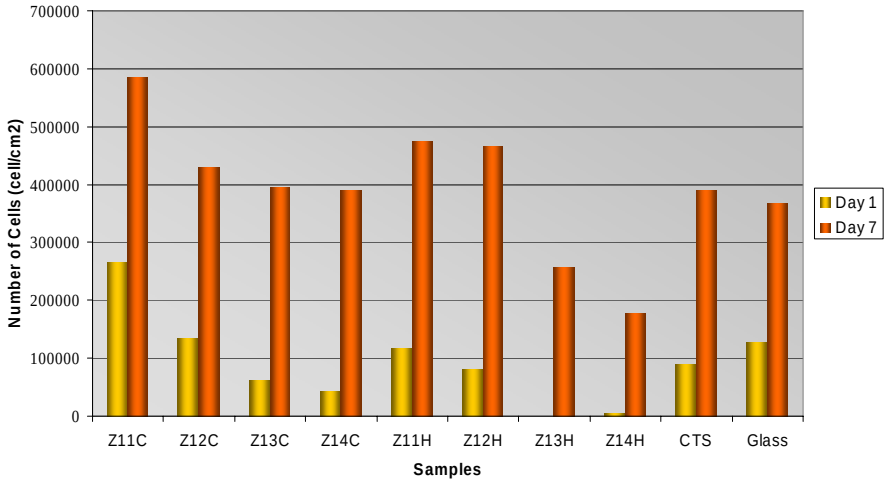

Figure 1. Cell growth of Human epidermal keratinocytes onto polymers, chitosan (precursor) and glass (positive control). From day 1 to day 7 there is significant cell growth onto our biomaterials.

The polymers of series C show a better behaviour compared with the series H, being the best Z11C, which shows the greater growth after seven days. Statistical correlation analysis, T-test, shows that the results have no significant difference between most of polymers.

Figure 2 shows the results for laminin antibody after 5 days of cell culture. As in the results for E-cadherin and fibronectin, the stain for laminin antibody is appropriate since keratinocytes do not show alterations or abnormal behavior for most of polymers. Due to the

importance of laminin promoting the neurite regeneration, this result could be considered important since if the laminin antibody stain is correct for HEK, onto our biomaterials, it is plausible to expect a similar behavior for nerve cells.

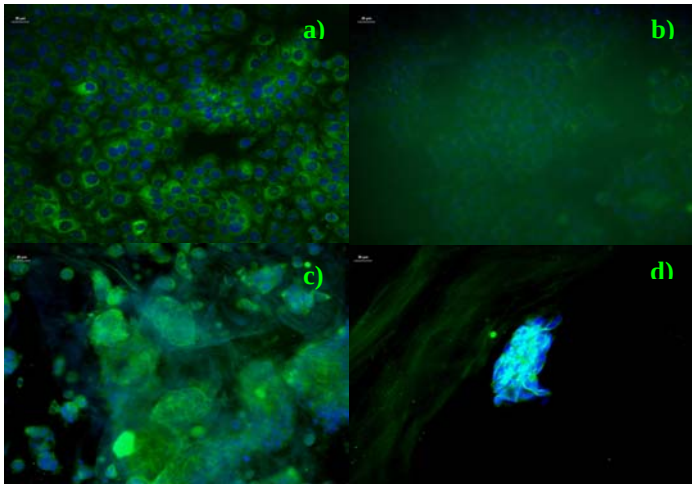

Figure 2. Representative images for laminin antibody stain on a) glass, b) chitosan, c) Z11H, and d) Z14C; after 5 days of incubation. The blue regions are the cells nucleus

DNA assay results for nerve cells shows the growth onto biomaterials, the results indicate that most of the samples have a smaller growth compared with the positive, there is enough evidence for the growth viability of this type of cell onto the biomaterials.

5. Conclusiones

HEK's DNA assay shows high cell preservation and growth in most samples and can be compared satisfactory with the positive control, in addition all antibodies probed in this study show an adequate stain for HEK identification and can be compared with positive control. These results indicate that the biopolymers do not affect the normal growth and proliferation of this type of cells.

6. Referencias

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