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Chitosan hydrogels enriched with nanostructured carbon materials for use as cell culture scaffolds

PhD dissertation abstract

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In order to perform the physicochemical characterisation of hydrogels, the sol-gel transition temperature of the colloidal systems was first determined based on rheological measurements and experiments using nuclear magnetic resonance (NMR) spectroscopy. Hydrogels were also analysed using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and differential scanning calorimetry (DSC).

As part of the biological studies, the cytotoxicity (resazurin assay) and genotoxicity (alkaline version of the comet assay) of the hydrogels against the human colon adenocarcinoma cell line (HT-29) and the normal human BJ fibroblasts were evaluated.

It has been shown that it is possible to develop the thermosensitive hydrogels from low-concentration colloidal chitosan solutions using the uridine 5'-monophosphate disodium salt.

The research made it possible to conclude that a sol-gel transition occurs for the chitosan systems with β-GP, whereas a gel-sol-gel transition was observed for the colloids with UMP. SEM microimages showed that the type of crosslinking agent used had a much greater impact on the structure of the chitosan systems than the type of solvent used. The scaffolds with UMP had a highly porous, interconnected structure compared to the systems with β-GP. The type of crosslinking agent used also affected the shape of the DSC thermograms for the first heating cycle and the number of endothermic peaks.

The analysis of FTIR spectra and XRD diffraction patterns of hydrogels conditioned in deionised water showed that changes in the structure of the systems occur along with the lengthening of the conditioning time. The shape of the spectra and diffractograms became similar to the spectrum and the XRD diffraction pattern of chitosan powder, indicating the release of β-GP and UMP from the structure of the biomaterials. However, comparing the dynamics of the observed changes, it was found that the biomaterials with UMP released the crosslinking agent faster than the hydrogels with β-GP.

Based on the interpretation of the results of the conducted research, proprietary mechanisms of forming thermosensitive hydrogels were proposed.

Biological studies have shown that the developed hydrogels with the uridine 5'-monophosphate disodium salt can be, in terms of biocompatibility, a solution competitive to the classical systems with the β-glycerophosphate disodium salt pentahydrate. None of the considered variants of hydrogels induced cytotoxic and genotoxic effects on cells of both lines, and the system prepared from the chitosan lactate solution with UMP even showed a pro-fibroblastic effect.

On the other hand, the introduction of a nanofiller into the polymer matrix resulted in a decrease in the sol-gel transition temperature of chitosan colloids, while improving the mechanical properties of the systems. The structure of systems with β-GP with the addition of graphene oxide was characterised by greater porosity than hydrogels without nanofiller, and no significant changes were noted for systems modified with UMP.

The use of low concentrations of nanofiller, in the case of systems prepared from chitosan lactate solutions with β-GP and UMP, promoted the survival of BJ cells at a level above 100%, without inducing genotoxic effects.

In conclusion, it can be stated that the chosen direction of research is promising, and the developed chitosan hydrogels are an attractive material for potential use as scaffolds in tissue engineering.