Hydrogels from chemically modified PVA with chondroitine sulfate: chemical characterization and biocompatibility.

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Statement of Purpose: Hydrogels may be widely applied in biomaterials as cell scaffold or drug release systems due to biocompatibility and/or low cytotoxicity.[1] The poly(vinyl alcohol) (or PVA) is used in pharmaceutical products due to its low oral toxicity.[2] PVA-based hydrogels may be obtained by chemical or physical cross-links. The chemical modification of PVA by insertion of unsaturated groups, for instance, methacryloil, could help the cross-link process without the use of glutaraldehyde that could unable the biocompatibility. Thus, the gelation of modified PVA could be done using light (UV) or other no-toxic initiator.[3] The association of modified PVA with the mucopolysaccharide chondroitine sulfate (SC) could form a semi-interpenetrated network (semi-IPN). Compared to PVA hydrogels, the biocompatibility of such semi-IPN may be enhanced and the material could be used as drug delivery system. In this work, insertion of glycidyl methacrylate (GMA) in PVA produced the chemically modified PVA-Ma and semi-IPN hydrogels of PVA-Ma were obtained in presence of SC. The goals are to characterize the mechanical properties and water uptake capability of hydrogels of PVA-Ma and semi-IPN hydrogels of PVA-Ma with SC and also evaluate their cytotoxicity to potential uses as drug delivery system.

Methods: PVA, Mw 13-23 kg Mol^{-1} was dissolved in DMSO by stirring at ~70 °C to obtain a 10 % (w-v) solution. The required amount of GMA was poured into the solution to obtain the %-molar [GMA/-OH(PVA)]: 2.5, 3.5, 5.0. TEMED was used as a catalyst in 1.0 mol-% relative to the PVA hydroxyl groups. Aqueous solution of modified polymer (PVA-Ma) and SC was prepared in several ratios PVA-Ma/SC and 0.1 mMol sodium persulfate was added, stirred for 15 min, transferred to a test tube and TEMED was added. After 24 hours a consistent cylindrical gel was formed. The gel was taken out and immersed in water. The water was renewed every 8 h for 72 h, after that the hydrogels were dried at room temperature. Hydrogels without SC were prepared for control. The raw polymer (PVA), modified polymer (PVA-Ma) and the hydrogels were lyophilized and FT-IR, ^{1}H NMR and ^{13}C NMR analyses were carried out. FT-IR spectra were taken on a Bomem FT-IR model MB100 spectrometer. Water uptake (or Wu) as a function of pH, in several ionic strengths, was measured. Measures of Wu in two pH conditions were performed: Gastric Simulation Fluid (GSF) pH = 2.0 and Intestinal Simulation Fluid (ISF) pH = 6.8. The mechanical properties of hydrogels were measures by use of Texturometer (TA.XT2i) in compression mode. Cytotoxicity of hydrogels was evaluated using VERO cells using two controls surfaces: silicon (positive cytotoxic) and polystyrene plaques (negative cytotoxic).

Results / Discussion: FTIR spectra of AG-MA presents a band at 1711 cm^{-1} attributed to the C=O stretching frequency of the conjugated ester groups from GMA. This band is an evidence of the modification of AG. The NMR spectra of AG-MA show signals at δ 6.30 ppm and δ 5.80 ppm attributed to the vinyl hydrogen from GMA. The mechanism of water transport into the hydrogels was evaluated by the use of equation $ln(M^t/M_o) = k\ n\ ln(t)$, being M^t the mass of absorbed water at time t and M_o the absorbed water in equilibrium swollen hydrogel, **k** is a constant that depends of hydrogel and **n** is a diffusional exponent from which the mechanism of transport solvent into the hydrogel is determined. According to the literature[3], the value of **n** depends on hydrogel geometry. In this work, cylindrical hydrogels were used. So, **n** = 0.45, Fickian diffusion (case I); $0.45 < n < 0.89$ non-Fickian transport (or anomalous), n=0.89 for zero order (case II) and $n > 0.89$ for super case II. In zero order (case II) the transport is governed by the polymer chains relaxation and the anomalous transport is due to the both Fickian diffusion and polymer chains relaxation. In this work, the obtained values for **n** ranges from 0.53 to 0.69. So it means that the water transport mechanism is anomalous. Also, the values of **n** do not change with the pH of hydrogel environment ISF or GSF: The pH does not alter the water uptake mechanism. The values of E increase as the DS in PVA-Ma raises. So, E increases with the density of cross-linking (υ^*_e). The value of E decreases in hydrogels of PVA-Ma with DS = 3.5% if the amount of SC is changed from 20 to 33%. This could be due to the hydrophilic character of SC. Compared to the positive control (silicon) and to the negative control (polystyrene) the hydrogels surfaces are less cytotoxic. The inhibition to cell growth is higher in hydrogels of PVA-Ma without SC. Finally, the density of cross-linking in PVA-Ma hydrogels affects slightly, and negatively, the cell compatibility

Conclusions: Chemically modified PVA can be obtained by insertion methacryloil groups into the side hydroxyl polymer chain in the following degree of substitution (DS): [GMA/ (-OH PVA) mol-%]: a 2.5, 3.5 and 5.0. The hydrogels of PVA-Ma and PVA-Ma with SC presented good mechanical properties. The elastic modulus ranges from 5 (33 wt-% in SC and DS = 2.5%) to 48 kPa (0 wt-% in SC and DS = 5.0%). The mechanism of water uptake is anomalous and it is not influenced by the pH. The hydrogels containing SC presented less inhibition to VERO cell growth as compared to polystyrene surfaces (a negative cytotoxic control). The inhibition to cell growth is higher in hydrogels of PVA-Ma without SC. The density of cross-linking in PVA-Ma hydrogels affects slightly, and negatively, the cell compatibility

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