Study on Cellulose/Chondroitin Sulfate Hydrogel Used in Drug Release Systems

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Abstract— **The aim of this study was to combine the properties of cellulose and chondroitin sulfate in mixed hydrogels in order to obtain new materials for medical and pharmaceutical applications as drug delivery systems. Different swelling profiles and active drug release rates were observed for the proposed formulation compared with pure cellulose hydrogels. Along with this aim, hemotoxicity and subcutaneous implantation experiments had been performed showing a good biocompatibility of the studied hydrogels.**

Keywords— **drug delivery, codeine, cellulose, chondroitin sulfate, hydrogel.**

I. Introduction

Hydrogels are water-swollen, crosslinked polymeric networks produced by reticulation involving chemical reactions or physical interactions (ionic and hydrogen bonding, hydrophobic interaction, micellar packing). Hydrogels can absorb and retain large amounts of water[1]. Since the pioneering work of Wichterle and Lim[2] of 1960 on hydrogels based on 2-hydroxyethyl methacrylate (HEMA), the field has developed continuously because of multiple applications that hydrogels have especially in medicine and pharmacy[3,5]. The main uses of hydrogels are in the biosensors field, artificially dressing for burns[4], controlled drug release[6] and tissue regeneration due to the remarkable properties that they have[7] in mimicking the extracellular matrix of natural tissues. Their water content and elasticity are similar with natural biological interactions at the molecular level[8]. Natural polymer (e.g. polysaccharide) based hydrogels constitute a very promissing class of substances mainly due to their high biocompatibility.

Chondroitin sulfate (CS) is a glycosaminoglycan (GAG) made by repeating disaccharide units of D-glucuronic (GlcUA) (β1-3) N-acetyl-galatosamine (GalNAc). Both monosaccharides can be sulfated (in position 2 for GlacUA and in positions 4' and 6' for GalNAc) (Figure 1). The impact of this sulfation diversity is very important for molecular biology and not completely understood.

Chondroitin sulphate it is used in medical applications[9], as dietary supplements (capsules and tablets)[10], eye drops[11]. CS can be found in humans[12,13] demonstrating it's important role biological processes[14, 15].

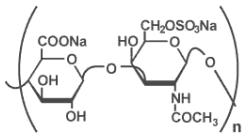

Fig. 1 The chemical structure of Chondroitin 6-sulfate

Chondroitin and Glucosamine[16] are well known for the benefic effects on joint pain, improving joint mobility, increasing and protecting the cartilage. This supplement is recommended for elderly people, athletes and active people maintaining joint health and function.

Cellulose (Figure2) is an organic compound with the formula $(C_6H_{10}O_5)_n$. Cellulose is a β-D-Glucose polymer which, in contrast to starch, is oriented with the $-CH_2OH$ groups alternating above and below the plane of the cellulose molecule thus producing long, unbranched chains.

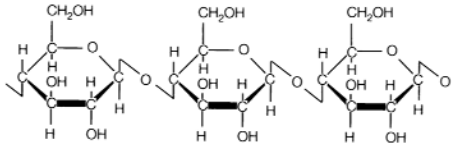

Fig. 2 Cellulose structure

Cellulose is the world's most abundant natural, renewable and biodegradable polymer. Cellulose is hygroscopic, retaining water by hydrogen bonding.

Bacterial cellulose has unique properties. Due to its high water absorbance, biocompatibility, high porosity, mechanical properties, high cristallinity and an ultra-fine and highly pure fibre network structure, bacterial cellulose has become popular in biomedical applications[17,18].

Codeine is a natural alkaloid of opium. Industrial synthesis of codeine is made by O-methylation of morphine. It has much weaker analgesic than morphine. Of all the receptors for opioids, codeine has the highest affinity for μ, subtype μ_2. Linking to subtype μ_1, somewhat weak, is responsible for analgesic effects. Codeine 6-glucuronide is metabolized in the liver by conjugation with glucuronic acid up to 80%. Codeine (methylmorphine) is well-known for the

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antitussive, analgesic and antidiarrheal effects, having a broad safety margin and 8%-12% of the strength of morphin.

The most recent advances in cellulose-based hydrogels aim not only at the sustained release of a bioactive molecule over a long time period, ranging from hours to weeks, but also at a space-controlled delivery, directly at the site of interest. The need to encapsulate bioactive molecules into a hydrogel matrix or other delivery devices (e.g., microspheres) is also related to the short half-life displayed by many biomolecules *in vivo*[19].

Controlled release through oral drug delivery is usually based on the strong pH variations encountered when transitioning from the stomach to the intestine. Cellulose-based polyelectrolyte hydrogels (e.g., hydrogels containing NaCMC) are particularly suitable for this application. For instance, anionic hydrogels based on carboxymethyl cellulose have been investigated recently for colon-targeted drug delivery[20].

The readily water-soluble nature of chondroitin sulfate limits its application as a solid-state drug delivery vehicle. Therefore, it is usual to carry out a crosslinking treatment to tailor the properties of chondroitin sulfate as reported in several works[21], or to combine it with other polymers, such as chitosan[22], gelatin and hyaluronan[23], collagen[24], poly(vinyl alcohol)[25] or poly-(lactic-co-glycolic acid)[26] in order to produce more stable materials.

The present study aims to investigate the properties of a newly developed hydrogel type[27] with a double network made of cellulose and CS as a potential transport matrix for biologically active substances. Along with the release evaluation of the loaded active molecule from the hydrogel matrix, also hemotoxicity tests and *in vivo* implantation studies were performed in order to characterize the biocompatibility of the formulations. The cellulose/ chondroitin sulfate hydrogels combine the biodegradation capacity, the biocompatibility, the transparency and lack of toxicity of cellulose with high water absorption, and biodegradability characteristics of chondroitin sulfate in order to obtain new biomaterials with special applications in processes that do not harm biological environment.

Up to now, after our knowledge, the combination of cellulose and chondroitin sulfate, for obtaining new hydrogels and their potential for biomedical applications, such as drug delivery, has not been yet exploited.

II. Materials and methods

A. Material Synthesis and Swelling

Microcrystalline cellulose (C) Avicel PH-101 with the polymerization degree of 183 was purchased from Sigma-Aldrich. Chondroitin sulfate (CS) was purchased from Roth, Germany and obtained from bovine tracheal cartilage.

The hydrogel samples were prepared as described in Oprea et al (2009)[27]. In short a crosslinking technique was used to prepare pure cellulose (used in this study as reference composition) and mixed hydrogel matrix with a mass ratio of 70/30 C/CS. The obtained composition were purified by washing with water and dried at room temperature (this will be further refered as C/CS). The kinetics of swelling was measured by placing the sample in pH=7.4 Phosphate Buffer Solution (PBS) and measuring the weigths of samples at different time intervals.

B. Codeine Loading

The drug loading method was performed by immersing the hydrogels samples in a pH=7.4 PBS-codeine solution with a concentration of $2{,}35*10^{-2}$, and left 48 hour for their complete loading.

C. Codeine Release

During the drug release study, the release medium (pH=7.4 PBS solution) was maintained at 37 ± 0.5 ^{0}C. At predetermined time intervals, aliquots of the medium of 2 ml were withdrawn from the release medium and analyzed at λ_{max}=285 nm using a RAYLEIGH 1800 UV-VIS spectrophotometer (China).

Codeine concentrations were calculated based on calibration curve determined at specific maximum absorption wavelengths. In order to maintain the solution concentration the sample is reintroduced in the circuit after analysis.

D. Percent Hemolysis Test

The used blood was collected from healthy patients. Before testing, the hydrogels were sterilized by ultraviolet light trans-illumination for 4 minutes. Each hydrogels sample was tested with the blood collected from the same patient. Copper powder was used as positive control and low density poly-ethylene (LDPE) as negative control[28]. From each blood sample were withdrawn 0.6mL, placed in Eppendorf containers with copper powder, LDPE, cellulose-based and C/CS hydrogels and incubated at 37°C for 3 h. After the incubation time the samples were centrifuged at 4000 rpm for 10 min. The separated plasmas were diluted 11 fold with Tris (62.5 mmol/L, pH 8.0 adjusted with HCl) prior to spectrophotometrical measurements.

The method used for measuring plasma hemoglobin concentration was the polychromatic method of Noe et al.[29]. Absorbance was measured at 380nm, 415nm and 470nm and the formula used was:

$$C(mg/L) = 1.65 \times m_{A415} - 0.93 \times m_{A380} - 0.73 \times m_{A470} \quad (1)$$

where C - hemoglobin concentration (mg/L); m_{A380}, m_{A415} and m_{A470} - absorbances at 380nm, 415nm and 470nm (miliabsorbance units). The results were expressed as:

$$H\ (\%) = (C_s - C_n)/(C_p - C_n) \times 100 \qquad (2)$$

where H – hemolysis percent, C_S - concentration of hemoglobin in the sample, C_n - concentration of hemoglobin in the negative control and C_p - concentration of hemoglobin in the positive control.

E. Subcutaneous Implantation

The *in vivo* experiments were performed on 4 groups of 6 animals each (Wistar male rats, average weight ~250g) kept under standard conditions, at 20-23 ^oC, with food and water *ad libitum*. The dimensions of each implant were 6x4mm (after swelling). Prior to the implantation procedure, the samples were swollen into pH=7.4 PBS solution. The implants have been sterilized with UV radiation and packed individually. Each animal was anesthetized with a mixture of ketamin (80-100 mg/kg) and xilazin (5-10mg/kg) injected intramuscular. The samples were implanted paravertebral subcutaneously by making a 1cm incision on disinfected and newly-shaved skin. The incision was sutured in 2 points with nonresorbable thread with a 3.0 needle and cleaned with ethyl alcohol. The implants were collected after 30 days, the examination being performed with an Olympus BX40 microscope and a digital camera. The implants sections were examined for the presence of inflammatory cells, fibrosis, and abnormal cell morphology.

III. Results and Discussions

A. Swelling Study

The swollen weights at equilibrium in PBS at 37^oC compared with the dryed samples were 183% for C and 317% for C/CS. This fact motivates the choice of the C/CS hydrogel over the C. The difference is due to the negative charges in the CS structure (-OSO_3^- and –COO^-, 2 charges/disaccharide repeating unit) which creates affinity for the codeine binding. Codeine molecules link to the CS matrix, but in low concentrations that can be neglected in comparison with the swollen solution.

B. Drug Release Profiles

From the release profiles depicted in Figure 3 it can be observed a decrease of the released amount of Codeine by adding CS in hydrogel composition. Also the rate of the loaded substance release is higher in the Cellulose hydrogel. This can be explained by the fact that the addition of the CS to the cellulose matrix draws it into a polyelectrolyte hydrogel.

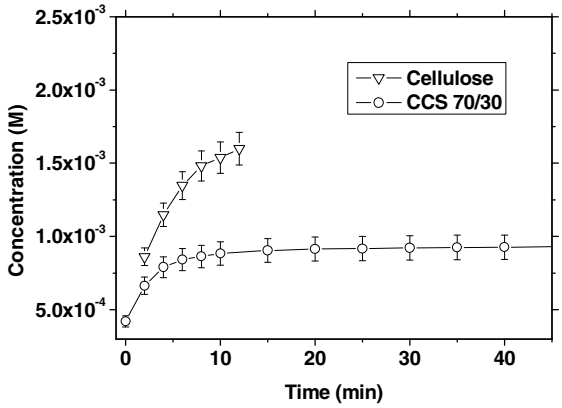

Fig. 3 Codeine release profiles from the Cellulose and C/CS hydrogel matrice

Negative charges are present into the matrix from the carboxylic –COO^- group of the glucuronic acid and from the sulfate moiety –O-SO_3^- of the N-acetylgalactosamine residues. In solution Codeine Phosphate is dissociated, the codeine bearing a net positive charge which triggers the ionic interaction between the drug and the polymer matrix, increasing in this way the retention of the drug inside the matrix.

C. Hemolysis Test

The hemolysis percent for the C/CS hydrogel sample was Hc/cs= -5,79%. The values reflect a high biocompatibility, the negative value obtained suggesting that the variations in the levels of free hemoglobin due to the contact with the material are lower in magnitude than the experimental errors.

D. Subcutaneous Implantation

The implants were easily absorbed without side-effects and proved the theoretical hypothesis of biocompatibility and effectiveness of the C/CS hydrogel.

Figure 4 shows the histological response to the implantation of C/CS. During the first 2 weeks moderate numbers of inflammatory cells were infiltrated into the hydrogel which integrates into the surrounding tissue, this behavior being characteristic to remodeling processes appeared in the implantation sites (figure 4a).

Figures 4b and 4c show the biomaterial-tissue interface, the presence of few inflammatory cells and a reduced infiltration. In this area it can be observed an increased basophilia of fibroblasts to the material interface, which prove the existence of some intense synthesis processes. The presence of inflammatory cells (neutrophils) and a mild chronic inflammatory reaction with multinucleated giant cells in hydrogel mesh (arrow) are presented in figure 4d.

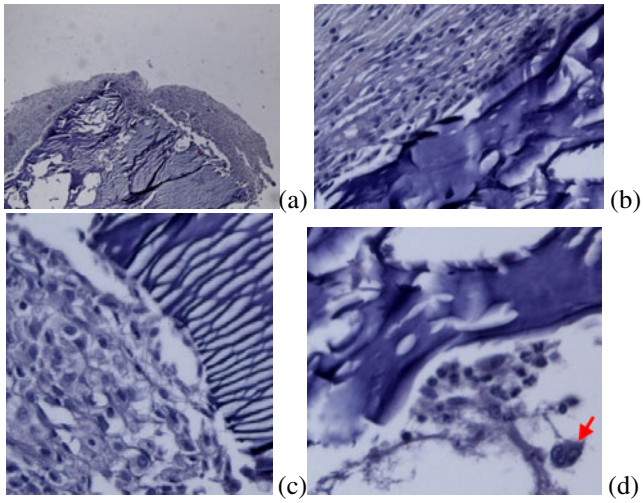

Fig. 4 Microscopic investigations of the inflammatory reactions after subcutaneous implantation of C/CS hydrogel in rats after 4 weeks. Images from 4 (a)(10X), (b)(40X) and (c)(40X) present the detailed zones of biomaterial-tissue interface and image 4 (d)(40X) present a detailed zone with the inflammatory cells (arrow) found at the edge of the hydrogel

The C/CS hydrogel was well tolerated for 1 month after implantation but should also be noted that usually the changes and tissues remodeling occur within the first 4-8 weeks after implantation, which can justify the presence of inflammatory infiltrate into the hydrogel mesh.

IV. Conclusions

The C/CS hydrogel loaded with codeine showed potential as drug delivery system. Its high biocompatibility along with the possibility of controlling the ratio of the released active substance over the retained one, by altering the amount of the negatively charged CS, makes it an attractive target for subsequent studies as a drug carrier.

Acknowledgements

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