Polysaccharides Based Hydrogels for Biological Applications

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Summary: Polysaccharides based hydrogels show several peculiar properties which can be so reassumed:

- Capability to absorb a great amount of water once immersed in biological fluids, assuming, consequently, a structure similar to extracellular matrix or biological tissue
- Tissotropic property, i.e. possibility to be injected through a needle without lose of their rheological properties.

These fundamental properties make them ideal materials for several biomedical applications, such as cellular scaffold, coatings for biomedical disposals, treatments for different diseases, controlled release of drugs, etc.
Hyaluronane, Carboxymethyl cellulose and Alginic acid based 50% hydrogels (i.e. 50% of the carboxylate groups present in the macromolecule chain were involved in the cross-linking reaction) are synthesised. Their effectiveness in promoting cells adhesion and proliferation was verified. Furthermore the possibility of injecting and sterilising hydrogels permitted to test the effect of Hyal 50% in the osteoarthritis therapy. It was found that the in vivo effect of Hyal 50% in the treatment of surgically created chondral defect in the rabbit knee was positive.
These materials can be both chemically and morphologically modified. In fact, the insertion of sulphate groups increase their hemocompatibility as demonstred by the increase of TT (time necessary to turn the fibrinogen to thrombin). Furthermore microporous hydrogels were obtained and tested as drug controlled release systems.

Keywords: adhesion; hydrogels; mechanical properties; microporosity; polysaccharides

Introduction

Hydrogels have been largely used in medicine, pharmacy and life sciences.[1-3] Their morphology and physico-chemical properties make them suitable for several applications and in particular as drug controlled release systems or scaffolds for tissue engineering.[4,5] Several polysaccharides have been utilised as materials for hydrogel production. The preference

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accorded to polysaccharides was due, first of all, to the critical role played by saccharide moieties in cell signalling schemes and in immune recognition. Furthermore their chemical structure can be easily modify to introduce new biological properties.[6] In the last decades different types of crosslinking procedures have been developed. Two main approaches were followed, physical or chemical processes. Among the physical methods UV irradiation was the most utilized.[7,8,9] This technique does not permit to exercise a control on the stoichiometry of the reaction and consequently on the physico-chemical properties of the obtained hydrogel. Consequently, a chemical procedure turned out to be preferable. The carboxylate functional groups, present in great number along the most part of polysaccharides chain, offer suitable chemical sites for modification from a linear polymer to a polymeric network. We obtained Hyaluronane, Alginate (natural polysaccharides) and Carboxymethylcellulose (semisynthetic polysaccharide) based chemical hydrogels by crosslinking the polysaccharide chains with an alchylic bridge.[10] An amidic bond between the carboxylate groups of the polysaccharide chain and the aminic group of the 1,3 diamine propane, used as crosslinking agent, was formed. That technique permitted a strict control of the crosslinking degree, expressed as percentage (i.e. the amount of COO^- groups involved in the formation of amidic bonds). Hydrogels were characterised in terms of swelling behaviour, rheological behaviour, protein adsorption. Their effectiveness as cellular scaffold was also verified. An ideal cellular matrix ought to have sufficient porosity to guarantee the easy diffusion of nutrients and the clearance of wastes, as well as an adequate mechanical stability to support and transfer loads. The morphology of the hydrogels was thus modified in order to obtain microporous matrices. Our technique differs from the others reported in literature because the porous hydrogels were obtained starting from an already synthesised hydrogel whereas the others were mainly based on addition of all the components during the synthesis phase.[11-16] Furthermore it permits a strict control of pore size and distribution. The porous hydrogels were utilised as drug release systems because the presence of pores affects the release kinetics of the substance uptaken by the matrix. The polysaccharides were also modified by inserting a new functional group: the sulphate groups. The presence of sulphate groups increases the amount of fluid uptaken by the hydrogel assuming both a consistence similar to the soft tissues and a chemical structure similar to that of some components of the human tissues (i.e. chondroitin sulphate and Keratin sulphate are the

main components of the cartilagineous tissue). Furthermore the presence of sulphate groups increases the hemocompatibility of the hydrogel.[17,18]

Materials

The sodium salt of hyaluronic acid (≅150-200 kDa) was kindly supplied by Biophil S.p.A. (Milan, Italy). The sodium salt of alginic acid (viscosity: 350-550 mPa.s in 1% water solution at 20°C), consisting of 60% β-D mannuronic acid and 40% α-L-guluronic, was supplied by Roth (Germany). The sodium salt of carboxymethylcellulose (NaCMC, viscosity 402 mPa.s in 2% w/v aqueous solution at 25°C and carboxymethylation degree of 0.9±0.1 per monosaccharide unit, Mw=100000) was supplied by Hercules Italia S.p.A (Italy). The polysaccharides were used as received after a check by FT-IR spectroscopy. Hyaluronic acid, alginic acid and carboxymethylcellulose based hydrogels with a cross-linking degree of 50% (i.e. 50% of the carboxylate groups of the polysaccharide chain were cross-linked) were obtained as previously described[10] and titrated by potentiometric titration to determine the cross-linking degree.[19]
All the other reagents were purchased from Fluka Chemie AG (Switzerland) and used without further purification.

Methods

Hydrogel Synthesis and Characterisation

The polysaccharide based 50% hydrogels were synthesised as previously described.[10] Briefly, a solution of sodium salt of polysaccharide (1.0% w/v) was subjected to a sodium-hydrogen ionic exchange using Dowex 50WX8 resin and then was added to a 5% tetrabutylammonium hydroxide solution until a pH of 8-9 was reached. The solution was then lyophilised obtaing the tetrabutylammonium salt of the polysaccharide. The TBA salt was then dissolved in N-N' dimethylformamide (DMF) under stirring and nitrogen flow. The solution was kept at 0°C before addition of 2-chloro-methyl-piridinium iodide (CMP-J), the activating agent. The amount of CMP-J added depended on the quantity of carboxylate groups to be activated. 1,3 diaminopropane was used as cross-linking agent. The reaction was catalysed by a small amount of triethylamine as hydrogen iodide captor. The reaction was then left at room temperature for 3-4 hours. The hydrogel formed was then washed alternatively with bidistilled

water and ethanol until no more solvents or secondary products were found in the washing solution, as demonstrated by UV absorption measurements.

The ninhydrin assay was performed to check the presence of unreacted NH_2 groups of the 1,3 diaminepropane used as cross-linker.[20]

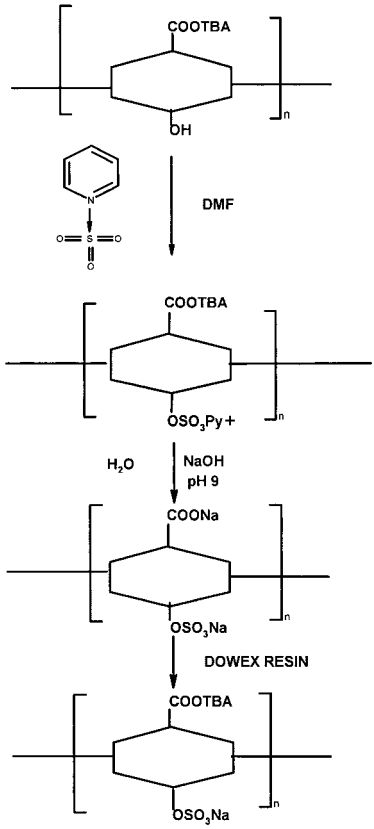


Fig. 1. Scheme of the sulphation reaction of soluble polysaccharides.

Sulphation of Polysaccharides

Sulphated derivatives can be obtained by the reaction showed in Figure 1. The reaction is carried out in the organic solvent N-N' dimethylformamide (DMF) in which the

tetrabutylammonium salt of polysaccharides, obtained by ion exchange resin (DOWEX) of the corrisponding sodium salt, was dissolved. The sulphating agent, trioxide pyridine SO_3-complex, was added, once dissolved in the minimun amount of DMF, in a molar ratio of 2:1 with the sulphatable groups. The mixture was left at 4°C for 2 hours and then the reaction was stopped by addition of H_2O and NaOH up to pH=9. The sulphated polymers were precipitated by addition of EtOH and leaving the mixture O/N at 4°C. The precipitate was dissolved in water and dialised against water for 5 days. The sulphated polysaccharides in Na^+ form was exchanged (Dowex resin) in TBA^+ form and the crosslinking reaction was conducted as previously reported. The sulphation degree of both free polysaccharides and sulphated hydrogels was determined by gravimetric analysis and by elementar analysis.

Immobilisation of Sulphated Polysaccharides onto Polyurethane

The sulphated derivatives were photoimmobilised on polyurethane (PU). The process needed the conjugation of polysaccharides with a photoreactive molecule (4-azidoaniline hydrochloride) through the formation of amidic bond using a water soluble carbodiimide as activating agent of the carboxylates of the polysaccharide [1ethyl3(3(dimethyl-amino)propyl(carbodiimmide)].[21] An aqueous solution of photoreactive polysaccharides (1 mg/ml) was dropped on the surfaces and air dried at room temperature. Subsequently, the surface was irradiated with UV lamp (W=150) at a distance of 5 cm for 90 seconds.

Microporous Structure Formation

The porous structure was obtained by stratifying the 50% hydrogel onto a cell-culture strainer with a defined and controlled porosity (40, 70 and 100 μm). Once obtained a film, it was rinsed with some drops of chloroform in order to maintain the matrix soft but, at the same time, preventing an excessive swelling which would alter the pore formation. The filter was placed on a beaker with the same diameter as the filter and containing in the bottom 1,5 g of a porogen salt $NaHCO_3$. By a drop by drop addition of 0,1M HCl solution with a sirynge, preventing direct contact between the hydrogel and the solution, violent effervescence was observed (Figure 2). The formation of CO_2 bubbles and their passage through the filter, first, and the matrix, then, induced the hydrogel to assume a porous morphology.

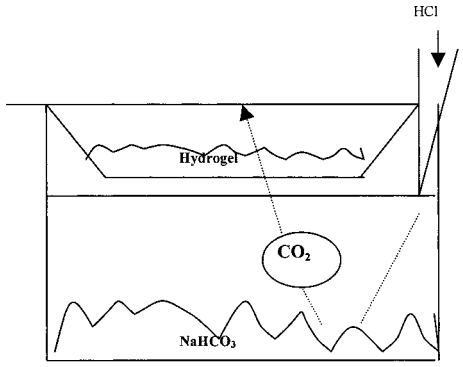


Fig. 2. Disposal utilised for the formation of porous structure.

Morphological and Physico-chemical Characterisation of the Hydrogels

Scanning Electron Microscopy (SEM) Analysis

Scanning Electron Microscopy (SEM) of cooled and dried gels were performed in order to analyse gel morphology and gel structure. Water swollen gels (2.5 mg) were put in cryotubes and cooled by liquid nitrogen. After cooling, gels were lyophilised, mounted on SEM stubs and gold-sputtered with an automatic sputter coater (BAL-TEC SCD 050, Balzer). The morphology and structure of the gels were displayed using an XL 20 SEM (Philips).

Water up-take Measurements

The water up-take (W.U.) was determined for each sample, as calculated with the following formula:

$$W.U. = [(W_s-W_d)/W_d] \times 100$$

where W_s and W_d are the weight of the swollen and dried hydrogels respectively. The procedure followed that one already reported.[19]

FT-IR ATR Analysis

ATR spectra of the samples in dry form were recorded on a Biorad FTS 6000 between 4000 and 750 cm^{-1}. A horizontal ATR accessory with a 456E zinc-selenide crystal was used. A MCT detector was used, and the apparatus was purged with nitrogen. Typically 50 scans at a

resolution of 1.0 cm^{-1} were averaged. The frequency scale was internally calibrated with a helium-neon reference laser to an accuracy of 0.01 cm^{-1}.

Rheological Characterisation

The rheological characterisation was performed on the cross-linked polymers by adding bi-distilled water to lyophilised samples to obtain a concentration of 10 mg/mL. A Bohlin VOR Rheometer (Bohlin Rheologi A B, Lund, Sweden) was used at a controlled temperature of 25°C. The procedure and the calculation were already reported.[10]

Controlled Release Kinetics Analysis

Hydrogels were emboded with Ibuprophen-lysine by swelling the dry hydrogels in a known volume of aqueous solution of the drug. The amount of drug captured by matrices was determined by degrading hydrogels in strongly alkaline environment (NaOH 1M) and analysing the absorbance of the obtained solutions by UV spectroscopy.

The disposal utilised for the kinetics studies was a suitable circuit which allows to maintain the washing solution (PBS pH 7,4, 37°C) in continuous flow (Figure 3). The emboded hydrogels were put in contact with the washing solution (PBS) and aliquotes of the solution were withdrawn at regular intervals and the absorption was ascertained. The amount of released drug and the release kinetics were determined by UV spectroscopy.

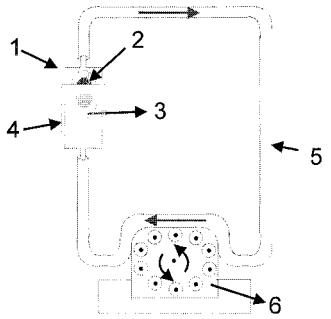


Fig. 3. Disposal utilised for the evaluation of the release kinetics in dinamic conditions: two parts of a syringe (1), containing 50 mg of hydrogel (2) enveloped in a nylon net (3) were closed by a sylicon tube (4). A peristaltic pomp (6) permitted to maintain the washing solution (PBS) flowing inside a sylicon tube (5).

Biological Characterisation

In vitro Test

Hydrogels were sterilised with 70% EtOH for 24 hours and then swollen in DMEM for the time necessary to reach the swelling equilibrium. 6.10^3 cells were seeded on each sample. To avoid the hydrogels floating, the materials were fixed by putting over them a filter. Growth curves were realised by counting the adhered cells at regular intervals: 24h, 48h, 72h and 96h.

Cell morphology was analysed after 96 hours of cell culture by Scanning Electron Microscopy.

In vivo Test

In vivo evaluation of the anti-arthritic activity of hyaluronane based hydrogels was performed as previously reported.[22]

Results and Discussion

Synthesis and Physico-chemical Characterisation of Polysaccharidic Hydrogels

Hyaluronane, Alginate (natural polysaccharides) and Carboxymethylcellulose (semisynthetic polysaccharide) hydrogels are obtained through the crosslinking of polysaccharidic chains by an alchylic bridge. The chemical reaction occurs via the formation of an amidic bond between the carboxylate groups of the polysaccharide chains and the aminic groups of the alchylic diamine (1,3 diamine propane) used as crosslinking agent. The utilised technique permits a strict control of the crosslinking degree, expressed as percentage (i.e. the amount of COO^- groups involved in the formation of amidic bonds). Consequently 25%, 50% and 100% hydrogels can be obtained. The physico-chemical properties, such as swelling property or rheological behaviour, change with the variation of the crosslinking degree. A good compromise between a good swelling behaviour and rheological properties was shown by 50% hydrogel. In fact, 25% hydrogel was able to absorb a very high amount of water guaranteeing an easy flow of substances trought the matrix but at the same time it did not show any mechanical resistance. The opposite situation was found for 100% hydrogel.

The swelling capability is a characterising property of an hydrogel and it depends on several factors such as temperature, ionic strenght, pH etc. All three polysaccharides based hydrogels showed a water up-take dependent on their different chemical nature. In fact, two of the most

important factors which affect the swelling behaviour of an hydrogel are the hydrophilicity and the flexibility of its chains. Hyal based hydrogel showed a very high water up-take (≅15000 in water), whereas alginate and carboxymethylcellulose based hydrogels showed a drastically reduced W.U. (AA ≅ 6000, CMC ≅6500 in water). The three different polysaccharides based hydrogels showed also a "marked" difference in terms of swelling kinetics (or the time necessary to reach the swelling equilibrium). Hyal hydrogel needed 24 hours whereas CMC 48 hours and AA 120 hours.[19,23] These hydrogels replay also to the environmental temperature changes. The W.U. increases with increasing the temperature.

Among the factors which affect the water up-take of an hydrogel, a first role is played by pH. According to the decrease of pH the free carboxylate groups became protonated causing contemporarily the "instauration" of hydrogen bonds and the decrease of the charge repulsion between the polysaccharide chains. These phenomena induce a structure compactation and consequently a progressive water up-take "reduction" (Figure 4).

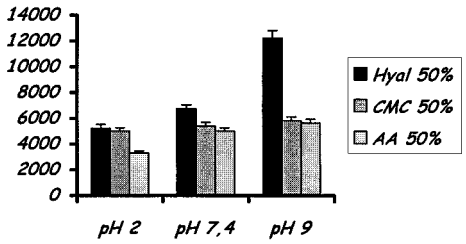


Fig. 4. Water up-take- pH relationship for all 50% hydrogels determined at three pH levels (pH 2: 6.5 mL 0.2M HCl mixed with 25 mL 0.2M KCl; pH 7.4: PBS (phosphate buffer solution); pH 9: 50 mL 0.1M TRIS with 5.7 mL 0.1M HCl).

The possibility to utilise an hydrogel as cellular scaffold is strictly dependent on its rheological behaviour, or its mechanical resistance in substaing cells. The mechanical properties can be expressed in terms of storage (G') and loss (G") modulus. The greater is the value of G' and G", the bigger is the hardness of the material. All three polysaccharides based hydrogels showed G' value greater than G" value, according to the typical "gel-like" behaviour. AA based hydrogel showed very high values of G' and G", in the order of 10^4 Pa for G' and of 10^3 Pa for G". In CMC based hydrogel a substantial decrease of both G' and G"(10^3 Pa for G' and

10^2 Pa for G") was observed. Further decrease was recorded for Hyal based hydrogel (G' 10^2 Pa and G" 10^1 Pa). In the graphs reported in Figure 5 the rheological behaviour of hydrogels was shown.

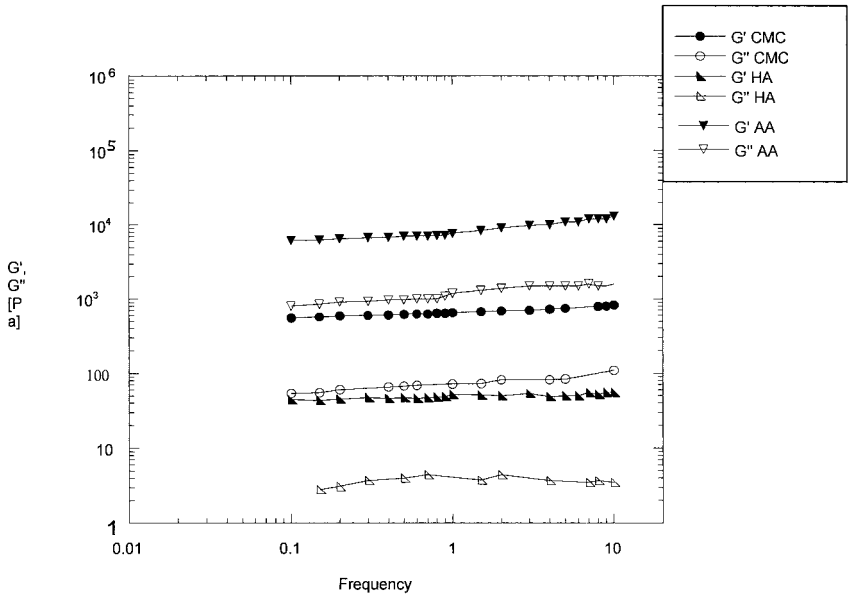


Fig. 5. Rheological behaviour of 50% polysaccharide hydrogels determined at 25°C.

This behaviour can be correlated both with the swelling properties of the three materials, in other words with the different hydrophilicity and flexibility of their polysaccharidic chains, and with their morphology. SEM analysis showed that the three materials assumed a different morphology. AA based hydrogel showed a very compact structure with the laminae strictly associated each other, whereas CMC, and in particular Hyal based hydrogels, showed a "soft" structure with large fissures among the laminae.

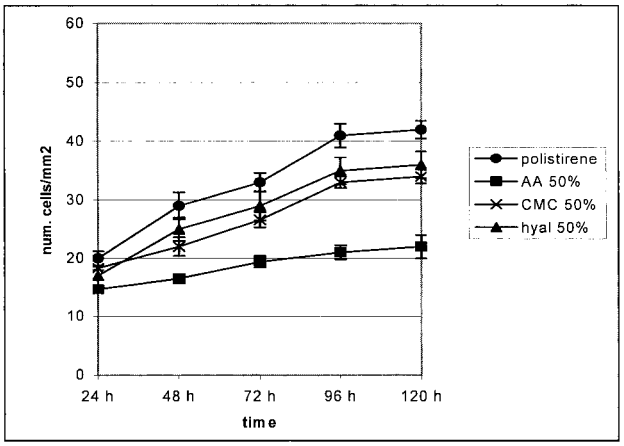
Biological Applications of Polysaccharidic Hydrogels

Scaffold for Tissue Engineering

These hydrogels have been tested as cellular scaffolds. It was evaluated their effectiveness in promoting adhesion and proliferation of several kinds of cells: endothelial cells, fibroblasts and

chondrocytes. The three hydrogels induced a different cellular response for all the cell lines analysed. Hyal based 50% hydrogel resulted the most effective in promoting cell adhesion and proliferation, whereas AA induced the lowest proliferation, CMC hydrogel showed an intermediate behaviour (Figure 6).

a)



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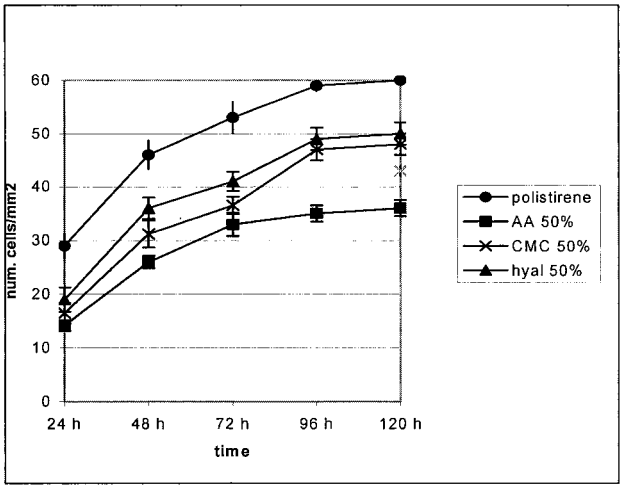

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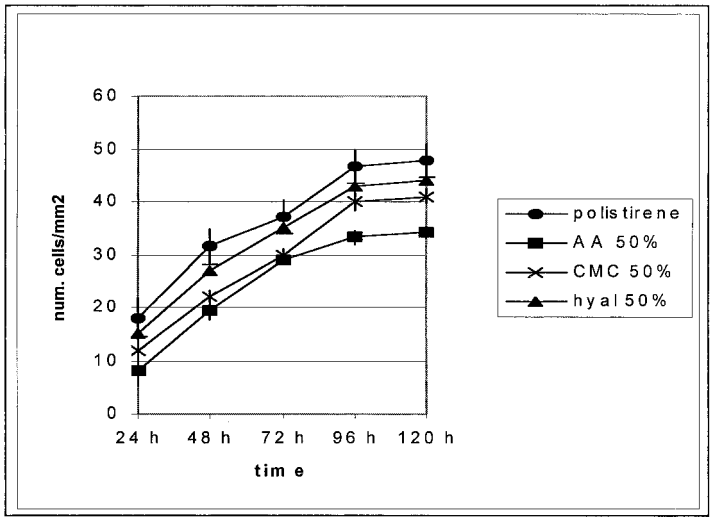


Fig. 6. a: growth curves of endothelial cells BAEC (bovine aortic endothelial cells) on polysaccharide 50% hydrogels; b: growth curves of human fibroblasts on polysaccharide 50% hydrogels; c: growth curves of human chondrocytes on polysaccharide 50% hydrogels.

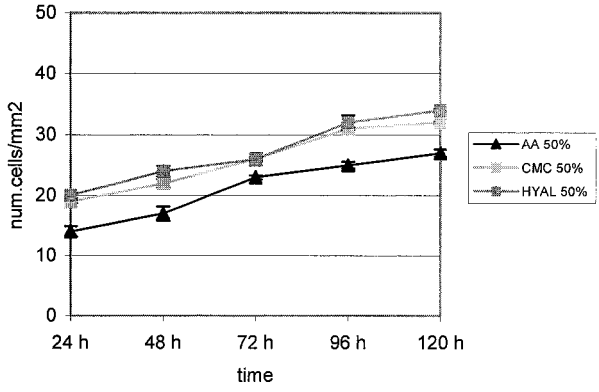


Fig. 7. Growth curve of 3T3 fibroblasts on polysaccharide 50% hydrogels without FCS. All the polysaccharide hydrogels induced low cellular proliferation. Negligible differences among the three hydrogels were observed.

The protein adsorption in terms of both conformational changes and amount of proteins adsorbed was evaluated to explain this different behaviour. In fact, proteins adsorption plays a critical role in modulating cellular response, as demonstred by the very low cell proliferation induced by all the matrices without FCS (Fetal Calf Serum) (Figure 7).

The amount of protein uptaken by the matrices was determined by Bradford assay.[24] The data obtained confirmed that the 3 hydrogels uptook a different amount of proteins. Hyal hydrogels was able to capture the greatest amount of proteins whereas CMC and AA hydrogels adsorbed a lower quantity. All the amounts were reported in Table 1.

Table 1. Amount of serum proteins adsorbed by polysaccharide 50% hydrogels (espressed as $\mu g/mg_{\text{dry gel}}$).

Hydrogel	FCS proteins ($\mu g/mg_{\text{dry gel}}$)
Hyal 50%	62.2±3.5
AA 50%	36.4±2.4
CMC 50%	11.2±1.8

To confirm the crucial role of serum protein-material interaction on the cellular behaviour, cell adhesion in presence of Fibronectin (Fn), one of the most important adhesive proteins, was evaluated. As shown by the growth curves reported in Figure 8, the presence of Fn was fundamental in modulating cellular behaviour.

Osteoarthritis Therapy

Hyal based hydrogel was also tested in the osteoarthritis therapy. The choice of this material depends on the fact that hyaluronane is one of the most important components of sinovial fluid. The therapy consisted in injecting soluble Hyal, but it did not appear effective for the very short resident time of the linear polysaccharide.[25-27] The same treatment was performed with our Hyal 50% hydrogel. The injection was possible since the 50% polysaccharide hydrogel shows tixotropic properties, i.e. in presence of an appropriate mechanical stimulus the hydrogel becomes fluid and can pass through a needle. Removing the stimulus it resumes the original consistence as demonstred by the similarity of G' and G" values before and after injection.[22] Hyal hydrogel resulted effective in osteoarthritis therapy. The alterated cartilage resumed a structure very similar to the native one after a 50 days treatment with Hyal hydrogel.[22]

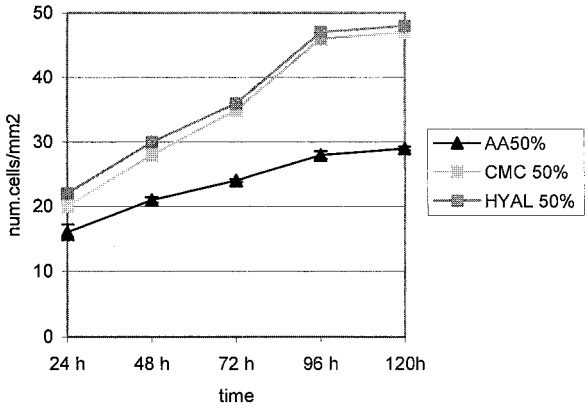


Fig. 8. Growth curves of endothelial cells on polysaccharide 50% hydrogels with Fibronectin. The presence of this protein induced a cellular response similar to that obtained in presence of FCS (Figure 6a).

Microporous Hydrogels

The formation of pores was obtained by stratifying the hydrogel on a cell strainer with a known porosity and by forcing the CO_2 bubbles to pass through the filter, first, and the matrix, then, inducing hydrogel to assume a porous morphology, as reported in the experimental section. The presence and distribution of pores in the polysaccharide hydrogels was confirmed by SEM analysis. A homogeneous distribution of pores was obtained for all the polysaccharide hydrogels. Independently of the chemical nature of the polysaccharide, there was a strict correspondence between the porosity of the filter and the pore diameter obtained in the hydrogel. The alginate based hydrogel with the smallest pore diameter (AA 13 μm) had the highest pore density (5 pores/mm^2). With increasing the pore diameter, the density decreased for all the polysaccharide hydrogels. All the porous matrices had a similar thickness; only the hyaluronane based hydrogel with the largest diameter (Hyal 40 μm) showed a much smaller thickness (i.e. 0.7 μm instead of ≅ 2 μm for all the other porous hydrogels).[28]

The presence of pores modified the water up-take of the native hydrogels: the porous hydrogels took up less water than the native ones at all the pH's investigated.[28] This can be explained by the mechanism of pore formation. Crossing the hydrogel, the CO_2 bubbles provoke an approach

of the hydrogel walls. The surface in contact with water is drastically reduced, causing a lower water up-take. Furthermore the compact material reduces the flexibility and freedom of the polysaccharide chains, decreasing their solvation power.

The AA matrices showed less swelling capability than the CMC and Hyal matrices under all the pH conditions. This behaviour was attributed to the greater rigidity of the chains which reduces not only the amount of the absorbed water, but also the time necessary to reach complete swelling in comparison with the other two polysaccharide based hydrogels.

The hyaluronane hydrogels showed the highest W.U. values. This can be explained by the high hydrophilicity, freedom and flexibility of the polysaccharide chain which permits consistent and easy solvation. This characteristic is also reflected in the high speed of swelling.

The CMC based matrices showed an intermediate behaviour, i.e. intermediate speed and degree of swelling. In fact, the flexibility of the polysaccharide chain is greater than that of AA, but lower than that of Hyal.

FT-IR analysis at all the investigated pHs was performed in order to evaluate whether the mechanism of pore formation induced chemical modification in the systems. A comparison between the native and the porous hydrogels was effectuated.

Hyal based hydrogels: The IR spectra were reported in Figure 9a. The mechanism of pore formation, or the passage of CO_2 bubbles through the matrix, induced an approach of the laminae. In consequence of that an appropriate distance between the functional groups able to form H bond was reached and H bonds were formed. In fact a new band at 1728 cm^{-1} relative to carboxylic groups H-bonded was found. The new H bonds were strong enough to resist all the pHs. Only the intensity of 1728 cm^{-1} band decreased with increasing of pH. The presence of such as strong H bonds, which persist at all the pHs, explains the consistent swelling decrease observed in the porous Hyal hydrogels.

CMC based Hydrogels: IR spectra recorded showed that no new H bonds were formed during the pore formation. Porous CMC hydrogel showed that, with increasing of pH the carboxylic group band progressively disappeared (Figure 9b).

AA based hydrogels: The AA based hydrogels showed a intermediate behaviour. H bonds were formed during the mechanism of pore formation, but with increasing of pH the intensity of the band was drastically reduced (Figure 9c).[28]

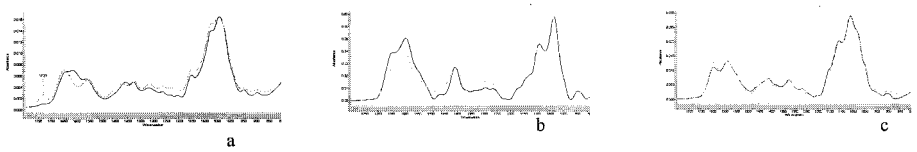


Fig. 9. a) I.R. spectra of Hyal (black) and porous Hyal (grey) recorded at pH 6; I.R. spectra of CMC (black) and porous CMC (grey) recorded at pH 6; I.R. spectra of AA (black) and porous AA (grey) recorded at pH 6.[28]

The rheological behaviour of microporous hydrogels was also evaluated. The presence of pores increases the mechanical characteristics (as demonstred by the increase of both G' and G") for both AA and Hyal hydrogel. A different situation was recorded for CMC based hydrogels. In fact in this case no significative differences were found between the native and porous CMC (Figure 10).

Porous Hydrogel as Systems for the Drug Controlled Release

These hydrogel have been utilised as system for the controlled release of drugs. Ibuprophen-lysine, which is a first-choice drug in the osteoarthritis therapy, has been chosen. The comparison between the release kinetics of native and porous hydrogel revealed a difference. The presence of pores slowed down the drug release. The total release of the drug was obtained for the 50% Hyal hydrogel in 24 hours whereas for the latter in 48 hours. By changing the procedure of enrichment of the matrix with the drug, a further slowing down was observed. In fact, by introducing the drug before the realisation of the porous structure, the drug was captured more steadily and its release resulted slower. In fact the total release of drug was obtained in 8 days. A similar result was found also for CMC based hydrogels (Figure 11).

Sulphated Polysaccharidic Hydrogel

The presence of sulphate groups were detected both by a qualitative assay (toluidine blue test) and Infrared Analysis. The appearence of a new peak at 1230 cm^{-1} (which generally shows two shoulders at 1220 cm^{-1} and 1240 cm^{-1}), relative to S=O, was a clear index of their presence (Figure 12).

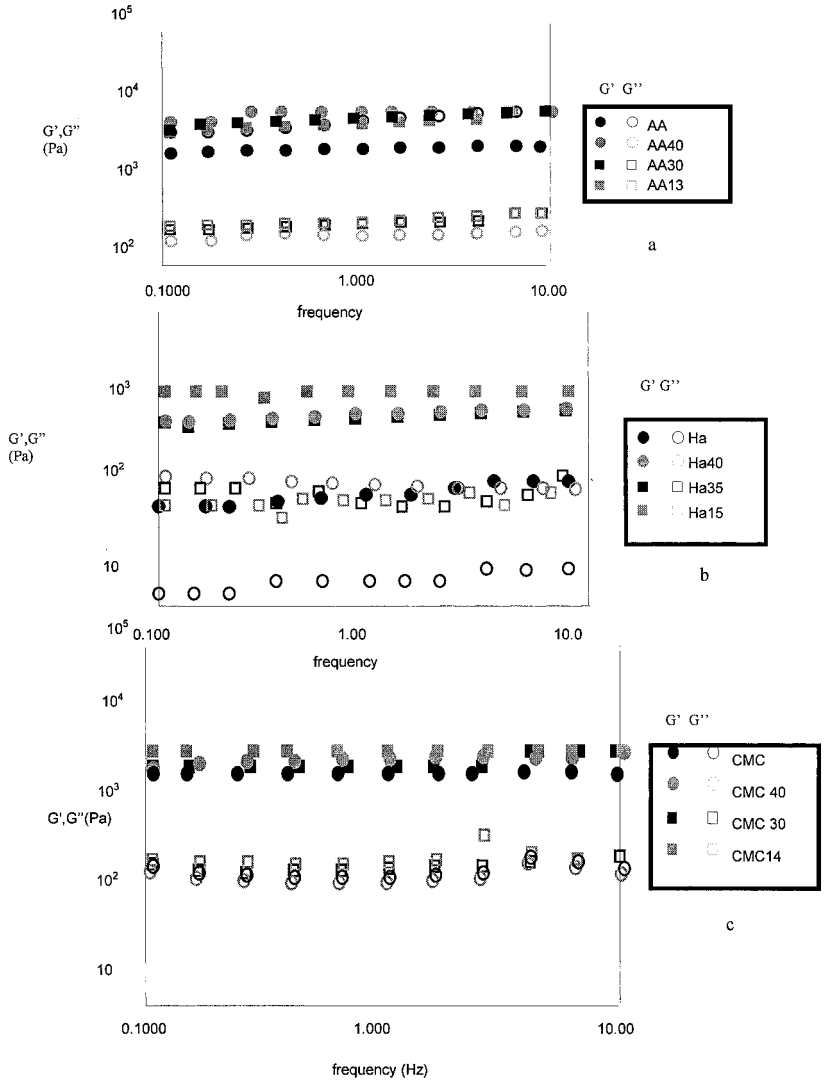


Fig. 10. Rheological behviour of a) native and porous AA hydrogels. The presence of pores increases the G'and G"values; b) native and porous hyal hydrogels. The presence of pores increases the G'and G"values;c) native and porous CMC hydrogels. The presence of pores does not affect G'and G"values.

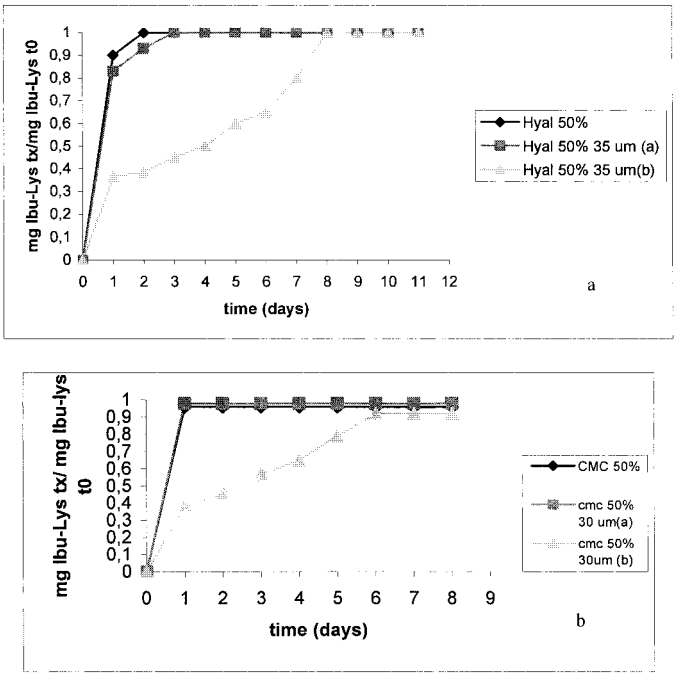


Fig. 11. a) Release kinetics of ibuprophen-lysine from 50% Hyal (black) and porous Hyal emboded with two different technques: pores formation and loading with the drug (dark grey);loading of gel with the drug and pores formation (light grey); b) Release kinetics of ibuprophen-lysine from 50% CMC (black) and porous CMC embodded with two different technques: pores formation and loading with the drug (dark grey); loading of gel with the drug and pores formation (light grey).

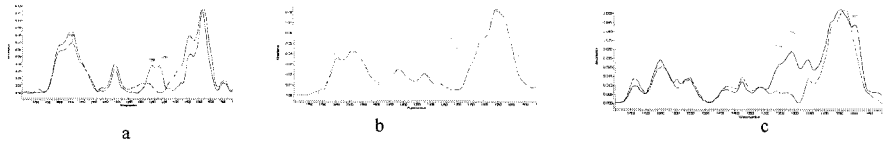


Fig. 12. IR spectra of: a) native and sulphated AA hydrogels. The sulphation was confirmed by the presence of the S=O peak at 1230 cm^{-1}; b) native and sulphated CMC hydrogels. The sulphation was confirmed by the presence of the S=O peak at 1230 cm^{-1}; c) native and sulphated Hyal hydrogels. The sulphation was confirmed by the presence of the S=O peak at 1230 cm^{-1}.

A quantitative evaluation of the sulphation degree was obtained by gravimetric analysis and elementar analysis both on the free and on the crosslinked polymer. A strict correspondence was found. In fact, the sulphation degree of the polymer was found also in the corrisponding hydrogel. The three different polysaccharides based hydrogels showed a different sulphation degree. In fact CMC showed one sulphate group per ripetitive unit whereas two sulphate groups per ripetitive unit were found in the other polysaccharides.

The sulphation affects both the swelling behaviour and morphology of hydrogels.

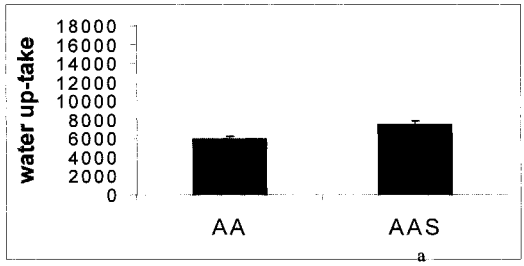
The water up-take of sulphated hydrogels was consistently greater than that of the native one as shown in Figure 13. This is due mainly to both the total hydrophilicity increase of the system after sulphation and the introduced negative charges which increase the repulsion among the polysaccharidic chains. The influence of pH on swelling behaviour was not affected by the presence of sulphate groups. With increasing the pH, the water up-take increased remarkably.

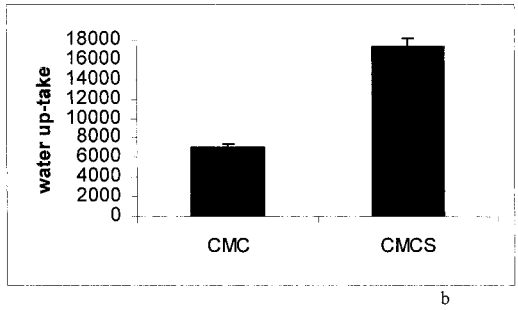
The influence of the sulphation on the hydrogel morphology was negligible. As shown by the pictures reported in Figure 14, no significative changes were observed. The laminae appeared slightly more compact and indented.

Biological Characterisation of Sulphated Polysaccharidic Hydrogels

One of the most important application of sulphated polysaccharide based hydrogel was the coating of catheters. In fact, these materials have to be in contact with blood and the presence of sulphate groups make them very similar to heparin, the most important anti-coagulant substance in human body. Both free and crosslinked polymers can be fixed to different surfaces throught the realisation of a photoreactive intermediate which, subsequently to the exposition to UV light, can react with functional groups of the substrate and a coating can be obtained. The antimicrobial properties of alginate were well-known so this polysaccharide was utilised in the realisation of this coating.

The effectiveness of the sulphatation on the increase of hemocompatibility was evaluated by the determination of the TT test. This analysis permits to determine whether the material is able to extend the time of fibrinogen turnig to fibrin. As shown by the data reported in Table 2 both the free and crosslinked sulphated polymer extend the time up to >180 seconds.





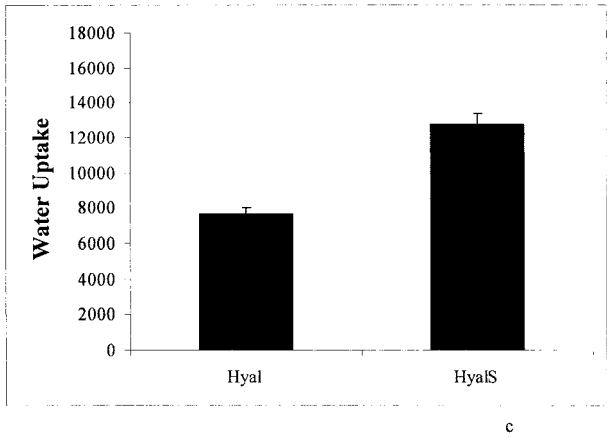
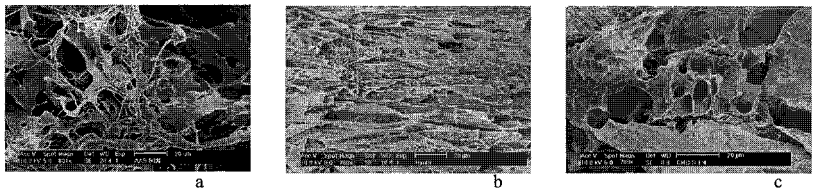


Fig. 13. Influence of sulphate groups on the water up-take of polysaccharide hydrogels (bidstilled water at R.T.): a) sulphated AA hydrogel absorbed a greater amount of fluid in comparison with the native AA hydrogel; b) sulphated CMC hydrogel absorbed a greater amount of fluid in comparison with the native CMC hydrogel; c) sulphated Hyal hydrogel absorbed a greater amount of fluid in comparison with the native Hyal hydrogel.



a b c

Fig. 14. SEM micrographs of sulphated polysaccharide hydrogels: a) AAS 50%; b) HyalS 50%; c) CMCS 50%. No significative morphological modifications in comparison with native polysaccharide hydrogels were observed.

Table 2. time (sec) necessary for sulphated and native polysaccharides and hydrogel to turn the fibrinogen to fibrin.

Materials	TT(sec)
Control	35.3±1.2
AA soluble polysaccharide	68.6±3.0
AAS soluble polysaccharide	> 180
AAS hydrogel	> 180

Conclusions

Polysaccharide based hydrogels were obtained by crosslinking macromolecule chains through an alchilic bridge deriving from the formation of an amidic bond between the carboxylate groups present in the polysaccharidic chains and the amine groups present in the crosslinking agent (alchylic diamine). For their physico-chemical properties, such as swelling behaviour and rheological properties, these hydrogels resulted good cellular scaffolds. Their morphology was changed to increase their effectiveness in promoting cell adhesion and proliferation. Microporous matrices were obtained by physically modification of crosslinked polymer. The morphological modification induced physico-chemical changes, such as the decrease of water up-take and an increase in the G' and G" values, or in their mechanical properties. That change was due to strong hydrogen bonds occurring in the process of pore formation. The microporous matrices were utilised also as drug controlled release systems. Also in this application the porous materials turned out to be more effective than the native matrices slowing down the release (8 days against 24 hours). To broaden the application fields of these materials, the starting polysaccharides were chemically modified by insertion of new functional group, sulphate group. This new functional group made the hydrogels hemocompatible as ascertained by the increase of the time necessary for fibrinogen turning to fibrin (TT test). Once modified they resulted optimal coating for different surfaces such as PU, acting as lubricating substance.

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