Rheology of mixed alginate-hyaluronan aqueous solutions

Andrea Travan[1], Simona Fiorentino[2], Mario Grassi[2], Massimiliano Borgogna[1], Eleonora Marsich[3], Sergio Paoletti[1], and Ivan Donati[1,*]

[1]Department of Life Sciences, Via Licio Giorgieri 5, [2]Department of Engineering and Architecture, Via Valerio 10, [3]Department of Medical, Surgical, and Health Sciences, Piazza dell'Ospitale 1, University of Trieste, I-34100 Trieste, Italy.

* Corresponding Author
Dr. Ivan Donati
e-mail: idonati@units.it
tel: +39 040 5588733

Abstract

The present manuscript addresses the description of binary systems of hyaluronan (HA) and alginate (Alg) in semi-concentrated solution. The two polysaccharides were completely miscible in the entire range of relative weight fraction explored at a total polymer concentration of up to 3 % (w/V). The rheological study encompassed steady flow and mechanical spectra for HA/Alg systems at different weight fractions with hyaluronan at different molecular weights. These extensive analyses allowed us to propose a model for the molecular arrangement in solution that envisages a mutual exclusion between the two polysaccharides even though a clear phase separation does not occur. This result may have profound implications when biomaterials based on the combination of alginate and hyaluronan are proposed in the field of biomedical materials.

1. Introduction

Alginate is a collective term to describe a family of polysaccharides isolated from brown seaweeds and bacteria [1]. Chemically, alginates are linear copolymers of 1→4 linked β-D-mannuronic acid (M) and its C-5 epimer, α-L-guluronic acid (G), arranged in a blockwise pattern, with homopolymeric regions of M- and G residues, indicated as M-blocks and G-blocks, respectively, interspersed with regions of alternating structure (MG blocks). At around neutral pH, alginate behaves as an anionic polyelectrolyte and as a semi-flexible chain upon reduction of the medium ionic strength. The ability to form stable hydrogels upon treatment with divalent ions, such as calcium ions [2], have resulted in alginates being widely used in industrial applications. In addition, alginates have relevant applications in the field of biomedical materials [3,4] due to their versatility in gel formation which has been exploited for the encapsulation of, for example, the insulin-producing Langerhans islets for the treatment of type I diabetes [5-8]. In fact, the availability of pure and well-characterised alginate samples makes this material an ideal choice for cell microencapsulation.

In addition to cell encapsulation, alginate has been proposed for several other applications in the biomedical field [3]. For example, alginate-based scaffolds containing hydroxyapatite showed structural features similar to those of the trabecular bone [9-12], and in combination with silver nanoparticles, as well as having antimicrobial properties, have shown good osteointegration in *in vivo* models [12].

However, it should be underlined that unmodified alginate shows non-adhesive properties and, as such, it does not favour cell adhesion [13], which is the first step towards cell proliferation and colonisation. For this reason, the addition of bioactive polymers to alginate has been considered as a way to improve its features [14,15].

Hyaluronan (HA), an alternating copolymer composed of β-D-glucuronic acid (GlcA) and β-D-N-acetylglucosamine (GlcNAc), linked in the sequence →4 GlcA1→3 GlcNAc1→ is the simplest

glycosaminoglycan and a major constituent of the extracellular matrix (ECM) as a high molar mass anionic polyelectrolyte component [16]. Apart from its well-known structural role due to the peculiar chain semi-flexibility, which gives the polymer exceptional viscoelastic behaviour, hyaluronan shows important biological features. HA has six known surface receptors [17], among which CD44 and RHAMM are the most well characterised. While both receptors bind HA through a common binding domain, they differ, to some extent, in the cellular functions: CD44 mediates cell attachment, HA uptake and degradation while RHAMM mediates cell locomotion in response to soluble HA [18].

The idea of exploiting mixtures of alginate and hyaluronan to develop novel biomaterials has been pursued by several authors for mesenchymal stem cell encapsulation [19], as a post-surgical tissue adhesion barrier [20] and for the encapsulation of chondrocytes [21]. The combination of alginate and hyaluronan has also been proposed for cartilage engineering by Lindenhayn and co-workers [22]. In this paper, it was noted that hyaluronan was, to some extent and depending on the alginate gelation conditions, excluded from the hydrogel network. In addition, Park *et al.* prepared hyaluronan beads starting from its mixture with alginate and exploiting the ability of the latter to form “reversible” calcium hydrogels [23]. Most of the above mentioned papers focused on the biological properties exerted by the mixed construct, but they overlook the physical-chemical implications incidental to the presence of two anionic polyelectrolytes in solution, which might impact properties such as gel formation kinetics, stability and mechanical performance.

2. Materials and Methods

Alginate (Alg) (LVG, M_W = 120000; F_G = 0.69; F_{GG} = 0.59; $N_{G>1}$ = 16.3; PI = 1.9) and hyaluronan (HA) with M_W of 1500000 (HA150; PI = 2.2) and of 800000 (HA80; PI = 2.4) were kindly provided by FMC, Drammen (Norway). Hyaluronan with a MW of 240000 (HA24) was kindly provided by Sigea S.r.L., Trieste (Italy). Hyaluronan with M_W of 400000 (HA40; PI = 2.7) was obtained by acid degradation of HA150 according to a procedure previously reported [24] and the molar mass was evaluated from intrinsic viscosity measurements [25]. In alginate characterization, F_G denotes the fraction of alginate consisting of guluronic acid, F_{GG} indicates the fraction of alginate consisting of guluronic acid in blocks and $N_{G>1}$ denotes the average length of guluronic acid blocks. Binary mixtures of HA and Alg were prepared in the presence of aqueous 0.15 M NaCl at a different weight ratio of HA (ϕ_{HA}) and of alginate ($\phi_{Alg} = 1 - \phi_{HA}$).

2.1 Viscometry

Specific viscosity was measured at 25 °C by means of a Schott-Geräte AVS/G automatic measuring apparatus and an Ubbelohde-type capillary viscometer in aqueous 0.15 M NaCl. For the polysaccharides used in the present study, the intrinsic viscosity ([η]) values were determined by analyzing the polymer concentration dependence of the reduced specific viscosity (η_{sp}/c) and of the logarithm of the reduced relative viscosity ($\ln(\eta_{rel})/c$) by means of the Huggins (eq. 1) and Kraemer (eq. 2) equations, respectively.

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]c \qquad (1)$$

$$\frac{\ln(\eta_{rel})}{c} = [\eta] - k''[\eta]c \qquad (2)$$

where k' and k" are the Huggins ad Kraemer constants, respectively. The specific viscosity of the Alg/HA mixed solutions was measured in aqueous 0.15 M NaCl at different polysaccharide weight fractions maintaining a constant total polymer concentration of 1 g/L.

2.2 Rheological determination

Rheological tests were performed on mixed solutions of alginate and hyaluronan under continuous shear conditions to determine steady viscosity values in the stress range 1 to 100 Pa, as well as under oscillatory shear conditions to determine the extension of the linear viscoelasticity regime (stress sweep tests at 1 Hz in the stress range $1 < \tau < 500$ Pa) and the mechanical spectra (frequency sweep, $\tau = 4$ Pa, within the linear viscoelastic range). The complex viscosity (η^*), the storage (G') and loss (G") moduli of the binary mixtures and of the hydrogels were recorded in the frequency range 0.1 – 50 Hz. All tests were carried out with the controlled stress rheometer Rheostress Haake RS 150 operating at 25 °C. A cone-plate CP60/1° geometry was used in all cases. A glass bell covering the measuring device was used to improve thermal control and limit evaporation.

3. Results and Discussion

3.1 Viscosity in dilute and semi-dilute conditions of binary Alg/HA mixtures

The (macroscopic) mutual compatibility of alginate (Alg) and hyaluronan in dilute binary solution was assessed by both transmittance measurements and viscometry. In the presence of aqueous NaCl (0.15 M), the former technique showed no notable variation in the transmittance of the binary system with respect to that displayed by the polysaccharides alone, thereby excluding the formation of macroscopic phase separation (data not reported). The complete macroscopic miscibility of the two polysaccharides was confirmed by the dependence of the specific viscosity of the HA/Alg dilute binary mixtures on their composition (Figure 1).

Figure 1

It can be noticed that the experimental results are in very good agreement with the theoretical prediction that was calculated assuming that no other interaction, apart from the hydrodynamic ones, occurred between the two polysaccharides [26].

The characterisation of HA/Alg binary mixtures was carried out in semi-concentrated solutions by means of a rheological measurements. The flow curves of the systems under analysis were recorded in 0.15 M NaCl aqueous solution while varying the weight fraction (ϕ) of the two polysaccharides and the molecular weight of the hyaluronan samples. In all cases, a pseudo-plastic behavior was detected with a clear Newtonian plateau for low values of the shear stress applied. The experimental curves were fitted with a simplified version of the Cross equation (eq. 3):

$$\eta = \eta_{\dot{\gamma}\to\infty} + \frac{\eta_0 - \eta_{\dot{\gamma}\to\infty}}{1+(k\dot{\gamma})^n} = \frac{\eta_0}{1+(k\dot{\gamma})^n} \qquad (3)$$

where $\eta_{\dot{\gamma}\to\infty}$, the viscosity at infinite shear rate, was set equal to zero. η_0 is the zero-shear viscosity, corresponding to the limiting Newtonian plateau for $\dot{\gamma}\to 0$, and *k* is the characteristic polymer relaxation time, often indicated with τ [27]. τ is the time associated with large-scale motion in the structure of the polymer. *n* is known as the Cross Rate Constant and it can be considered as a "*pseudoplasticity* index" [28]. The dependence of the zero-shear viscosity on the molecular weight of hyaluronan for both the polysaccharide alone and for binary HA/Alg mixtures is reported in Figure 2a, showing a power-law dependence ($\eta_0 \propto MW^{\beta}$) when the total polysaccharide concentration was maintained equal to 30 g/L.

Figure 2

In particular, when hyaluronan alone was considered, the power-law dependence showed a scaling factor of approximately 3.1. This is close to the value (3.4) predicted [29] and reported for different flexible polysaccharides including hyaluronan [30] and alginate [31] in the semi-dilute regime. Upon addition of alginate to the system, the scaling factor β showed a non-linear decrease upon reduction of the fraction of HA in the mixture (Figure 2, inset). This result likely stemmed from the dilution of hyaluronan due to the addition of the second polysaccharide, *i.e.*, alginate. In fact, the β exponent decreases for all (saccharidic) polymers at lower concentrations, down to the value of 1 in very dilute conditions. The effect on overall viscosity of the binary system due to the presence of alginate was also evident from the zero-shear viscosity which was 36.2 Pa·s for C_{HA}=15 g/L and increased to 84.9 Pa·s in the ϕ_{HA} = 0.5 mixture (still with C_{HA}=15 g/L).

In Figure 2b, the dependence of the relaxation time, τ, on the molecular weight of HA is reported for both hyaluronan alone and for HA/Alg binary mixtures. In the first case, a power-law of $\tau \propto MW^{3.5}$ was found, which is very close to the theoretically expected relationship (3.4) [32-34].

Similarly, in the case of the binary hyaluronan/alginate mixtures, the exponent of the power law was 3.2, which is even closer to some reported experimental values of τ for HA (3.0) [32-34]. In Figure 3a the dependence of the zero-shear viscosity for the binary Alg/HA mixtures on the weight fraction of HA (ϕ_{HA}) is reported for HA at different values of the molecular weight.

Figure 3

It can be seen that in all cases, a scaling law holds, at least in the ϕ_{HA} range explored. In Figure 3b and 3c, the dependence of the zero-shear viscosity and of the polymer relaxation time, τ, on the concentration of HA is reported for both a hyaluronan solution alone and a HA/Alg binary mixture with $\phi_{HA} = 0.50$. For both properties, the addition of alginate brings about an effect that differs from that of the pure dilution with the solvent. This provides an indication of the arrangement between the two components of the mixture at a molecular level. In fact, considering the onset of the shear-thinning behavior as a progressive decrease in the steady-state entanglements density of the hyaluronan chains [33], the higher τ in the presence of alginate seems to point to an effective higher concentration of HA which might arise from a (at least partial) segregation, rather than its homogeneous mixing with alginate.

3.2 Mechanical spectroscopy

The stress sweep curves for the samples were recorded and a type I behavior was found in all cases. A typical curve is reported in Figure 4 together with the best fit of the experimental data according to the Soskey-Winter equation (eq. 4).

$$G' = G'_0 \frac{1}{1+(b\gamma)^n} \quad (4)$$

Where γ is the strain, G'_0 is the limiting value of the storage modulus for $\gamma \rightarrow 0$, while b and n are adjustable parameters. The critical strain, γ_c, marking the limit of the linear viscoelastic regime, was arbitrarily assumed to correspond to $G'/G'_0 = 0.95$. The limit of the linear viscoelastic response is reported in table 1.

Figure 4

Table 1

The mechanical response of hyaluronan alone and of its binary mixtures with alginate was recorded as a function of pulsation, ω (= $2\pi\nu$). Depending on the molecular weight of hyaluronan, a transition from viscous (G" > G') to elastic (G' > G") behaviour took place in the frequency range explored (Figure 5a, for the sample case of HA150) with a cross-over point.

Figure 5

The experimental data were fitted using a generalised Maxwell model composed of a sequence of elements in parallel (spring and dashpot). The frequency dependence of the viscous (G') and elastic (G") response is then described with the following equations (eqs. 5 and 6):

$$G' = \sum_{i=1}^{n} G_i \frac{(\lambda_i \omega)^2}{1+(\lambda_i \omega)^2} \quad (5)$$

$$G'' = \sum_{i=1}^{n} G_i \frac{\lambda_i \omega}{1+(\lambda_i \omega)^2} \quad \text{with} \quad G_i = \eta_i / \lambda_i \qquad (6)$$

where n is the number of Maxwell elements considered, G_i, η_i and λ_i represent the spring constant, the dashpot viscosity and the relaxation time of the *i*th Maxwell element, respectively [35]. The fitting of the experimental data was performed assuming that the relaxation times were not independent of each other but they were scaled by a factor 10. The number of Maxwell elements was selected, based on a statistical procedure, to minimise the product $\chi^2 N_p$, where χ^2 is the sum of the squared errors, while N_p (= 2 + n) indicates the number of fitting parameters (table 2).

Table 2

Figure 5b reports the dependence of the frequency corresponding to the cross-over point, ω_{cross} (= $2\pi\nu_{cross}$), on the molecular weight of hyaluronan for both HA alone and for its binary mixtures with alginate. In both cases, a very similar power-law relation holds for HA alone and for the HA/Alg mixture, namely $\omega_{cross} \propto MW^{-3.1}$ and $\omega_{cross} \propto MW^{-3.2}$, respectively. This is in good agreement with the experimental findings reported in the literature for hyaluronan solutions [36]. Despite the increase in ω_{cross} determined by the dilution of hyaluronan with alginate, it appears that the entangled structure was not altered, with respect to the dilution of hyaluronan alone, by the presence of alginate. However, in the case of the HA sample with the highest molecular weight, the mechanical response of the binary mixture was also compared with a solution containing only hyaluronan at the same concentration as in the mixture (Figure 5b, triangle). It can be noted that in this latter case, the ω_{cross} is higher than that of the mixture.

Figure 5c reports the dependence of the modulus-at-cross-over, G_{cross} $(G_{cross} \equiv G'_{cross} = G''_{cross})$, on the molecular weight of HA. It can be seen that in the case of hyaluronan alone, a power-law

dependence $G_{cross} \propto MW_{HA}^{-0.1}$ holds, which is in good accordance with the experimental results previously reported for hyaluronan, *i.e.* $G_{cross} \propto MW^{0}$ [36]. In contrast, when alginate is added to HA to form the binary system, G' and G" show a non-zero (negative) dependence on the molecular weight of hyaluronan - more specifically, $G_{cross} \propto MW^{-1.2}$. It follows that the presence of the second polysaccharide brings about interference on the viscoelastic properties of the binary system whose extent is dependent on the molecular weight of the hyaluronan used in the system.

Figure 6 shows the relative effect on η_0 and ω_{cross} due to the dilution of a hyaluronan solution with the solvent and of a HA/Alg binary mixture with $\phi_{HA} = 0.5$.

Figure 6

In relative terms, the variation of ω_{cross} was more marked in the case of the mixture than of the HA alone while basically no effect was noted on η_0. This behaviour indicates that alginate lacks specific interactions with hyaluronan and contributes to the viscosity in simply additive terms.At variance, alginate markedly affects the formation of the complex network of entanglement which is the basis for the mechanical response of HA containing systems.

4. Conclusion

The biotechnological relevance of polysaccharide mixtures as ECM mimics has gained attention over recent years. However, while mixed systems have been exploited for *in-vitro* experiments, extended physical-chemical analyses are still partially lacking.

The present manuscript deals with the experimental physical-chemical characterisation by rotational rheometry of binary mixtures of alginate and hyaluronan. These analyses allow us to propose a model for the mutual arrangement of the two polysaccharides in solution that envisages a situation

where the polyanions tend to segregate instead of mixing freely, but without leading to macroscopic phase separation. These results may impact systems in which a three-dimensional arrangement (hydrogel), for biological purposes where a controlled release of the biologically active component might affect the final outcome. Rheological measurements in semi-dilute solution provide new information on the system at the macroscopic level and have shown that, although the combined presence of the two polysaccharides does not give rise to marked synergistic effects, alginate and hyaluronan tends to exclude each other, thus forming microdomains enriched in the two separate components instead of a true homogeneous one-phase solution. This conclusion has notable implications for systems using hydrogel formations. In fact, the treatment of the binary mixtures with calcium ions, although leading to a three dimensional architecture due to the presence of alginate, could result in an uneven distribution of the two components where hyaluronan might be excluded from the three dimensional structure and may be confined at the borders of the hydrogel [22]. The present manuscript will be followed by another work where the formation and mechanical properties of hydrogels from mixed systems of alginate and hyaluronan, together with their biological properties, will be explored.

Acknowledgments

This study was supported by the EU-FP7 Project “Development of a resorbable sealing patch for the prevention of anastomotic leakage after colorectal cancer surgical treatment - AnastomoSEAL” (Contract number 280929) and by the Italian Ministry of Education (PRIN 2010-11 (20109PLMH2)).

Captions to Figures

Figure 1. Dependence of the reduced specific viscosity from mixture composition for HA/Alg systems at total polymer concentration of 1 g/L in aqueous 0.15M NaCl. The dotted line represents the theoretical dependence based on Donati et al [26].

Figure 2. Dependence of the zero-shear viscosity (η_0, **a**) and relaxation time (*k*, **b**) on HA molecular weight (MW) for hyaluronan alone (■) and for HA-Alg binary mixtures with ϕ_{HA} = 0.5 (□). In **a**, ▲ represents a hyaluronan solution at a total polymer concentration of 15 g/L and △ represents the theoretical value of zero-shear viscosity calculated for a HA-Alg binary mixtures with ϕ_{HA} = 0.5 according to [26]. Inset in **a**: Dependence of the scaling factor β ($\eta_0 \propto MW^{\beta}$) on the fraction of HA in the HA/Alg binary mixture. In all cases, total polymer concentration = 30 g/L.

Figure 3. **a**) Dependence of the zero-shear viscosity, η_0, on hyaluronan fraction (ϕ_{HA}) for binary mixtures with alginate of HA150 (■), HA80 (□) and HA40 (•). **b**) Dependence of the zero shear viscosity, η_0, on hyaluronan concentration (C_{HA}) of a HA solution upon addition of alginate at constant total polymer concentration of 30 g/L (■), or upon dilution (□). **c**) Dependence of the polymer-chain relaxation time, *k*, on hyaluronan concentration (C_{HA}) of a HA solution upon addition of alginate at constant total polymer concentration of 30 g/L (■), or upon dilution (□).

Figure 4. Example of a stress sweep (G') test for a mixture of hyaluronan and alginate (■). Dotted line represents the best fit of the experimental data according to the Soskey-Winter equation (eq. 5).

Figure 5. **a**) Dependence of the storage (G', ■) and loss (G", □) moduli from the pulsation, ω (= 2πν), for a HA/Alg binary solution, with hyaluronan weight fraction ϕ_{HA} = 0.5 (sample HA150). Solid and dotted lines represent generalised Maxwell model best fitting (eq. 5 and 6). **b**) Dependence of the cross-over pulsation on molecular weight of hyaluronan for HA alone (■) and HA/Alg binary solutions with ϕ_{HA} = 0.5 (□). In both cases, total polysaccharide concentration was 30 g/L. Parenthesis indicate ω_{cross} values outside the frequency range explored and calculated with eqs. 5 and 6. In **b**), ▲ represents the pulsation corresponding to cross-over for a HA solution of 15 g/L. **c**) Dependence on the molecular weight of HA of the elastic (G') and viscous (G") modulus at the cross-over point for a hyaluronan solution alone (■) and for a HA/Alg binary solution with ϕ_{HA} = 0.5 (□). All solutions were prepared in aqueous 0.15 M NaCl.

Figure 6. Relative variation of zero shear viscosity ($\eta_{0c}/\eta_{0c=30g/L}$) with total polymer concentration ($C_{Tot} = C_{HA} + C_{Alg}$) (right y-scale) for HA (■) and HA/Alg mixture (□, ϕ_{HA} = 0.5) upon dilution with solvent (0.15 M NaCl). Relative dependence of the pulsation corresponding to the crossover ($\omega_{cross,c}/\omega_{cross,c=30g/L}$) on total polymer concentration (left y-scale) for HA (●) and for the HA/Alg mixture (○, ϕ_{HA} = 0.5).

References

1. I. Donati, S. Paoletti, Material Properties of Alginates, in: B.H.A. Rhem (Eds), Alginates; Biology and Applications, Springer-Verlag, Berlin Heidelberg, 2009, pp. 1-53.

2. K.I. Draget, O. Smidsrød, G. Skjåk-Bræk, Alginates from algae, in: A. Steinbüchel, S.K. Rhee (Eds), Polysaccharides and Polyamides in the Food Industry. Properties, Production, and Patents., Wiley-VCH Verlag GmbH & Co., Weinheim, 2005, pp. 1-30.

3. T.H. Silva, A. Alves, B.M. Ferreira, J.M. Oliveira, L.L. Reys, R.J.F. Ferreira, R.A. Sousa, S.S. Silva, J.F. Mano, R.L. Reis, Materials of marine origin: A review on polymers and ceramics of biomedical interest, Int. Mater. Rev. 57 (2012) 276-307.

4. S. Van Vlierberghe, P. Dubruel, E. Schacht, Biopolymer-based hydrogels as scaffolds for tissue engineering applications: A review, Biomacromolecules 12 (2011) 1387-1408.

5. R. Calafiore, G. Basta, G. Luca, A. Lemmi, M.P. Montanucci, G. Calabrese, L. Racanicchi, F. Mancuso, P. Brunetti, Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases, Diabetes Care 29 (2006) 137-138.

6. P. de Vos, M.M. Faas, B. Strand, R. Calafiore, Alginate-based microcapsules for immunoisolation of pancreatic islets, Biomaterials 27 (2006) 5603-5617.

7. P. de Vos, M. Bucko, P. Gemeiner, M. Navrátil, J. Švitel, M. Faas, B.L. Strand, G. Skjåk-Bræk, Y.A. Mørch, A. Vikartovská, I. Lacík, G. Kolláriková, G. Orive, D. Poncelet, J.L. Pedraz, M.B. Ansorge-Schumacher, Multiscale requirements for bioencapsulation in medicine and biotechnology, Biomaterials 30 (2009) 2559-2570.

8. F. LimA.M. Sun, Microencapsulated islets as bioartificial endocrine pancreas, Science 210 (1980) 908-910.

9. G. Turco, E. Marsich, F. Bellomo, S. Semeraro, I. Donati, F. Brun, M. Grandolfo, A. Accardo, S. Paoletti, Alginate/hydroxyapatite biocomposite for bone ingrowth: A trabecular structure with high and isotropic connectivity, Biomacromolecules 10 (2009) 1575-1583.

10. G. Jin, G. Kim, Multi-layered polycaprolactone-alginate-fucoidan biocomposites supplemented with controlled release of fucoidan for bone tissue regeneration: Fabrication, physical properties, and cellular activities, Soft Matter 8 (2012) 6264-6272.

11. C.C. Yu, J.J. Chang, Y.H. Lee, Y.C. Lin, M.H. Wu, M.C. Yang, C.T. Chien, Electrospun scaffolds composing of alginate, chitosan, collagen and hydroxyapatite for applying in bone tissue engineering, Mater. Lett. 93 (2013) 133-136.

12. E. Marsich, F. Bellomo, G. Turco, A. Travan, I. Donati, S. Paoletti, Nano-composite scaffolds for bone tissue engineering containing silver nanoparticles - preparation,

characterization and biological properties, J. Mater. Sci-Mater. M. 24 (2013) 1799-1807.

13. A.D. Augst, H.J. Kong, D.J. Mooney, Alginate Hydrogels as Biomaterials, Macromol. Biosci. 6 (2006) 623-633.

14. E. Marsich, M. Borgogna, I. Donati, P. Mozetic, B.L. Strand, S.G. Salvador, F. Vittur, S. Paoletti, Alginate/lactose-modified chitosan hydrogels: a bioactive biomaterial for chondrocyte encapsulation, J. Biomed. Mater Res. A 84 (2008) 364-376.

15. E. Marsich, A. Travan, M. Feresini, R. Lapasin, S. Paoletti, I. Donati, Polysaccharide-Based Polyanion-Polycation-Polyanion Ternary Systems in the Concentrated Regime and Hydrogel Form, Macromol. Chem. Phys. 214 (2013) 1309-1320.

16. C.B. KnudsonW. Knudson, Cartilage proteoglycans, Semin. Cell Dev. Biol. 12 (2001) 69-78.

17. D.E. Ingber, Mechanical control of tissue morphogenesis during embryological development, Int. J. Dev. Biol. 50 (2006) 255-266.

18. J. Gailit, R.A.F. Clark, Wound repair in the context of extracellular matrix, Curr. Opin. Cell Biol. 6 (1994) 717-725.

19. E.E. Coates, C.N. Riggin, J.P. Fisher, Photocrosslinked alginate with hyaluronic acid hydrogels as vehicles for mesenchymal stem cell encapsulation and chondrogenesis, J. Biomed. Mater. Res. 101A (2013) 1962-1970.

20. S. Oh, S. Na, K. Song, J. Lee, Sprayable powder of hyaluronate embedded in mildly cross-linked alginate as a post-surgical tissue adhesion barrier, Macromol. Res.2013) 1-7.

21. C. Gigant-Huselstein, P. Hubert, D. Dumas, E. Dellacherie, P. Netter, E. Payan, J.F. Stoltz, Expression of adhesion molecules and collagen on rat chondrocyte seeded into alginate and hyaluronate based 3D biosystems. Influence of mechanical stresses, Biorheology 41 (2004) 423-431.

22. K. Lindenhayn, C. Perka, R.S. Spitzer, H.H. Heilmann, K. Pommerening, J. Mennicke, M. Sittinger, Retention of hyaluronic acid in alginate beads: Aspects for in vitro cartilage engineering, J. Biomed. Mater. Res. 44 (1999) 149-155.

23. K.H. Bae, J.J. Yoon, T.G. Park, Fabrication of hyaluronan acid hydrogel beads for cell encapsulation, Biotechnol. Prog. 22 (2006) 297-302.

24. K. TømmeraasC. Melander, Kinetics of Hyaluronan Hydrolysis in Acidic Solution at Various pH Values, Biomacromolecules 9 (2008) 1535-1540.

25. A. Gamini, S. Paoletti, F. Zanetti, Chain rigidity of polyuronates: static light scattering of aqueous solutions of hyaluronate and alginate, in: S.E. Harding, D.B. Sattelle, V.A. Bloomfield (Eds), Laser Light Scattering in Biochemistry, Royal Society of Chemistry, Cambrige, 1992, pp. 294-311.

26. I. Donati, M. Borgogna, E. Turello, A. Cesáro, S. Paoletti, Tuning Supramolecular Structuring at the Nanoscale Level: Nonstoichiometric Soluble Complexes in Dilute Mixed Solutions of Alginate and Lactose-Modified Chitosan (Chitlac), Biomacromolecules 8 (2007) 1471-1479.

27. M.M. Cross, Relation between viscoelasticity and shear-thinning behaviour in liquids, Rheol. Acta 18 (1979) 609-614.

28. M.M. Cross, Rheology of non-Newtonian fluids: a new flow equation for psedoplastic systems, J. Colloid. Sci. 20 (1965) 417-437.

29. P.G. De Gennes, Dynamics of Entangled Polymer Solutions. I. The Rouse Model, Macromolecules 9 (1976) 587-593.

30. D. Calciu-Rusu, E. Rothfuss, J. Eckelt, T. Haase, H.B. Dick, B.A. Wolf, Rheology of Sodium Hyaluronate Saline Solutions for Ophthalmic Use, Biomacromolecules 8 (2007) 1287-1292.

31. T. Matsumoto, M. Kawai, T. Masuda, influence of concentration and mannuronate/guluronate [correction of gluronate] ratio on steady flow properties of alginate aqueous systems, Biorheology 29 (1992) 411-417.

32. M.K. Cowman, S. Matsuoka, Experimental approaches to hyaluronan structure, Carbohydr. Res. 340 (2005) 791-809.

33. E. Fouissac, M. Milas, M. Rinaudo, Shear-rate, concentration, molecular weight, and temperature viscosity dependences of hyaluronate, a wormlike polyelectrolyte, Macromolecules 26 (1993) 6945-6951.

34. W.E. Krause, E.G. Bellomo, R.H. Colby, Rheology of Sodium Hyaluronate under Physiological Conditions, Biomacromolecules 2 (2001) 65-69.

35. R. Lapasin, S. Pricl, Rheology of Industrial Polysaccharides, Theory and Applications, Chapman and Hall, London, 1995.

36. M. Milas, M. Rinaudo, I. Roure, S. Al-Assaf, G.O. Phillips, P.A. Williams, Comparative rheological behavior of hyaluronan from bacterial and animal sources with cross-linked hyaluronan (hylan) in aqueous solution, Biopolymers 59 (2001) 191-204.

Table 1. Composition, critical strain and critical stress (τ_c) for binary HA/Alg mixtures in NaCl 0.15 M. ϕHA represents the weight fraction of hyaluronan in the mixture.

Sample	ϕ_{HA}	γ_c	τ_c (Pa)
HA150 30 g/L	1	0.44	123.3
HA150 22g/L	1	0.50	72.6
HA150 18g/L	1	0.51	45.5
HA150 15g/L	1	0.99	48.3
HA80 30 g/L	1	0.55	107.3
HA40 30 g/L	1	0.79	38.2
HA150/Alg 30 g/L	0.73	0.7	79.5
HA150/Alg 30 g/L	0.6	0.68	77.1
HA150/Alg 30 g/L	0.5	0.58	46.5
HA150/Alg 30 g/L	0.4	0.62	30.3
HA80/Alg 30 g/L	0.73	0.54	61.4
HA80/Alg 30 g/L	0.6	0.64	33.9
HA80/Alg 30 g/L	0.5	0.91	47.0
HA80/Alg 30 g/L	0.4	0.68	21.1
HA40/Alg 30 g/L	0.73	1.0	26.8
HA40/Alg 30 g/L	0.6	0.86	13.2
HA40/Alg 30 g/L	0.5	0.85	16.8
HA150/Alg 25 g/L	0.5	0.59	29.2
HA150/Alg 20 g/L	0.5	0.65	15.0
HA150/Alg 15 g/L	0.5	0.65	6.8

Table 2. Parameters obtained from the fitting of the experimental datapoints of the mechanical spectra for the hyaluronan/alginate binary mixture (G_i, λ_i, η_i, eqs. 5 and 6).

Sample	ϕ_{HA}	G_i (Pa)	λ_i (s)	η_i (Pa·s)
HA150 30g/L	1	281.2	0.0041	1.2
		288.9	0.0414	11.9
		189.9	0.414	78.6
		62.1	4.14	257.1
HA150 22 g/L	1	202.0	0.0074	1.49
		165.2	0.074	12.2
		77.0	0.739	56.9
		12.7	7.388	93.7
HA150 18 g/L	1	153.2	0.005	0.8
		126.1	0.0504	6.4
		53.1	0.5041	26.8
		9.2	5.0414	46.2
HA150 15 g/L	1	91.8	0.0011	0.1
		94.2	0.0109	1.0
		64.1	0.1093	7.0
		17.3	1.0935	18.9
HA80 30 g/L	1	432.2	0.0045	1.9
		313.3	0.0451	14.1
		110.8	0.4513	49.9
		14.3	4.5513	64.7
HA40 30g/L	1	589.6	0.0006	0.4
		388.4	0.0061	2.4
		85.6	0.0615	5.3
		4.8	0.6147	3.0
HA24 30g/L	1	351.9	0.0001	0.04
		290.8	0.0012	0.3
		54.1	0.012	0.6
		2.8	0.12	0.3

Table A.2 (continued)

Sample	ϕ_{HA}	G_i (Pa)	λ_i (s)	η_i (Pa·s)
HA150/Alg 30 g/L	0.73	281.1	0.004	1.1
		179.9	0.040	7.2
		112.6	0.399	44.9
		32.1	3.986	128.0
HA150/Alg 30 g/L	0.6	325.4	0.0015	0.5
		168.1	0.0153	2.6
		112.0	0.1533	17.2
		43.3	1.5333	66.3
HA150/Alg 30 g/L	0.5	256.4	0.004	1.1
		107.2	0.041	4.4
		53.4	0.412	22.0
		11.2	4.119	46.2
HA150/Alg 30 g/L	0.4	385.5	0.0013	0.5
		96.3	0.0129	1.2
		51.7	0.1292	6.7
		15.4	1.2924	19.8
HA80/Alg 30 g/L	0.73	506.1	0.001	0.5
		295.9	0.009	2.9
		157.3	0.098	15.4
		29.6	0.976	28.9
HA80/Alg 30 g/L	0.6	325.3	0.003	1.0
		124.0	0.031	3.9
		29.6	0.313	9.3
		1.9	3.129	6.0
HA80/Alg 30 g/L	0.5	329.6	0.003	1.1
		120.4	0.032	3.9
		30.5	0.322	9.8
		2.3	3.218	7.4
HA80/Alg 30 g/L	0.4	393.2	0.0006	0.3
		172.8	0.006	1.1
		51.7	0.064	3.3
		6.9	0.644	4.5

Table A.2 (continued)

Sample	ϕ_{HA}	G_i (Pa)	λ_i (s)	η_i (Pa·s)
HA40/Alg 30 g/L	0.73	478.1	0.002	1.0
		111.3	0.021	2.3
		8.8	0.206	1.8
		0.2	2.064	0.4
HA40/Alg 30 g/L	0.6	386.9	0.002	0.9
		54.5	0.023	1.3
		3.2	0.236	0.7
		0.01	2.357	0.03
HA40/Alg 30 g/L	0.5	481.7	0.002	1.2
		66.3	0.024	1.6
		4.1	0.244	1.0
		0.07	2.436	0.2
HA150/Alg 25 g/L	0.5	287.0	0.001	0.4
		95.0	0.012	1.2
		55.7	0.122	6.8
		16.4	1.222	20.1
HA150/Alg 20 g/L	0.5	280.9	0.0008	0.2
		46.3	0.008	0.4
		33.3	0.082	2.7
		8.2	0.825	6.7

Figure 1

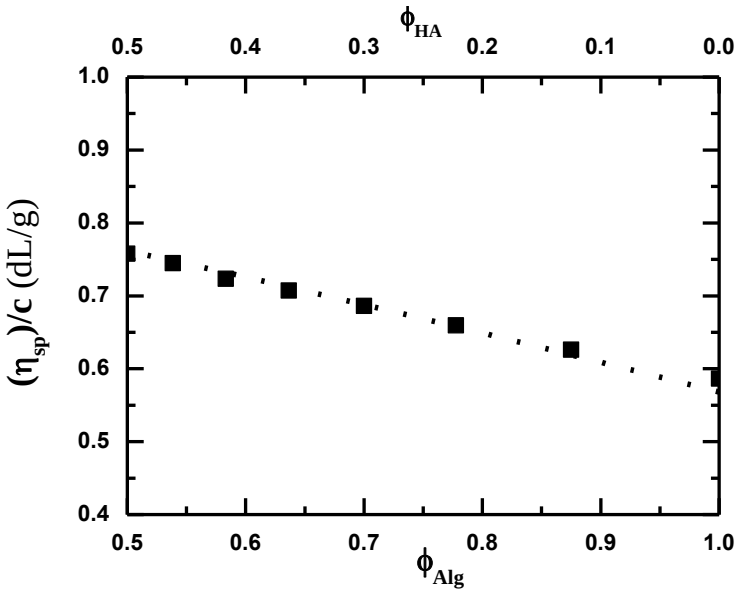

ϕ_{HA}
0.5
0.4
0.3
0.2
0.1
0.0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
$(\eta_{sp})/c$ (dL/g)
0.5
0.6
0.7
0.8
0.9
1.0
ϕ_{Alg}

Figure 2

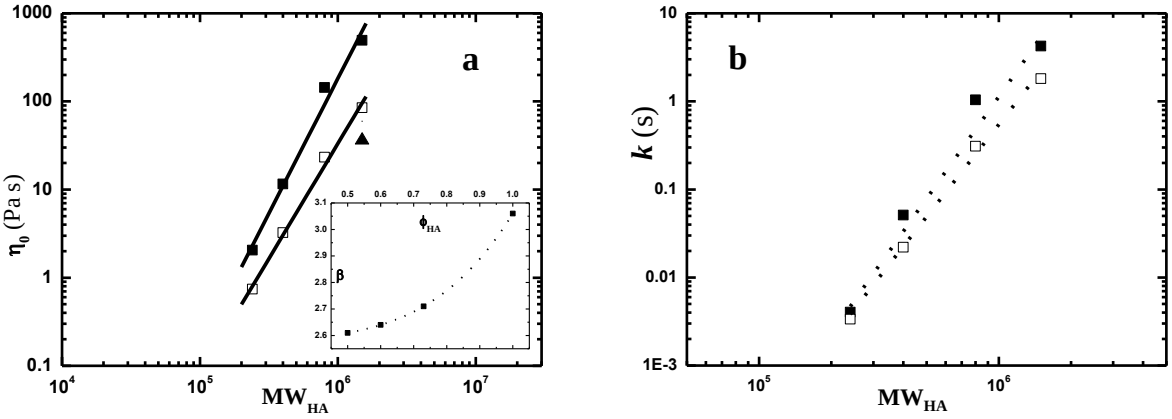


Figure 3

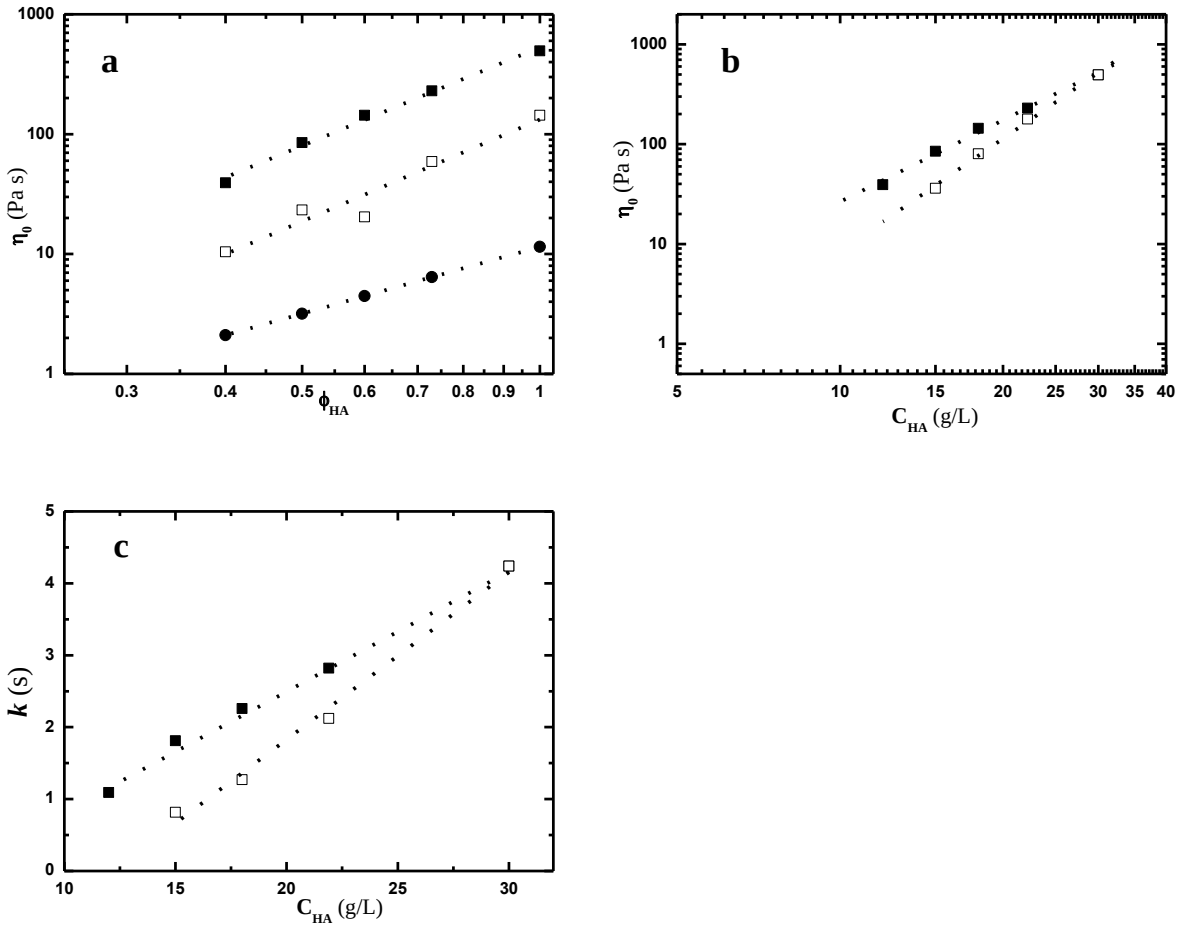


Figure 4

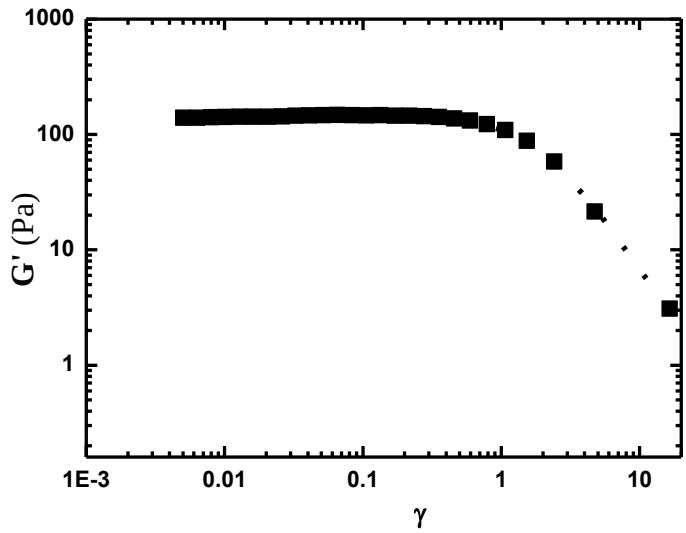


Figure 5

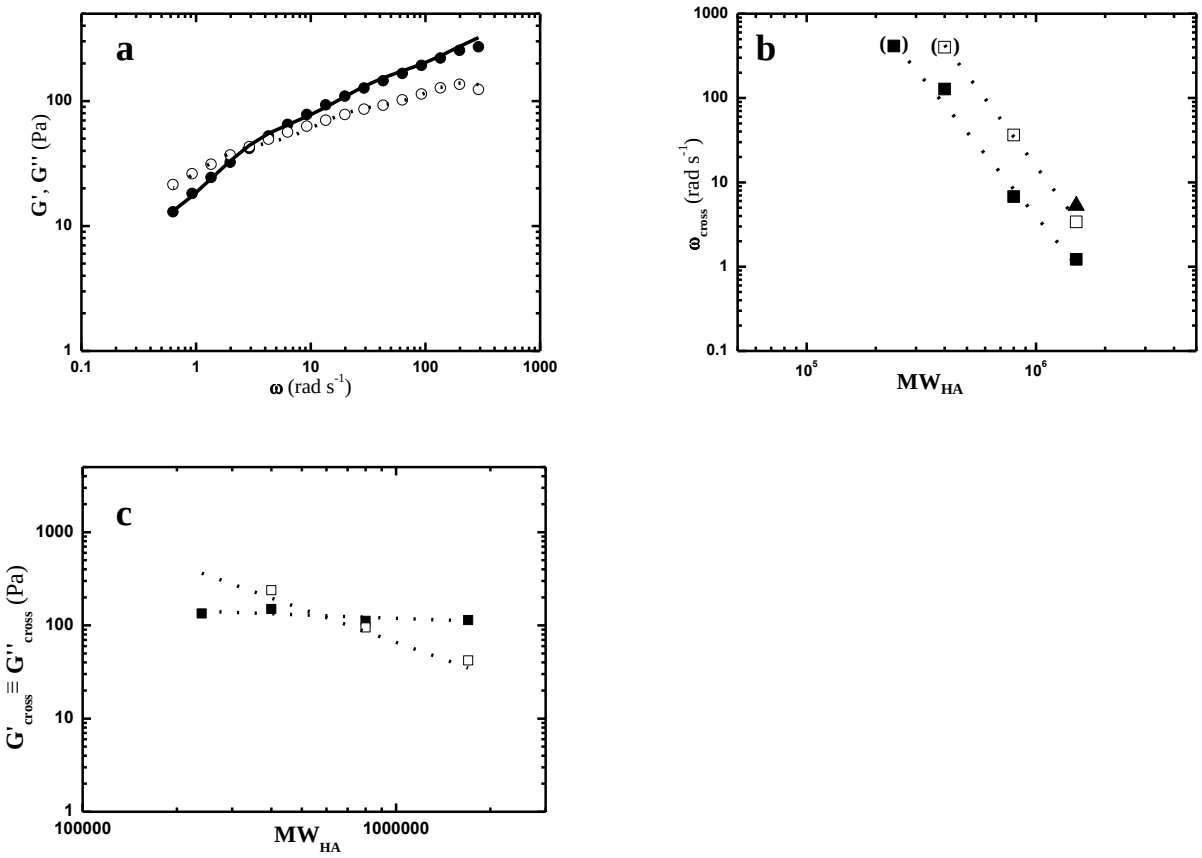
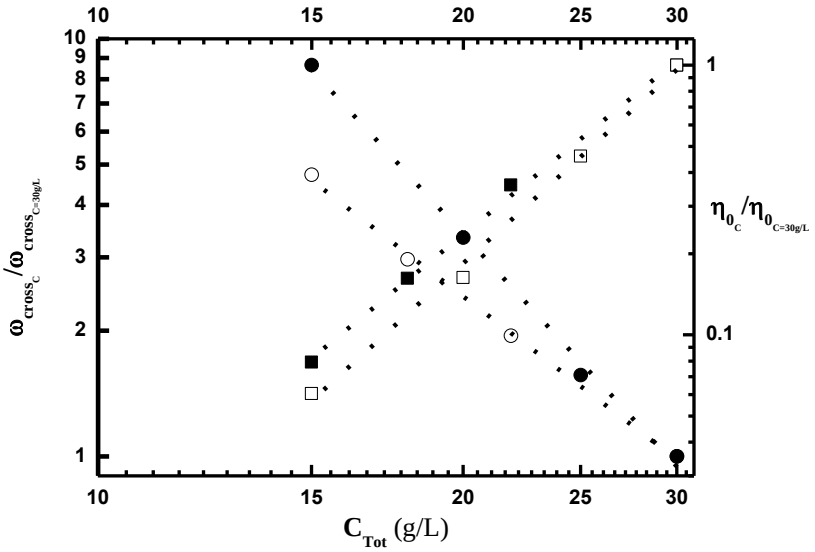


Figure 6


10
15
20
25
30
10
9
8
7
6
5
4
3
2
1
1
0.1
$\omega_{CROSS_C}/\omega_{CROSS_{C=30g/L}}$
$\eta_{0_C}/\eta_{0_{C=30g/L}}$
C_{Tot} (g/L)