

Nucleation, reorganization and disassembly of an active network from lactose-modified chitosan mimicking biological matrices

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ABSTRACT

Developing synthetic materials able to mimic micro- and macrorheological properties of natural networks opens up to novel applications and concepts in materials science. The present contribution describes an active network based on a semi-synthetic polymer, a lactitol-bearing chitosan derivative (Chitlac), and a transient inorganic cross-linker, boric acid. Due to the many and diverse anchoring points for boric acid on the flanking groups of Chitlac, the cross-links constantly break and reform in a highly dynamic fashion. The consequence is a network with unusual non-equilibrium and mechanical properties closely resembling the rheological behavior of natural three-dimensional arrangements and of cytoskeleton. Concepts like network nucleation, reorganization and disassembly are declined in terms of amount of the cross-linker, which acts as a putative motor for remodeling of the network upon application of energy. The out-of-equilibrium and non-linear behavior render the semi-synthetic system of great interest for tissue engineering and for developing *in-vitro* mimics of natural active matrices.

1. Introduction

In biological tissues, collagen, collagenous proteins, cytoskeletal network of actin and intermediate filaments, natural components of the extracellular matrix (ECM), contribute to activating/deactivating enzyme pathways and signaling cascades by means of forces exerted within a mechanoresponsive niche (Hoffman, Grashoff, & Schwartz, 2011; Orr, Helmke, Blackman, & Schwartz, 2006). In cells, in addition to passive response to stress, mechanical and dynamical properties of the cytoskeleton allow activating changes in shape and generating forces and movement in response to external signals (Deng et al., 2006).

Understanding and mimicking the interconversion into biochemical signaling of mechanical forces applied to cells, or of biologically generated forces (e.g. the contractile ones) within a meshwork, could be the hallmark to novel insight into tissue engineering (Chaudhuri, Gu, Darnell et al., 2015; Chaudhuri, Gu, Klumpers et al., 2015; Lee, Gu, Mooney, Levenston, & Chaudhuri, 2017). At present, intense research activities are carried out in the field of mechanotransduction which is becoming one of the pivotal focuses in clinical medicine and biomaterial design (Ingber, 2006). Indeed, it has been reported that cyclic strain and stress alter cytoskeleton assembly and orientation by

modulating Rho-ROCK pathway (Zhao et al., 2007). In addition, external mechanical stimuli are key mediators of cell fate both *in vitro* and *in vivo* (Mammoto & Ingber, 2009). Along this line, extended research is being conducted on natural proteins whether crosslinked or in the presence of molecular motors (Backouche, Haviv, Groswasser, & Bernheim-Groswasser, 2006; Broedersz & MacKintosh, 2011; Ideses, Sonn-Segev, Roichman, & Bernheim-Groswasser, 2013; Luan, Lieleg, Wagner, & Bausch, 2008). Ground-breaking research has also been conducted to decode the main features determining mechanical response by biological materials using synthetic polymers, although they might pose questions as to their biocompatibility and adequacy for their use in biomedical applications (Vatankhah-Varnosfaderani et al., 2017).

Here we present the peculiar role of boric acid as dynamic cross-linker in the formation, reorganization and fate of active networks obtained using Chitlac, a lactitol-bearing chitosan which has shown interesting biological properties and it is now commercially available in medical grade (Donati et al., 2005; Medelin et al., 2018; Travan et al., 2012).

Dynamic cross-linking molecules have attracted great interest for their implications and effects in active networks capable of a multi-

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scale self-organization (Ideses et al., 2013). Structure and dynamics of actin networks reticulated by actin binding proteins (ABP) have been recently reviewed and the relevance of ABP on global structure and mechanics recapitulated (Lieleg, Claessens, & Bausch, 2010). In the same work, it is underlined that the active network microstructure sets the local deformation mode and thus influence also the macroscopic network viscoelasticity in the linear and non-linear regime (Lieleg et al., 2010).

The transient nature of the interaction between the polysaccharide (Chitlac) and boric acid endows the network with the ability to reorganize and disassemble, providing in addition stress-stiffening features. Boric acid indeed displays three main abilities, *i.e.*: network-nucleator, network reorganizer and network disassembling agent. In this sense, boric acid exerts a role on a Chitlac-based network which is similar to the one of myosin II or ABP on actin (Ideses et al., 2013). Overall, the system containing boric acid and Chitlac shows all the main rheological features of the network proteins composing the cytoskeleton. A preliminary characterization of the Chitlac – boric acid system was presented in a previous work limitedly to the cases of a mainly viscous response (Cok et al., 2018). In the present contribution, the analyses of the transient system and its properties is extended to the gel-like condition.

2. Materials and methods

Lactose-modified chitosan (Chitlac or CTL)-hydrochloride form with fractions of: *N*-acetyl-glucosamine (GlcNAc; “acetylated”, A) (F_A) = 0.07; glucosamine (GlcNH₂; “deacetylated”, D) (F_D) = 0.36; lactitol-substituted D unit (*N*-alkylated GlcLac; “lactitol”, L) (F_L) = 0.57 was kindly provided by biopoLife S.r.l. (Trieste, Italy). The intrinsic viscosity, $[\eta]$, at 25 °C was 480 mL/g (Sacco, Cok, Asaro, Paoletti, & Donati, 2018) and the weight average molecular weight was $M_w^* = 770\,000$ g/mol with a PI of 2.68 (SEC-MALLS, data not shown). Residual boron in Chitlac was determined by means of a spectrophotometric method (Sacco et al., 2017). Phosphate-buffered saline (PBS), boric acid (H₃BO₃), sodium chloride, deuterium oxide, 4-chlorophenylboronic acid (pCB), sodium hydroxide (NaOH), and deuterium oxide were all purchased from Sigma Aldrich (USA). All other reagents were from Sigma-Aldrich. All reagents and chemicals were of high purity grade. Deionized (Milli-Q) water was used as solvent in all experiments.

2.1. Preparation of the Chitlac-boric acid system

Chitlac solutions were prepared starting from the hydrochloride form of the polymer, at different concentrations in PBS; the pH was adjusted to 7.4. Boric acid was added under vigorous magnetic stirring to the Chitlac solutions to avoid precipitation. Boric acid aqueous solutions were prepared in PBS and the pH was adjusted to 7.4.

2.2. Dynamic light scattering (DLS)

Dynamic light scattering measurements were made by means of a Zetasizer Nano ZS with 173° detection optics (Malvern Instruments) in PBS buffer at pH 7.4 using disposable cuvettes. Measured count rates were normalized for the attenuation factor to yield derived count rate. Measurements were performed on Chitlac alone and in the presence of boric acid at 8.8 mM at 25 °C and after a thermal curing during which the sample was measured at 25 °C, heated rapidly at 43 °C and measured at 43 °C, cooled at 25 °C and measured also at this latter temperature.

2.3. High-field NMR measurements

700 μ L of Chitlac solution (1% w/v, 90% v/v D₂O, PBS, pH = 7.4) placed in a quartz tube were titrated (70 μ L injections) using 4-

chlorophenylboronic acid (pCB) solution (20 mM, PBS, pH 7.4). After each pCB injection, the solution was vigorously shaken and allowed to equilibrate for few minutes before measurements. The ¹H-NMR, ¹³C-NMR and ¹H-¹³C-2D spectra were recorded on a Varian VNMRS (11.74 T) NMR spectrometer operating at 499.65 MHz for proton. All measurements were performed at 25 °C.

2.4. Rheological measurements

Rheological tests for the Chitlac-boric acid systems were performed under continuous shear conditions to determine the extent of the steady viscosity values in the stress (τ) range 0.1–1000 Pa, as well as under oscillatory shear conditions to determine the extension of the linear viscoelasticity regime (stress sweep tests at $\nu = 1$ Hz) and the mechanical spectrum (frequency sweep, $\tau = 1$ Pa, within the linear viscoelastic regime). The complex viscosity (η^*), the storage (G') and loss (G'') moduli were recorded in the frequency range 0.01–10 Hz. Continuous oscillatory shear tests were performed at $\nu = 1$ Hz and $\tau = 1$ Pa for 3 h (10 800 s). All tests were carried out with the controlled stress rheometer Haake Mars III operating at 25 °C. A glass bell covering the measuring device was used to improve thermal control and limit evaporation. In the case of the system composed of Chitlac and boric acid 8 mM, a short stress sweep test was recorded at 25 °C and after a thermal curing (25 °C \rightarrow 43 °C \rightarrow 25 °C). For comparison, the short stress sweep of Chitlac alone was used.

3. Results and discussion

3.1. Boron anchoring points on Chitlac

It has been reported that boric acid binds diols in Chitlac in a transient manner (Cok et al., 2018; Sacco et al., 2017). Indeed, the lactitol flanking groups in Chitlac (see Scheme S1 in Supporting information) bear a glucitol moiety to which an unmodified galactose is linked, each of which is endowed with a different binding constant towards boric acid when taken separately (Peters, 2014; Wu et al., 2013). This is addressed by high field NMR using *p*-chloro-phenylboronic acid (pCB) as a model divalent diol-binding molecule to prevent broadening of the peaks due to chain reticulation. The saturation of the binding sites in Chitlac is determined upon addition of increasing amounts of pCB (see Figs. S4 and S5 in Supporting information) and estimated to be 0.88, corresponding approximately to one boronic acid molecule per lactitol side chain.

The nature of the hydroxyl anchoring sites in Chitlac was further investigated, looking at the modifications of ¹H- and ¹³C-NMR spectra (see Supporting information) (D'Amelio et al., 2013). The schematic representation of Chitlac with the labelling of the different carbon atoms is reported in Scheme S1 of the Supporting information. Several variations in the intensity and position of the ¹H-NMR signals can be spotted in the chemical shift range from 3.3 to 4.0 ppm upon addition of pCB (Fig. 1).

Specifically, there is a decrease in the intensity of the signals assigned to hydrogens C7l, C8l, C9l and C10l upon increasing the amount of boronic acid. In addition, a shift of C11l from approximately 3.9 ppm to 4.2 ppm is noted. Thus, it appears that pCB is first involved in the interaction with the OH pair on vicinal carbons C10l and C11l of the side chain. The complexation in this site may be further stabilized by an electrostatic interaction between the negative boronate group and the positive ammonium one. Such an electrostatic stabilization has been invoked in the interaction of phenylboronic acid with glycated hemoglobin (Wu et al., 2013). Upon increasing pCB concentration, also C8l, C9l are interested by the binding. This is in line with the reported higher involvement in binding of inner CHOH groups of polyol compounds, which possess less freedom of rotation than the terminal CH₂OH groups (Dawber, Green, Dawber, & Gabrail, 1988). ¹H-NMR signals arising from the galactose moiety are not affected by the

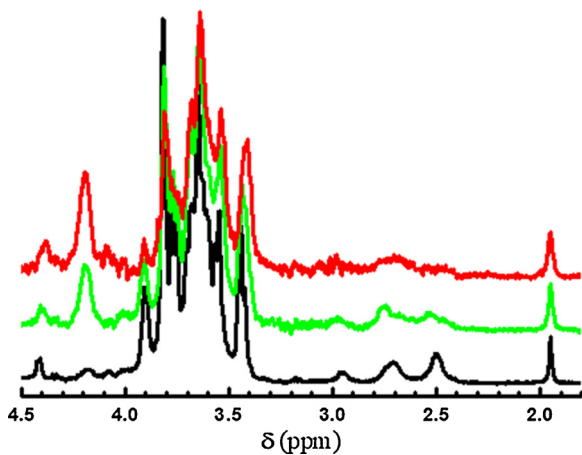


Fig. 1. Transient anchoring of the cross-linking molecule to Chitlac. ^1H -NMR spectrum of Chitlac in the absence (black line) and in the presence of pCB at a concentration of 5.6 mM (green line) and of 8.6 mM (red line) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

presence of boronic acid (Springsteen & Wang, 2002). This was supported by ^1H - ^{13}C 2D experiments (see Fig. S6 in Supporting information); the only cross-peaks clearly detectable after the addition of pCB are those belonging to the galactose part of the flanking groups of Chitlac. It therefore seems that the sole glucitol side chain offers two anchoring points for the binding of boron derivatives (Scheme 1) which, when the tetravalent boronic acid is used instead of the divalent boronic derivative, might end up in two different sites for polysaccharide reticulation. From the 1:1 stoichiometry between pCB and the lactitol side chains reported above, one could infer that boronic acid shifts continuously between the two conditions indicated by Scheme 1 which are expected to basically share the same thermodynamic features. Although these conclusions, in strict terms, are valid for pCB, it can be speculated that they are extendable also to the case of the use of boronic acid.

3.2. Chitlac-boric acid active network reorganization

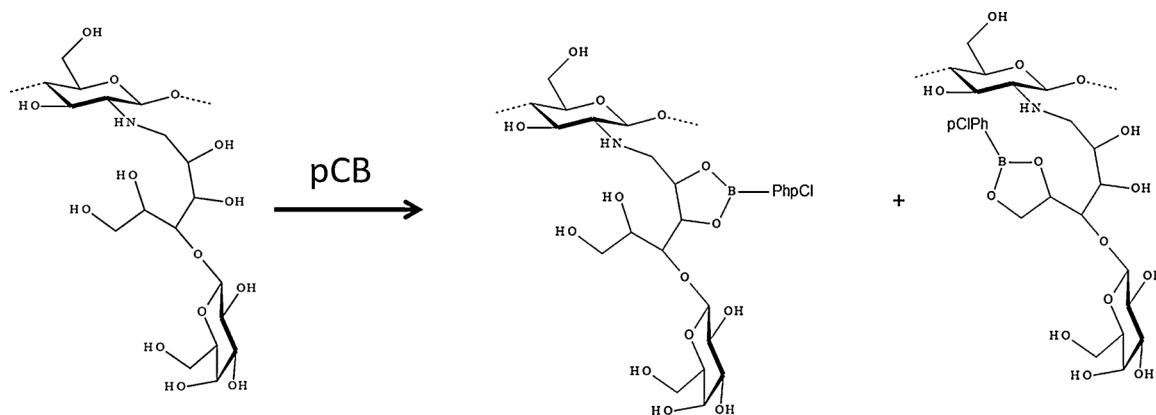
The presence of boric acid, at neutral pH, induces transient reticulations among Chitlac polymer chains (Cok et al., 2018; Sacco et al., 2017). As a consequence, a metastable network is formed with reticulation points distributed stochastically within a relative potential well. Given the plethora of hydroxyl groups to which it can anchor, boric acid is an active cross-linker, constantly breaking and reforming linkages. In this sense, Chitlac-boric acid network can be described as an out-of-equilibrium system constantly remodeling much like the

cytoskeleton in cells (Backouche et al., 2006). The reorganization of the Chitlac-boric acid network is induced by the application of energy, much like the activity of the molecular motors, such as myosin II for actin filaments, in the reorganization of natural networks is activated by ATP (Backouche et al., 2006).

Reorganization of hydrogel structures upon curing has been reported for synthetic and natural polymers and for different polymer-based biomedical materials (Cooke et al., 2018; Danielsen et al., 2018; Robert, Rossow, Hookway, Adam, & Gelfand, 2015). Temperature has been reported to cure metastable actin-based networks obtained with ABPs or polycations (Lieleg et al., 2009). In addition, reorganization upon continuous compression was noticed for Langmuir polymer films and for dynamic covalent cross-linked synthetic polymer gels showing interesting self-healing properties (Chandran et al., 2015; Liu et al., 2012).

When the energy is provided in the form of heat, the Chitlac-boric acid system undergoes a transition with a rearrangement of reticulations detected as an increase of the scattered light (Fig. 2a). In line with previous reports, the addition of boric acid (8 mM) to a solution of Chitlac brings about an increase in the derived count rate of the scattered light as a consequence of the reticulation points formed among polysaccharide chains (Sacco et al., 2017). Thermal curing raises the derived count rate even further, pointing at a molecular rearrangement characterized by an increase of the boric acid cross-links per unit volume (Scheme 2). In the absence of boric acid, the derived count rate for Chitlac is only minimally affected by the thermal cycle, likely stemming from the very small amount of residual boron from the synthesis (approximately 125 ppm). The network reorganization induced by temperature is also noticed, to some extent, from rheological measurements (Fig. S7 in Supporting information) where the thermal curing led to an increase of G' which was not noticed for the system composed of Chitlac without added boric acid. A different way to transfer energy to the system is by replacing heat with a continuous oscillatory shear (Moghimi, Jacob, Koumakis, & Petekidis, 2017). As to the present case, a continuous mechanical solicitation of Chitlac, in the presence of boric acid (8 mM), produces a 4-fold increase of both the elastic and of the viscous moduli in the time frame analyzed (Fig. S8 in Supporting information), arising from the reorganization of the cross-links. This is also confirmed by the stress sweep curves (Fig. 2b), which display a marked increase in moduli after the mechanical treatment of the sample.

A non-linear behavior in the mechanical response is found for Chitlac in the presence of boric acid (Fig. 2b and c) both prior to and after the application of the continuous oscillatory shear. In particular, both G' and G'' show a linear dependence on stress (τ) up to a critical value, followed by a marked strain-stiffening behavior. It is of striking evidence that the energy transferred through mechanical stimulus leads the Chitlac-boric acid system to a different potential energy well



Scheme 1. Schematic representation of the pCB binding sites in Chitlac.

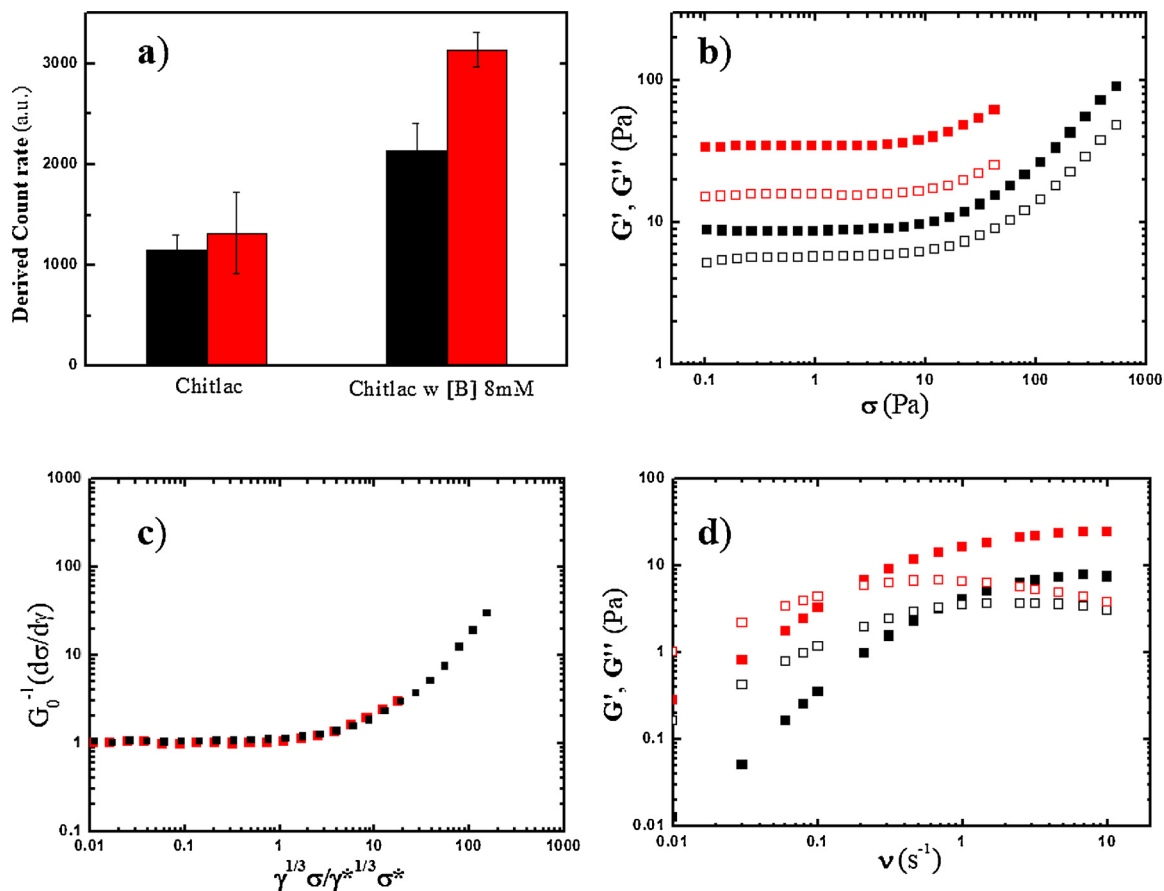
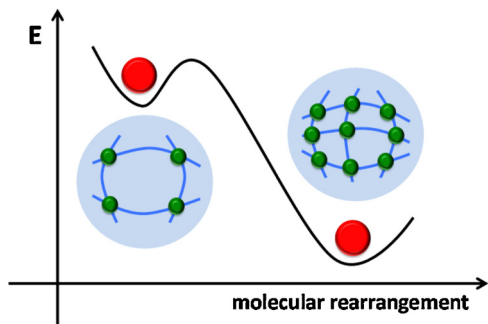


Fig. 2. Energy-induced reorganization of the active network based on Chitlac: effect of thermal and mechanical curing. a) Derived count rate for Chitlac and Chitlac in the presence of boric acid (“B”; 8 mM) at 25 °C (black) and after the thermal curing (25 °C → 43 °C → 25 °C, red). Total polysaccharide concentration = 2 g/L in PBS at pH 7.4. Data reported as mean ± SD (n = 4). Dependence of G' (filled symbols) and G'' (open symbols) on b) stress and d) frequency for Chitlac with boric acid 8 mM prior to (black) and after (red) continuous oscillatory shear. c) Reduced differential modulus for Chitlac with boric acid 8 mM prior to (black) and after (red) continuous oscillatory shear. In all cases analyzed from b) to d), the total polysaccharide concentration was 1% (w/v) in PBS at pH 7.4. SD in b, c and d is below 5% (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).



Scheme 2. Network rearrangement upon application of energy.

characterized by a higher mechanical performance (shear modulus, G , varies from 9.5 Pa to 35.3 Pa after mechanical treatment) but a reduced elasticity (maximum sample elongation, γ_{Max} , decreased from 3.2 to 2.5 after mechanical sollicitation, see Supporting information). This is also seen in Fig. 2c which shows that the normalized differential constants prior to and after the mechanically induced reorganization of the system are superimposable, with the latter having a lower chain extensibility. While this behavior has already been reported for the actin network (Yao et al., 2013), this is the first time such an effect is found for a system containing a polysaccharide and a diol-binding moiety.

The mechanical spectrum of Chitlac in the presence of boric acid prior and after the mechanical sollicitation is reported in Fig. 2d. The

unsolicited sample shows a maximum in the $G'' - \omega$ curve with a relaxation time, τ_{relax} , of 0.36 s, which can be related to the time-scale dynamics of the dissociation of the boric acid residues on the polysaccharide chains (Mayumi, Marcellan, Ducouret, Creton, & Narita, 2013).

The application of a continuous oscillatory shear to Chitlac and boric acid at 8 mM increases the stiffness of the network and extends the range of solid-like behavior. This is in contrast to the response of several materials where the application of an external stress leads to a fluidization, but it has been observed in the case of actin networks formed by the transient α -actinin-4 cross-linker (Yao et al., 2013). It is interesting to note that τ_{relax} for the mechanically treated sample resulted to be 0.45 s, only slightly different from the untreated sample (0.36 s), suggesting that the energy induced network reorganization might alter the binding-unbinding kinetics of boric acid. These aspects will be the focus of dedicated relaxation measurements that are ongoing. This result sets forth the similarity of the present network with the cytoskeleton of cells, where an effective remodeling can take place, the unbinding timescale (rates) of the cross-linking proteins from actin filaments being in the msec regime (Lieleg et al., 2010).

3.3. Chitlac-boric acid active network nucleation and disassembly

Boric acid induces the formation of reticulation points among Chitlac polymer chains, thus showing its nucleation ability. Using the two approaches reported in the Supporting information, the stress-strain curves were analyzed at different polymer concentration and in

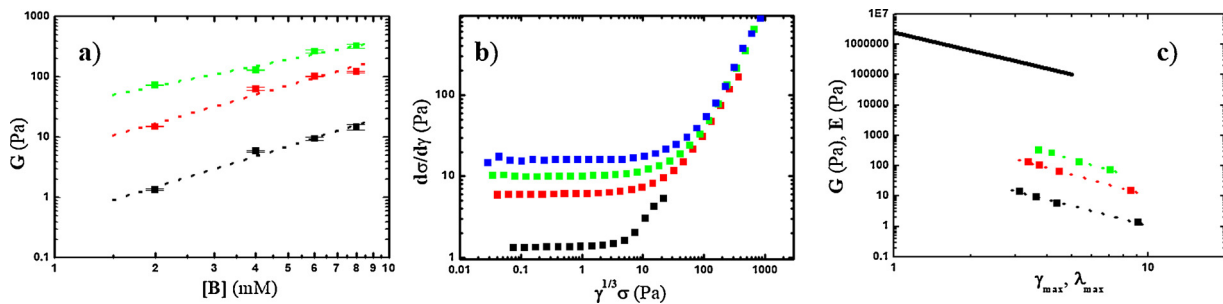


Fig. 3. Mimicking the biological properties of biological tissues with Chitlac and boric acid. a) Dependence of the shear modulus (G) on the concentration of boric acid for Chitlac at concentration of 1% (■), 2% (●) and 3% (▲) (w/v), respectively. Data reported as mean \pm s.d. of the G values obtained from eqs. 1 and 6 (see Supporting information). b) Differential modulus for Chitlac 1% (w/v) in the presence of boric acid at 2 mM (■), 4 mM (●), 6 mM (▲), 8 mM (◆). c) Dependence of the shear modulus on the maximum shear (γ_{\max}) in the presence of different amounts of boric acid for Chitlac at concentration of 1% (■), 2% (●) and 3% (▲), respectively. Dotted lines represent the fitting of the experimental data by means of a power law $G \propto \gamma_{\max}^a$, with a ranging from -2.2 to -2.4. For comparison, the dependence of the Young's modulus on the maximum strain for elastomers is also reported, taken from (Vatankhah-Varnosfaderani et al., 2017) (black solid line). SD in b and c is below 5%.

the presence of different amounts of boric acid. The first result to be underlined is that both approaches allowed to determine the shear modulus G with convergent results, as seen in Fig. 3a. In all cases considered, the increase in the amount of boric acid at the same concentration of Chitlac, leads to an increase of the shear modulus, G , and the power law $G \propto [B]^\alpha$ holds. This is easily explained considering that the increase of boric acid increases the numbers of cross-linking points (nucleation) between the Chitlac chains. However, it should also be kept in mind that boric acid could lead to the formation of polymer bundles, on one side, and to intrachain binding on the other side. The exponent α decreases upon increasing the concentration of the polysaccharide used, being equal to 1.7, 1.5 and 1.1 for Chitlac concentrations of 1%, 2% and 3% (w/v), respectively. When the same amount of boric acid in solution is used, the higher the polysaccharide concentration the higher the shear modulus due to the shifting of the transient cross-linker equilibrium towards the interchain bound state. In addition, at given boric acid concentration, approximately the following scaling law was found: $G \propto C_{\text{Chitlac}}^{2.5}$. These values are in good agreement with the predictions expected for a composite system containing point-like reticulations and polymer bundles under affine deformation. It should also be noted that the G values obtained in the present case are quite similar to those of the actin-ABP system for the same composite network arrangement (Lieleg et al., 2010).

The stress-strain relationship was studied for the Chitlac samples at a total concentration of 1%, 2% and 3% (w/v), respectively, in the presence of different boric acid concentration. Fig. 3b reports, as an example, the differential modulus for Chitlac at a concentration of 1% (w/v) for increasing concentrations of boric acid (additional data at higher values of the concentration of Chitlac are reported in Fig. S9 in the Supporting information). There is a linear extended part of the curve followed by an increase of the differential modulus, stemming from the non-linear stress-stiffening behavior, which might be correlated with the presence of polymer bundles within the composite chitlac-boric acid system. The analysis of the non-linear stress-strain allowed for calculating the critical strain at which a deviation from linearity is noticed, *i.e.* γ^* , and exploring its dependence from the concentration of boric acid (Fig. S10 in Supporting information). Regardless of the total polysaccharide concentration, the critical strain shows a rather good scaling law with the molar ratio between boric acid and Chitlac repeating units ($[B]/[\text{Chitlac}]_{\text{ru}}$) over a twenty-fold range of the ratio, at least in the $[B]/[\text{Chitlac}]_{\text{ru}}$ range between 0.02–0.4. This seems to indicate that the sole parameter influencing the deviation from the linearity is the total amount of reticulating agent *per* number of reticulation sites within the chain network, but also that a significant fraction of the reticulating agents are ineffective as to the dramatic onset of non-linearity.

The shear modulus showed a power-law dependence on the

maximum elongation network, γ_{\max} , with a coefficient ranging between -2.2 and -2.4, thus in substantial agreement with the theoretical expectations (*i.e.* -2) (Fig. 3c). For the sake of comparison, Fig. 3c reports also the $E - \lambda_{\max}$ relation expected for elastomers (Vatankhah-Varnosfaderani et al., 2017). Although the conventional decrease of G with the maximum strain is maintained also in the present case, the experimental data span over an area of the graph characterized by both a lower mechanical performance and a much higher maximum elongation of the network. The latter can be traced back to the higher flexibility of the Chitlac chain with respect to structural proteins such as collagen. It is of no surprise that the general trend shown by the polysaccharide is similar to that of synthetic bottlebrush polymers (Vatankhah-Varnosfaderani et al., 2017). The values reached for γ_{\max} are quite high (they extend almost up to the value of 10), showing that the material based on Chitlac and boric acid can be described as “super-soft”, opening up potential applications in the field of biomaterials for soft tissue engineering. In the present case, it is conceivable that a contribution to softness arises from the transient features of the cross-linking agent. The peculiar non-linear mechanical behavior of the system composed by chitlac and boric acid at 8 mM is further analyzed and it compares surprisingly well with that shown by two other reticulated biopolymers of the natural extracellular matrix, *i.e.* collagen and neurofilaments, as reported by Storm et al. (Fig. S11 in the Supporting information) (Storm, Pastore, MacKintosh, Lubensky, & Janmey, 2005).

The disassembly features of boric acid at high concentrations is evidenced from the decrease of G in both stress and frequency sweep upon increasing the concentration of the inorganic component up to 20 mM (Fig. S12 in Supporting information), in agreement with previous reports in dilute solutions (Sacco et al., 2017). Focusing on the stress sweep curves, one should note that the increase of the boric acid to 20 mM, besides lowering the storage modulus, results in deviation from linearity for lower τ values. This could be traced back to the fact that boric acid disassembles more easily the single cross-linking points leaving a higher fraction of bundles in the composite network.

The effect of boric acid at different concentrations on steady flow properties of Chitlac has also been explored (see Fig. S2a in Supporting information). In view of the response of Chitlac under shear, one could speculate that the molecular basis of the thickening resides in the shear-induced reorganization of (transiently) bound boric acid with the formation of additional reticulation points leading to increasing viscosity. The higher is the initial number of cross-links as indicated by η_0 (*i.e.* the higher the initial concentration of boric acid), the more efficient is the shear induced formation of novel additional cross-links and the sudden uprise of the $\eta_0 - \dot{\gamma}$ curve (Fig. S2b). This parallels the effect of reorganization induced by the application of energy (thermal or mechanical) to the system as reported in Fig. 2. A similar effect was seen in

the case of hydrophobically modified urethane-ethoxylate where the application of stress induced the modification in the number of mechanically active chains among the micellar cores (Tam, Jenkins, Winnik, & Bassett, 1998).

4. Conclusions

The treatment of Chitlac with boric acid results in a very peculiar system, which displays several similarities with networks composed of natural polymers (proteins). It is interesting to note that the inorganic component can act like a motor for the formation of the network, thus providing nucleation, reorganization and disassembly depending on curing and concentration. The possibility of using these tunable semi-synthetic networks opens up for novel potential interpretations for the mechanical behavior of the natural tissues. The elucidation of the specific aspects endowing Chitlac with these peculiar properties is still ongoing; nevertheless, the peculiar biological and rheological features of the polysaccharide in the presence of diol binding and cross-linking molecules might pave the way to its use for novel and advanced applications in the field of mechanoresponding biomaterials for soft tissues engineering.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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