Biorheology 48 (2011) 263–275
DOI 10.3233/BIR-2011-0596
IOS Press

A new viscosupplement based on partially hydrophobic hyaluronic acid: A comparative study

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Received 19 September 2011
Accepted in revised form 9 November 2011

Abstract. A novel partially hydrophobized derivative of hyaluronic acid (HYADD®4), containing a low number of C16 side-chains per polysaccharide backbone, provides injectable hydrogels stabilized by side-chain hydrophobic interactions. The rheological properties of Hymovis®, a physical hydrogel based on the hyaluronic acid derivative HYADD®4, were evaluated using as reference a solution of the parent natural polysaccharide, hyaluronic acid. The rheological measurements were performed both in flow and oscillation regimes at the physiological frequency values of the knee, typically spanning the range from 0.5 Hz (walking frequency) to 3 Hz (running frequency). Moreover, the viscoelastic features of Hymovis® were compared with the market-available viscosupplementation products in view of its use in joint diseases.

The different behavior of the investigated materials in crossover frequency measurements and in structure recovery experiments can be explained on the basis of the structural and dynamic properties of the polymeric systems.

Keywords: Hyaluronic acid, rheology, hydrogels, elastic modulus, viscosupplement, osteoarthritis

1. Introduction

Osteoarthritis is a major pathology in modern western societies, particularly affecting the elderly, who account for an increasing share of today's population. The disease is generally divided into primary osteoarthritis, related to age and genetic factors, and secondary osteoarthritis, related to different kinds of joint injuries, such as trauma, surgery and infections [2]. The highly repetitive loading or mechanical stress involved in sports and physically demanding activities can go towards increasing the risk of joint injuries. Injury-induced joint degeneration causes joint pain and dysfunction, the major symptoms of post-traumatic osteoarthritis [7]. The management of primary and secondary osteoarthritis is no minor social issue involving high costs for national health systems. The symptoms of osteoarthritis involve the articular junctions of knees, hips, distal phalanges and intervertebral joints and its end-stage is characterized by deterioration and detachment of the weight bearing joints. Usual treatments of this degenerative

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Table 1
Summary of the main features of the HA-based viscosupplements considered in this study

Trade name	Molecular weight (kDa)	HA source	Network type	Concentration (mg/ml)
Hyalgan®	500–700	Rooster combs	Physical	10
Hyalubrix®	1500	Bacterial	Physical	15
Synvisc®	6000–7000[a]	Bacterial	Hylan A (high molecular wt. HA) and Hylan B (cross-linked HA) ratio 80:20	8
Durolane®	1000[b]	Bacterial	Chemical cross-linked HA	20
Monovisc®	High	Bacterial	Slightly cross-linked HA	20

[a]Molecular weight of hylan A; [b]apparent molecular weight.

joint disease address pain relief and inflammation, using both physical therapy and/or anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. The intra-articular management of osteoarthritis is currently based on the injection of corticosteroids or hyaluronans, the latter being used as a viscosupplement for the deteriorated synovial fluid in the inflamed joint. As initially pointed out by Balazs and Denlinger [4] the main function of hyaluronic acid (HA)-based viscosupplementation is to restore the rheological features of synovial fluid. This application requires the formation of chain clusters causing a large deviation from Newtonian behavior, as is actually observed in most hyaluronan formulations. A way to accomplish such conditions without increasing the polymer concentration or its molecular weight is the partial modification of HA chains by grafting moieties that promote a reversible chain aggregation.

Today, a fairly large family of hyaluronan-based products with structural as well as functional differences is commercially available as viscosupplementing devices for the symptomatic management of knee and hip osteoarthritis. A summary of the main available information on the most relevant marketed viscosupplements is given in Table 1 (adapted from [18]). A quick review of the bibliography highlights the paucity of structural information at the supramolecular level provided to the end-user of these materials. With the available data we tried to rationalize the behavior of the marketed products as compared with Hymovis® (CE marked medical device, Fidia Farmaceutici SpA, Italy), a new hydrogel obtained from the HA derivative HYADD®4, for which a chemical and physical characterization has already been described in literature [12].

The understanding of the action of HA and HA derivatives injected in the osteoarthritic joint is somewhat complicated by the multifunctional biochemical role played by this polysaccharide in the extracellular matrix (ECM). The well documented HA recognition for cell receptor proteins as CD44 [23,24], RHAMM [22] and ICAM-1 [17] occurs in the ECM and shows that HA supports a variegated set of functions going beyond mere joint lubrication and shock absorption. Several studies have investigated the influence of HA molecular weight (MW) on its efficacy as an intra-articular treatment for osteoarthritis [25]. From a mechanical point of view, increasing the mean MW of hyaluronan clearly increases the elastoviscous properties of the solution or hydrogel, thus enhancing its shock absorption capacity. Any application of HA and HA derivatives as viscosupplements is based on their non-Newtonian behavior obtained by increasing either the polymer concentration or the polymer molecular weight. It is known [3] that an increase of the elastic component in the mechanical spectra of a HA preparation is directly linked to an increase of the molecular weight of the polymer. Nevertheless, the mechanism of hyaluronan action is likely to be based on several biological activities mediated through receptor-based processes.

Although there is no solid evidence of a difference between hyaluronan products in clinical efficacy as for pain reduction, it should be considered that preclinical studies performed on animal models of osteoarthritis indicate that low- to mid-MW (500–1000 kDa) hyaluronans may have a higher disease modification potential. Furthermore, in the choice of the preferred product for injection, it should perhaps be considered that a potential association between chemically cross-linked polymer products and an increased risk of adverse events has been reported [25].

On the other hand, several comparative clinical studies involving the commercial products listed in Table 1 versus placebo were carried out in order to assess a relationship between structural features and clinical effectiveness in the intra-articular treatment of the osteoarthritic knee [6,13,16]. The picture emerging from these studies is complex and does not allow for unambiguous conclusions. In most cases, the HA products proved efficacious in their relief of pain linked to the pathology and showed therapeutic efficacy and safety when tested against placebo [26].

In this scenario, a new HA-based product, Hymovis®, was essayed in comparison with the most used viscosupplements with regard to several rheological features. The hydrogel Hymovis® consists of an 8 mg/ml formulation in phosphate buffer solution (PBS) of HYADD®4, the novel HA derivative where 2–3% of the disaccharide repeating units have been modified by introducing hexadecylamine as side-chain on the CDI-activated carboxyl group of the glucuronic unit (see Fig. 1).

HYADD®4 sterile aqueous formulations are stable and transparent hydrogels whose relaxation processes occurring within the network have been studied by dynamic light scattering (DLS), fluorescence recovery after photobleaching (FRAP) and molecular dynamic simulations (MD) [12]. On the basis of such studies, these physical hydrogels have been described as networks with an average mesh size ranging from 25 to 13 nm when concentration is varied from 3 to 8 mg/ml. The structure is stabilized by the hydrophobic interactions introduced by the alkyl side-chains grafted onto the HA backbone and the characteristic relaxation time has been estimated in the range of hundreds of microseconds. This timescale is related to several collective processes involving the reptation of polysaccharide chains within the hydrogel as well as the reversible association of the hydrophobic side-chains causing a slowing down of the main chain diffusion, usually termed sticky reptation. MD simulations were useful in the identification of the alkyl side-chains as the major cause of the slower reptation of the chains with a life-time of several tens of nanoseconds.

The characterization of HYADD®4 [12] hydrogels is preliminary to the comparative analysis of the behavior in flow and oscillation regimes of Hymovis®, Synvisc®, Monovisc®, Durolane® and Hyalubrix®. In this paper we report on the main features of Hymovis® with specific reference to the frequencies of walking and running, i.e., 0.5 and 3 Hz, respectively, in comparison with the above mentioned marketed viscosupplements. As these materials are used for the intra-articular treatment of knee osteoarthritis by injecting the viscosupplement through 18- to 22-gauge needles, we also investigated their response to shear stress as well as their shear thinning properties. Moreover, we also considered the ability of the hydrogels to recover their structure after mechanical shock. The comparison with other viscosupplements will help provide a rationale in the physicians choice.

2. Materials

Hyaluronic acid tetrabutylammonium salt (HATBA), obtained from the hyaluronic acid sodium salt of bacterial source ($M_W = 7 \times 10^5$ Da) after resin-catalyzed ion exchange, was used as a freeze-dried powder for the synthesis of the hexadecyl derivative of HA (HYADD®4, see Section 3). Methanesul-

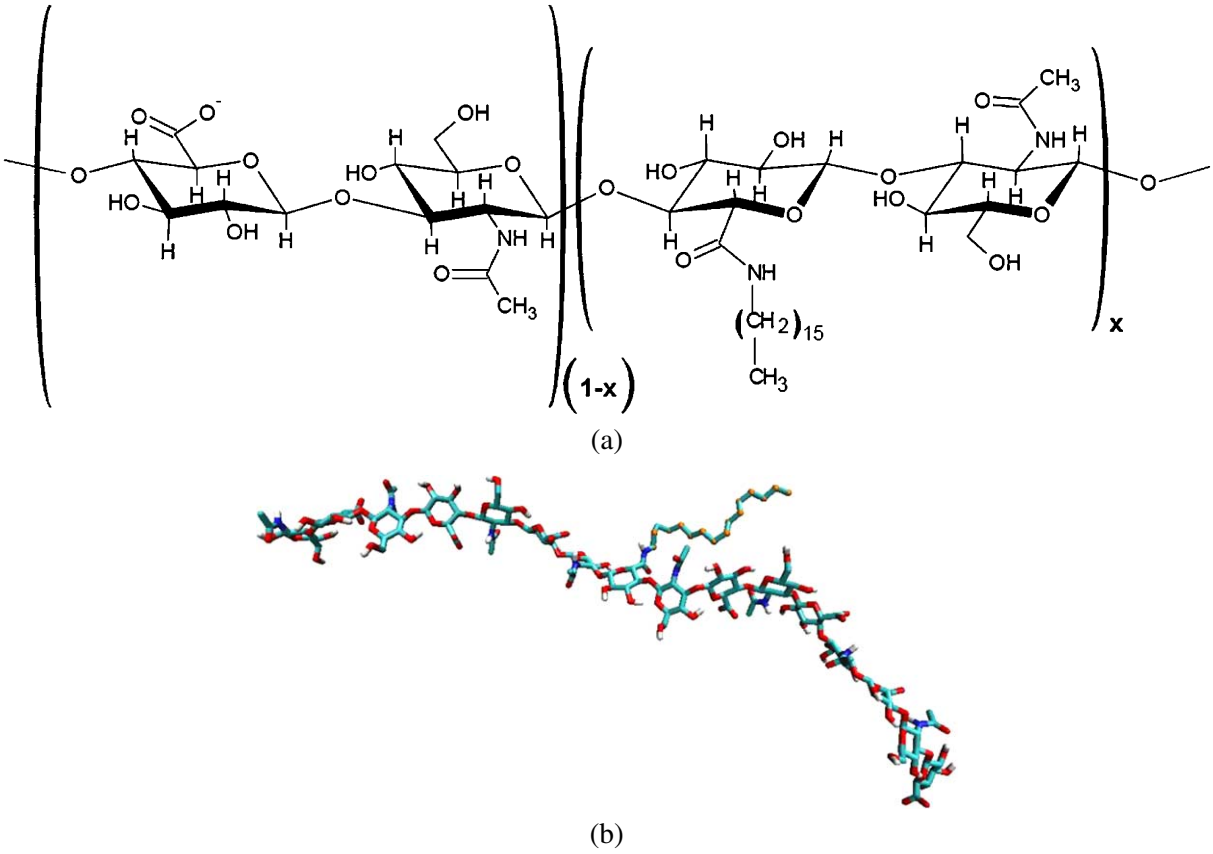

Fig. 1. Chemical structure (a) and graphical representation (b) of HYADD®4. The degree of substitution of hexadecyl chains, x, ranges from 2 to 3% (mol/mol of repeating unit). (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/BIR-2011-0596.)

fonic acid, 1,1′-carbonyldiimidazole, hexadecylamine, o-phthaldialdehyde were supplied by Sigma and used without further purification. Dimethylsulfoxide (DMSO), methanol (MeOH) and ethanol (EtOH), phosphate-buffered solution (PBS) were Carlo Erba, RPE grade products. MilliQ water was used throughout this study. Hyalubrix® was a kind gift by FIDIA Farmaceutici SpA; Monovisc®, Synvisc®, Durolane® were purchased from VitaResearch, Genzyme and Keryos, respectively. Commercial samples were used as provided by the suppliers. Their chemical and physical characteristics are based on the description provided by the suppliers and briefly summarized in Table 1.

3. Methods

3.1. Synthesis of HYADD®4

The synthesis of the hexadecyl derivative of hyaluronic acid, HYADD®4, has been reported elsewhere [12]. Typically, 1 g of HATBA was dissolved at room temperature in 100 ml of DMSO, then a catalytic amount of methanesulfonic acid and 26 mg of 1,1′-carbonyldiimidazole were added slowly. This activation phase was performed at room temperature while stirring for 60 min. 272 mg of hexadecylamine

was then added and the amidation reaction was performed at 42°C overnight while stirring. A saturated aqueous NaCl solution was added and the product was recovered by pouring 200 ml of EtOH into the solution and filtering the precipitate. The powder was then washed several times using a mixture of EtOH and water and finally dried in an oven. Following this procedure, 600 mg of hexadecyl substituted HA was obtained.

The degree of substitution was determined by carrying out an HPLC/fluorimetry analysis of total hexadecylamine (HPLC system Perkin Elmer series 200, detector Luminescent Spectr. LS30, column Versapack C18 10µ 25 cm, mobile phase MeOH/H_2O 95/5, flow rate 1.3 ml/min) after alkaline hydrolysis (NaOH 2 M at 70°C for 3 h while stirring), neutralization, extraction with MeOH and derivatization of the amine with o-phthaldialdehyde (OPA). The fluorimetric detector was set at an excitation wavelength of 330 nm and emission wavelength of 440 nm.

The absence of free hexadecylamine as an indicator of purity in the reaction was confirmed by resuspending a sample of polymer in MeOH for 30 min, in order to extract non-bound amine. The collected supernatant was then treated with OPA for amine derivatization and the hexadecylamine-OPA product was quantified using the HPLC/fluorimetry method, as described above.

The HYADD®4 sample used for this study showed a degree of substitution of 2.4% (mol/mol repeating unit). The chemical structure was further investigated by FT-IR, mono-dimensional and two-dimensional ^{1}H- and ^{13}C-NMR spectroscopy techniques (data not shown), which confirmed the presence of the amidic bond between the HA backbone and hexadecylamine and the absence of covalent cross-links. Furthermore, dilute aqueous solutions of the sodium hyaluronate used as substrate and the corresponding HYADD®4 polymer in 0.1 M NaCl were analyzed by means of gel permeation chromatography using a refractive index detection. By comparing the eluted peaks it was possible to assess that the polysaccharide molecular weight remains nearly unaltered during the side-chain grafting reaction.

3.2. HYADD®4 hydrogels and Hymovis® preparation

HYADD®4 dispersions were prepared by dissolving the hexadecyl HA derivative in phosphate buffered saline (PBS, pH 6.9) at concentrations ranging between 3 and 8 mg/ml. The polymer was dispersed in PBS by overnight stirring and the milky dispersions obtained were then autoclaved for 10 min at 121°C in a stoppered cylindrical glass vial. The resulting hydrogels cooled at room temperature were visibly clear, without any opacity due to macroscopic inhomogeneities. Hymovis® is the 8 mg/ml HYADD®4 hydrogel.

3.3. Rheology

An AR 2000 Advanced stress-controlled rheometer (TA Instruments) was used to study the mechanical response of the samples in oscillatory shear and in steady shear experiments. In these studies a 20-mm parallel plate geometry with a solvent trap was used. A gap of 500 µm was sufficient to distribute the sample evenly over the entire surface of the plate. The rheometer is equipped with a Peltier plate to regulate the sample temperature to 25°C. In all measurements, an initial strain sweep with an oscillatory shear strain of increasing amplitude, γ, at a constant frequency of $\omega = 1$ Hz was applied to determine the region of linear response of the sample. On this basis, a value of applied strain within the linear regime was used in subsequent frequency sweeps. In addition, steady shear flow experiments were carried out for shear rates up to 10^3 s^{-1}. Continuous flow measurements were carried out leaving the

sample at rest for 10 min on the rheometer plate. The shear stress curve was measured as a function of a shear rate ramp from 0 to 5 s^{-1} in 5 min in linear mode, followed by a decreasing ramp to zero shear rate in 5 min. Increasing the time between sampling intervals from 1 point/s to 1 point/10 s did not change the features of the curve. Also, we verified that changing the equilibration time after shearing from 10 to 20 min did not change the behavior of the sample.

The self-healing process, i.e., recovery of the mechanical properties after a shock, was investigated as a function of time by oscillatory measurements after destruction of the gel network. The structure recovery process of the hydrogel was assessed as follows. At first, a low-amplitude oscillation ($\gamma = 1\%$, $\omega = 1$ Hz) was applied for 2.5 min to measure the equilibrium gel strength (phase 1). A high shear rate ($\dot{\gamma} = 3000\ s^{-1}$), corresponding to 150 rad $\cdot\ s^{-1}$, was then applied to the gel for 1 min in order to unstructure it (phase 2). Finally, a $\gamma = 1\%$ amplitude oscillation at 1 Hz was applied for 5 min to measure the G' recovery (phase 3). The whole cycle was repeated 6 times.

The viscoelastic behavior of HYADD®4 hydrogels was preliminarily investigated at room temperature and at 37°C without recording any significant difference. Although the reference temperature for viscosupplementing materials is 37°C, we decided to carry out most of the experiments at room temperature for the sake of comparison with other studies on the viscoelasticity of different alkylated derivatives of hyaluronan [11,12].

4. Results and discussion

HYADD®4 hydrogels are hyaluronan-based biomaterials stabilized by the hydrophobic interactions of the hexadecyl side-chains grafted on the backbone at a low extent, i.e., 2 to 3 every 100 HA repeating units. The network is physically connected and the interactions taking place therein are transient, giving rise to an average dynamic structure described recently in the literature [12].

The parameters characterizing the dynamics of these hydrogels were previously obtained by dynamic light scattering (DLS). Table 2 gives a summary of the values of the characteristic hydrogel lengths, δ, and relaxation times, τ, for HYADD®4 at different concentration and for Hymovis®. The correlation length δ, i.e., the network pore size at the nanoscale level, decreases as concentration of the polymer increases. Values reported in Table 2 highlight the finding that the structural dynamic behavior of Hymovis® is homogeneous with the other HYADD®4 hydrogels at lower polymer concentrations. The network dynamics probed in the DLS experiment, with correlation times ranging from some hundreds to tens of microseconds, can be associated with the motion of chain segments between cross-links around the equilibrium position.

Table 2
Characteristic hydrogel lengths, δ, and relaxation times, τ, of HYADD®4 hydrogels and Hymovis® from dynamic light scattering experiments at $\theta = 90°$

C (w/v)	δ (nm)	τ (μs)
0.3%	24 ± 2	210 ± 3
0.4%	18 ± 2	190 ± 4
0.5%	16 ± 1	130 ± 3
0.6%	14 ± 1	110 ± 2
0.7%	15 ± 1	100 ± 2
Hymovis®	13 ± 1	85 ± 2

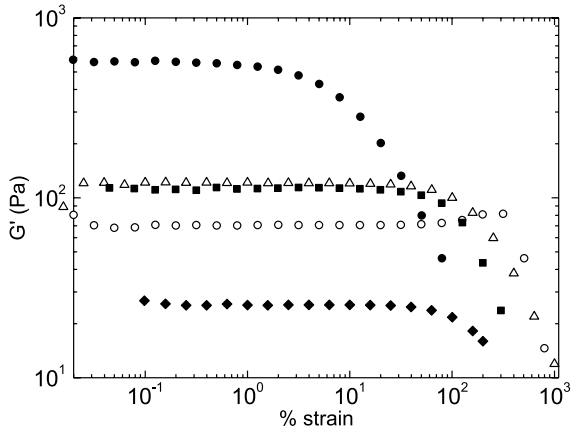

Fig. 2. Storage modulus as a function of strain at $\omega = 1$ Hz. Durolane® (●), Monovisc® (△), Synvisc® (■), Hymovis® (○), Hyalubrix® (◆).

Along with this micro-structural information, we probed the macroscopic viscoelastic behavior of Hymovis® in comparison with other marketed viscosupplements based on hyaluronic acid, as Synvisc®, Monovisc®, Durolane® and Hyalubrix®. It is important to underline that the viscoelastic properties of the different viscosupplements were deliberately investigated on the commercially available samples in order to monitor their behavior in the conditions of use. These samples were tested with a strain-sweep at constant frequency of 1 Hz. All products showed the independence of the storage modulus, G' (see Fig. 2), from strain in a broad region, ranging from 0.1 to 50%.

It should be pointed out that for Durolane® the independence of G' from the strain is observed in a much more limited region. This result is a clear feature of the prevailing physical nature of the majority of the investigated networks, including Synvisc®. On the contrary Durolane® behaves as a real chemically cross-linked hydrogel [10,15]. Consistently, the high value of G' of Durolane® was an additional indication of the enhanced solid-like character of this viscosupplement due to the presence of covalent cross-links. Among the physical hydrogels, Hymovis® provides a linear viscoelastic regime extending for several decades of strain values. This feature can be regarded as a useful advantage for the use of this hydrogel as synovial fluid substitute in joints subjected to high-strain activities.

In Fig. 3 the viscoelastic behavior of Hymovis® is compared with the parent polymer HA at 8 mg/ml. A marked increase in elastic and viscous moduli is evident for Hymovis®. Moreover, hyaluronic acid at the same concentration displays a solution behavior, i.e., $G'' > G'$ in the investigated frequency range.

This viscoelastic behavior does not change when Hymovis® is tested at higher deformations than 1%, consistent with those occurring in the articular joints. Frequency sweeps at a deformation of 1 and 30% were almost coincident.

After the assessment of the linear strain region of the storage and loss moduli, these viscosupplements were examined in frequency sweeps. In Fig. 4(a) and (b), the storage and loss moduli of Hymovis® and the commercial hydrogels are reported as a function of frequency. The dashed region indicates the frequency of a knee joint corresponding to a walk (0.5 Hz, lower limit) and to a run (3 Hz, higher limit), respectively.

Only Hymovis®, Durolane® and Synvisc® display viscoelastic properties typical of a gel, i.e., $G' > G''$, in the frequency region of interest (Fig. 4(a)), whereas Hyalubrix® and Monovisc® display similar values of G' and G'' (Fig. 4(b)). This is a consequence of the fact that the crossover point for Hymovis®,

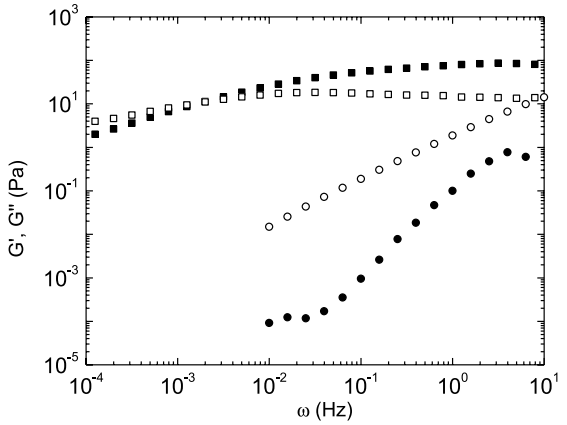

Fig. 3. Storage, G' (filled symbols) and loss, G'' (empty symbols) moduli for Hymovis® (■ and □) and HA (● and ○) as a function of the frequency (Hz).

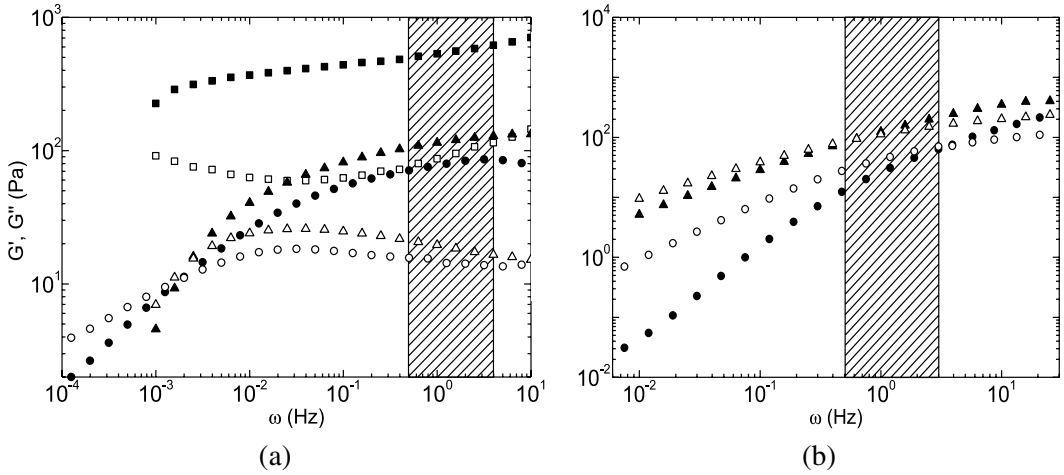

Fig. 4. (a) Storage G' (full symbols) and loss G'' (empty symbols) moduli for Hymovis® (●, ○), Synvisc® (▲, △) and Durolane® (■, □); (b) as in (a) moduli for Hyalubrix® (●, ○) and Monovisc® (▲, △). Shaded areas indicate the frequency range of the knee joint for walking (lower frequency) and running (higher frequency).

Durolane® and Synvisc®, i.e., the frequency ω_c at which $G' = G''$, falls well below the range of frequency at which the knee usually works [5].

The higher resistance of gel structures to OH radicals in the inflamed environment of a chronic osteoarthritic joint had already been reported in literature [1] for cross-linked Hylan (see Table 1) and non-cross-linked hyaluronate. This feature is an advantage as it limits the number of intra-articular administrations for synovial fluid replacement.

Interestingly, a map of the crossover points of the examined viscosupplements clearly reveals their different rheological behavior (see Fig. 5), allowing the investigated materials to be sorted into two classes: gel-like materials, with lower ω_c values, and solution-like materials, placed in the map region with higher ω_c.

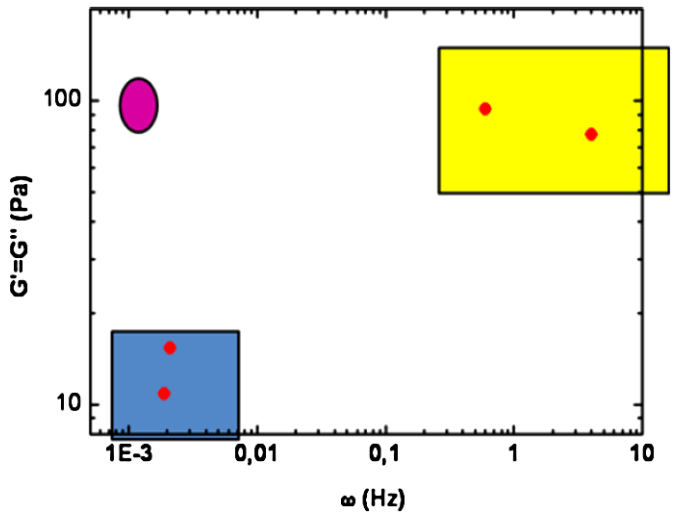

Fig. 5. Map of the crossover points for different viscosupplements. Physical networks belong to the blue region (Hymovis® and Synvisc®). Solutions are positioned in the yellow area (Hyalubrix® and Monovisc®). Violet spot indicates the approximate position of Durolane® crossover point. (The colors are visible in the online version of the article; http://dx.doi.org/10.3233/BIR-2011-0596.)

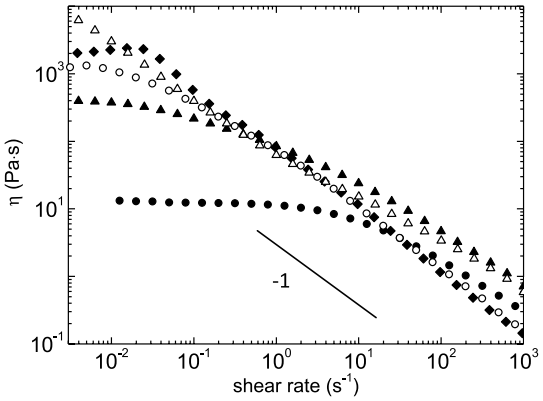

Fig. 6. Shear thinning behavior of Hymovis® (◆), Durolane® (△), Synvisc® (○), Monovisc® (▲) and Hyalubrix (●). A line with the slope −1 (limiting behavior of a gel) is also reported as reference.

The Durolane crossover frequency was too low to be detected with conventional rheometers as well as the values of storage and loss moduli. The broad violet spot on the map indicates a gross evaluation of Durolane crossover point. Regarding the general viscoelastic profile of the compared products, it should be stressed that Hymovis® is the only non-chemically-cross-linked HA derivative showing gel-like behavior and a marked predominance of the elastic character.

A major source of degradation of the hydrogel is the shear stress applied during the intra-articular injection. The synovial fluid substitutes studied in this work show a shear thinning behavior in steady shear flow experiments, see Fig. 6. In the case of Hymovis®, the plateau at low shear rates was studied by means of a "stress vs. shear rate" test in continuous flow, as reported in Fig. 7. The trend of the

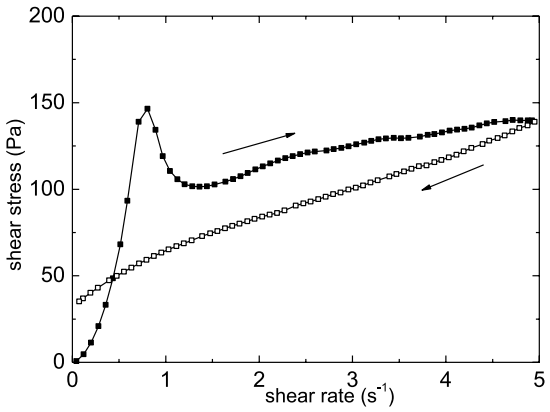

Fig. 7. Hymovis® shear stress as a function of the shear rate. Total experiment duration 10 min: 5 min for increasing and 5 min for decreasing shear rates, respectively.

shear stress as a function of increasing deformation shows a critical stress of 140 Pa for a deformation rate of about 0.4 s^{-1}. This is clear evidence of the presence of domains with an organized structure capable of contrasting the deformation applied to the sample at the investigated rates. According to this experiment, Hymovis® shows a very similar profile to other partially rendered hydrophobic hyaluronic acid derivatives reported in the literature [11]. For Durolane®, within the range of investigated shear rates, only the shear-thinning region was observed, indicating a prevailing contribution of chemically cross-linked chains to the overall viscoelasticity of the hydrogel.

Shear thinning behavior is indicative of strongly entangled polymer systems [20], a condition obtained at low concentrations with high and intermediate molecular weight HA grafted with associating side-chains [8,9,11,12,19].

Today, the use of synovial fluid substitutes is aimed at protecting and lubricating the load-bearing joints as efficiently as possible during their routine activity. In consideration of the stresses normally applied to knee joints and shear stress related to the gauge passage during intra-articular administration, we submitted the HA-based materials to structure recovery tests.

After the mechanical shock, the elastic modulus of hydrogels based on derivatized HA (Fig. 8(a)) is completely recovered only for Hymovis® on a timescale of a few hundred seconds. Synvisc® and Durolane® lose about 30% of their initial elastic properties, most probably due to the partial cleavage of the chemical cross-links of the network. In Fig. 8(b), the structure recovery of Hyalubrix® and Monovisc® is fully accomplished in a shorter time, as it is expected for a liquid system. The characteristic structure recovery time constant for Hymovis® can be evaluated with an exponential fit of the data and it is satisfactorily fitted with a biexponential function (Fig. 9).

This aspect reflects the influence of the heterogeneous structure of Hymovis® on the elastic behavior of the material, probably due to at least two different processes: a faster association of the hydrophobic side-chains and a slower chain entangling process. This slow relaxation process has a macroscopic characteristic time, τ_c, of about 20 s (Fig. 9). The recovery of the mechanical properties in hydrogels is a process involving several time scales and the oversimplification of the treatment of the above data does not allow an insight at molecular level. However, the overall behavior of Hymovis®, unlike other viscosupplements, is characterized by a slower but complete recovery of the mechanical properties after repeated mechanical shocks.

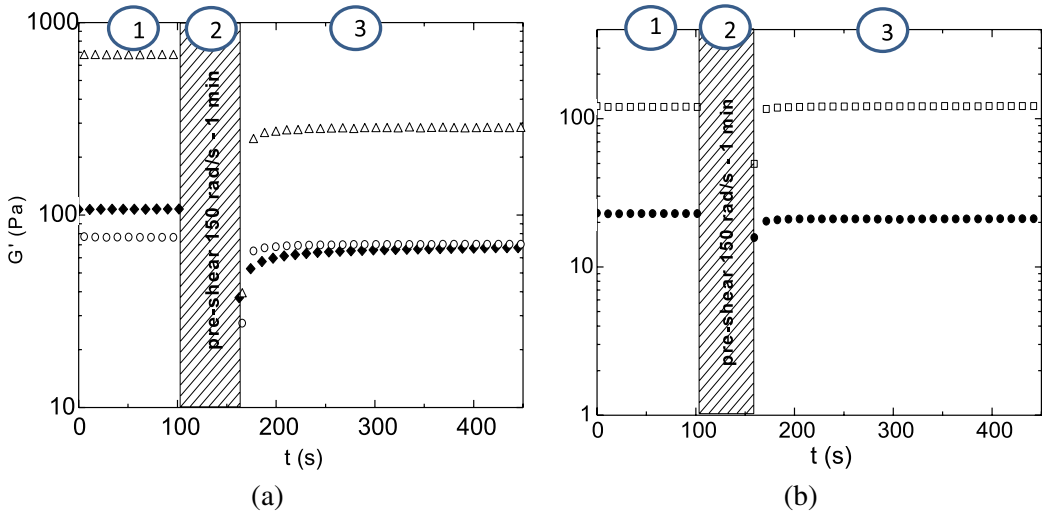

Fig. 8. (a) Structure recovery of hydrogels: Hymovis® (○), Durolane® (△), Synvisc® (◆); (b) structure recovery of HA solutions: Hyalubrix® (●), Monovisc® (□). Numbers refer to the phase of the experiments, only the last cycle is reported. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/BIR-2011-0596.)

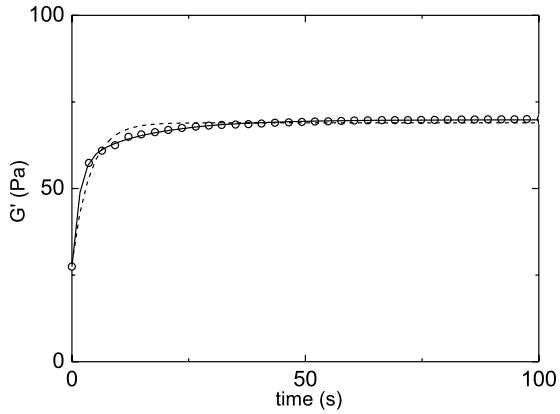

Fig. 9. Structure recovery as a function of the time of Hymovis®: monoexponential fit: dashed line; biexponential fit: continuous line.

5. Concluding remarks

We investigated the viscoelastic behavior of solutions and hydrogels obtained with HA or linear and cross-linked HA derivatives designed for viscosupplementation in the treatment of osteoarticular pathologies. The scenario emerging from this analysis shows that the systems examined are characterized by different behavior in terms of shear thinning and crossover frequency. To the best of our knowledge, Hymovis®, a product based on a novel linear amide HA derivative, is the only non-chemically cross-linked hyaluronan-based marketed viscosupplement showing gel-like behavior. Also the structure recovery experiments denote a different behavior mainly due to the chemical characteristics of the hydrogels. Among the systems displaying gel behavior in the investigated range of frequencies, the capability

to recover completely the viscoelastic properties after several cycles of mechanical stress is observed only in the case of Hymovis®.

The problems related to osteoarticular pathologies involve patients with different ages and life styles, requiring an addressed use of viscosupplements. From this point of view, as for the specific rheological properties discussed before, Hymovis® proves to be potentially suitable for the treatment of patients with an active life style, characterized also by activities such as intense walking and running. This potentiality, validated with clinical studies, should be further investigated with biomechanical evaluations to assess suitable therapeutic indications.

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