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Cartilage adhesive and mechanical properties of enzymatically crosslinked polysaccharide tyramine conjugate hydrogels†

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Using a home-built tensile tester, adhesion and mechanical properties of injectable enzymatically crosslinkable hydrogels were determined by placing the hydrogels in between cartilage surfaces. Dextran–tyramine (Dex-TA) and hyaluronic acid–tyramine (HA-TA) conjugates as well as a 50/50 composite material of these polysaccharide conjugates were tested. To integrate the injectable hydrogels with the cartilage tissue, pretreatment of the tissue with a Dex-TA conjugate solution strongly improved the adhesion. Only failure of the crosslinked hydrogel was observed and not at the hydrogel–tissue interface. Moduli of a Dex-TA hydrogel are higher than those of a HA-TA hydrogel, whereas the ultimate strain of the HA-TA hydrogel was at least three times higher. The Dex-TA/HA-TA hydrogel has similar storage and elastic moduli as the Dex-TA gel and also an ultimate strain of ~30%, similarly as found for the HA-TA gel. The controlled biodegradability and gelation time of the Dex-TA/HA-TA hydrogel, the developed method for strong tissue adhesion of the gel particularly in comparison with fibrin glue, makes this material applicable as an injectable hydrogel for tissue regeneration applications. Copyright © 2014 John Wiley & Sons, Ltd.
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Keywords: hydrogel; injectable; cartilage; adhesion; tensile properties

INTRODUCTION

Cartilage damage due to trauma, wear, and diseases such as osteoarthritis are the most common reasons for joint disability. The limited regenerative capacity of cartilage mostly leads to further degeneration of the tissue in time. Treatment of degenerated cartilage nowadays can be performed by techniques such as microfracture or mosaicplasty; however, results are not satisfying. With a population that becomes increasingly active and older, it is expected that the prevalence of cartilage damage will increase, and development of new strategies for cartilage repair becomes necessary.[1]

Hydrogels, three-dimensional elastic networks, mimic hydrated native cartilage tissue and are considered suitable scaffolds for cartilage tissue engineering. Although preformed hydrogels may be applied, their main disadvantage is the need for invasive surgical interventions. *In situ* forming hydrogels, also called injectable hydrogels, have attracted attention in recent years because they offer various advantages. In contrast to preformed hydrogels, they can be applied in a minimally invasive surgical procedure, can fill irregular-shaped defects, and can be co-injected with cells and bioactive molecules mixed in the precursor solution. Approaches currently used to fill defects focus on cell-free hydrogels or hydrogels containing autologous cells. Although much progress has been made in the development of new materials for cartilage regeneration, several considerations have to be made to effect new cartilage formation in a proper way revealing the complex nature of this tissue.[2–5]

In a review paper of Khan *et al.*,[6] several factors impeding repair of cartilage were described. Cell death at the hydrogel–tissue interface, impaired differentiation of chondrocytes in constructs containing cells, donor age-related effects of cells used to repair the defect, and pretreatment of the interface with collagenase to improve chondrocyte outgrowth may all contribute to implant failure. In this respect, the success of the integration of graft materials with the host tissue is dependent on the viability of chondrocytes at the edges of the cartilage. Also, maintaining the chondrocyte phenotype is important in the function and deposition of a new matrix and contributes to the success of the formation of new cartilage and integration of new and existing tissue. Inhibition of chondrocyte cell death and stimulation of cell density together with facilitating cell migration are considered to contribute to cartilage healing. Adhesive properties of implants such as injectable hydrogels most likely critically determine integration of the gel with the

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cartilage.[7] In order to clinically transform the use of injectable hydrogels in cartilage repair, it is necessary to integrate the hydrogel in a stable way in the surrounding tissue. Although some studies have addressed tissue integration, only a limited number of studies are related to the firm adhesion of hydrogels to the surrounding tissue. The group of Elisseeff[8] has performed most work in this area, and translation of their materials to clinic is in progress.[9] To firmly adhere hydrogels to the existing tissue, they used a strategy by first priming the tissue with reactive groups that subsequently can be copolymerized with a photopolymerizable hydrogel precursor solution. They used functionalized chondroitin sulfate with methacrylate groups and aldehyde groups. Aldehyde groups were introduced by oxidizing part of the rings. This material was used as a primer. By formation of Schiff base groups upon reaction of the aldehyde groups of the polysaccharide with free amine groups from, e.g. lysine residues of the collagen, the chondroitin sulfate conjugate was covalently attached to the tissue. Upon filling the defect with the photopolymerizable hydrogel precursor, the acrylate groups of the chondroitin sulfate were co-crosslinked thereby attaching the hydrogel to the tissue interface. Tensile and shear forces applied to the tissue–gel constructs revealed failure in the hydrogel showing the proper fixation of the hydrogel at the interface.

The deposition of a new matrix by cells in hydrogels may well contribute to the adhesion of the gel to the tissue. Erickson *et al.*[10] studied the integration and compressive properties of a photocrosslinkable methacrylated hyaluronic acid hydrogel containing mesenchymal stem cells in cartilage explants. It was shown that the hydrogel mesenchymal stem cell's constructs afforded higher equilibrium moduli in a push-out test than materials without cells. It was hypothesized that the deposition of new matrix contributed to the better integration and thereby adhesion of the hydrogel to the cartilage. Maher *et al.*[11] prepared gels containing chondrocytes using Puramatrix™ (Waltham, MA, USA). Puramatrix is a peptide-based hydrogel having a nanometer scale fibrous structure. By addition of transforming growth factor-β3, integration of the gels with cartilage appears to be enhanced as measured by a push-out method. Although no explanation was presented, it is believed that matrix deposition at the interface can be regarded as the main reason for the improved adhesion.

Recently, an injectable hydrogel has been described that is based on the use of oxidized carboxymethyl cellulose, borax, and gelatin. Such a gel can be applied to cartilage defects using a double syringe. In this method, the borax provided linkages with remaining hydroxyl groups, while the aldehyde groups on the partially oxidized polysaccharide were used to induce crosslinking via Schiff base formation with the gelatin free amine groups.[12] The *in situ* gelating material was tested for its integration with goat cartilage explants. Good integration was shown, and the adhesion to cartilage was determined via its burst strength. The formation of Schiff base groups between collagen fibers located at the defect interface and oxidized carboxymethyl cellulose in the hydrogel was reported a reason for the adhesive nature of the hydrogel.

In previous research, we have shown that *in situ* forming hydrogels can be prepared by the horseradish peroxidase (HRP)-mediated (co)-crosslinking of dextran–tyramine (Dex-TA) and hyaluronic acid–tyramine (HA-TA) conjugates.[13–15] Initial results on placing such *in situ* forming hydrogels into a cartilage defect indicated adhesion to the surrounding tissue. We hypothesized that the reaction results in covalent binding of the hydroxyphenyl groups in the polysaccharide tyramine conjugate to collagen tyrosine functional groups in the cartilage matrix. In this paper, we describe a method to measure the adhesion strength of *in situ* forming injectable and enzymatically crosslinkable hydrogels to cartilage tissue. We used a sandwich model in which the hydrogels were injected in between two cartilage explants. Applying a tensile force, disruption in the hydrogels or at the hydrogels cartilage interface could be determined. Moreover, this method also allowed for measuring the hydrogel's mechanical properties. These properties were compared with mechanical properties of the hydrogels by rheology.

MATERIALS AND METHODS

Dextran (M_w = 15 to 25 kg/mol) was obtained from Sigma-Aldrich (St Louis, MI, USA) and dried by lyophilization. Hyaluronic acid sodium salt (M_w = 15 to 25 kg/mol) was purchased from CPN Shop (Dolni Dubrouc, Czech Republic). Fibrin gel with two components (Tissucol Duo 500) was purchased from Baxter AG (Volkitswil, Switzerland). *N*-Hydroxysuccinimide (NHS), tyramine, *p*-nitrophenyl chloroformate (PNC), anhydrous dimethylformamide (DMF), pyridine (anhydrous), hydrogen peroxide (H_2O_2, 30 wt% in H_2O), lithium chloride, HRP (253 purpurogallin units/mg solid) were obtained from Sigma-Aldrich and used without further purification. *N*-Ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydroxide (EDAC) was obtained from Fluka. Phosphate buffered saline (PBS, pH 7,4) was purchased from B. Braun Co. The Dex-TA conjugate and HA-TA conjugate were synthesized using slightly modified procedures as described previously.[13,14]

SYNTHESIS

Dextran–tyramine conjugate

Typically, dextran (3.0 g, 56.3 mmol OH groups) was dissolved in DMF (120 ml, containing 2.4 g of LiCl) at 90°C under nitrogen. After the dextran was dissolved, the mixture was cooled and thermostated at 0°C. Pyridine (1.5 ml, 18.6 mmol) and 4-nitrophenyl chloroformate (PNC, 2.6 g, 12.9 mmol) were added to the solution while stirring. The reaction was conducted for 1 h, and the product (denoted as Dex-PNC) was precipitated in cold ethanol and filtered and washed with ethanol and diethyl ether. Degree of substitution (DS) (^{1}H NMR): 24. Yield: 3.7 g (99%). ^{1}H NMR (DMSO-d_6): 3.0–4.0 (m, dextran glucosidic protons), 4.4–5.8 (m, dextran anomeric protons and OH), and 7.58 and 8.32 (d, aromatic protons).

Subsequently, Dex-PNC DS 24 (3.7 g, 4.4 mmol of 4-nitrophenyl carbonate groups) was dissolved in 57 ml of DMF, and tyramine (1.2 g, 8.9 mmol) was added under nitrogen. The reaction was conducted for 1 h. The product was precipitated in cold ethanol and filtered and washed with ethanol and diethyl ether. The Dex-TA conjugate was purified by ultrafiltration (MWCO 3000) against deionized water and isolated after lyophilization. DS (^{1}H NMR): 12. Yield: 2.7 g (82%). ^{1}H NMR (D_2O): δ 2.75 and 3.05 (m, —CH_2—CH_2—), 3.3–4.1 (m, dextran glucosidic protons), 4.2–5.6 (s, dextran anomeric protons), and 6.86 and 7.17 (d, tyramine aromatic protons).

Hyaluronic acid–tyramine conjugate

Sodium hyaluronate (1.0 g) was dissolved in 20 ml of MES buffer (0.1 M, pH 6.0), EDAC (240 mg, 1.25 mmol) and NHS (144 mg, 1.25 mmol) both separately dissolved in 5.0 ml of MES buffer were added. After 30 min of stirring, a solution of tyramine (342 mg, 2.50 mmol) in 6 ml of DMF was added, and the mixture

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was stirred for 3 days. The mixture was neutralized with 1 M NaOH and ultrafiltrated (MWCO 1000), first with PBS and then Milli-Q water. The resultant HA-Tyr conjugate was obtained after lyophilization as a white foam. Yield: 0.72 g (71%). DS (UV–Vis): 2.2. ^{1}H NMR (400 MHz, D_2O): δ 1,93 (s, methyl protons), 2.4–2.8 (m, polysaccharide ring protons), 3.2–4.1 (m, polysaccharide ring protons), 4.2–4,6 (d, 1H, polysaccharide ring protons), 4.69 (s, H_2O), and 6.86 and 7.17 (d, tyramine aromatic protons).

CHARACTERIZATION

^{1}H NMR

^{1}H (400 MHz) NMR spectra were recorded using a Bruker AVANCE III 400 MHz (Nanobay) spectrometer (Billerica, MA, USA). Polymers were dissolved in D_2O or DMSO at a concentration of 0.020 g/ml. The DS of tyramine groups was calculated using the integral ratio between the aromatic protons of the tyramine groups and anomeric and OH protons of the polysaccharide backbone.

Ultraviolet–visible

The DS of tyramine residues in Dex-TA and HA-TA conjugates was determined by an UV measurement. The HA-TA conjugate was dissolved in PBS at a concentration of 5 μg/ml, and the absorbance was measured at 275 nm using a Cary 300 Bio spectrometer (Varian). The absorbance was correlated to the DS of tyramine groups in the HA-TA using a calibration curve from tyramine in PBS.

Rheological analysis

Rheological experiments were carried out with a MCR 301 rheometer (Anton-Paar, Gentbrugge, Belgium) using parallel plates (25 mm diameter, 0°) configuration at 37°C in the oscillatory mode. In a typical experiment, a solution of HRP (25 μl, 150 units/ml stock solution) and H_2O_2 (25 μl, 0.3% stock solution) were mixed and added to the polymer dissolved in PBS (200 μl, 10 wt%). The resulting gel was placed at the ground plate, and the upper plate was lowered to a measuring gap size of 0.3 mm, and the measurement was started. To prevent water evaporation, a layer of oil was introduced around the polymer sample. The storage (G′) and loss (G″) modulus were recorded using a strain of 1% and a frequency of 1 Hz.

Tensile testing

A tensile test setup with a 9 N load cell was used to measure the material response on tensile forces. Bovine cartilage explants were obtained from fresh bovine knees using a Trukor drill (ø = 8 mm) sleeve. In a typical experiment, a 10 wt% Dex-TA hydrogel was injected between two bovine cartilage pieces. Mixing was achieved using a Mixpac™ (Winterthur, Switzerland) double syringe. A Dex-TA polymer solution (20 wt% in PBS) was mixed with an equal volume of HRP (150 unit/ml stock solution in PBS) and placed in one of the chambers. To the other chamber of the double syringe was added a mixture of the Dex-TA polymer solution (20 wt% in PBS) and an equal volume of H_2O_2 (0.3 wt% stock solution in PBS). Gels were formed *in situ* between the cartilage pieces by injection. After 5 min, the cartilage pieces were pulled apart at a uniform speed of 0.1 mm/s. Control fibrin gels (Tissucol, Baxter, Volketswil, Switzerland) were applied in a similar way. Cartilage surfaces were either nontreated or pretreated with a 10 wt% Dex-TA polymer solution in PBS for 5 min before injecting the *in situ* forming hydrogels.

RESULTS AND DISCUSSION

The synthesis of the Dex-TA and HA-TA conjugates (Fig. 1) was performed in an analogs way as described previously with some modifications. The activation of dextran hydroxyl functional groups with 4-nitrophenyl chloroformate was performed according to a paper of Schacht and Bruneel.[16] The reaction appears highly sensitive to impurities such as amines present in the solvent (DMF) and moisture. Moreover, a reaction time of 1 h appeared sufficient, and longer reaction times decreased the number of activated hydroxyl groups. The subsequent reaction with tyramine affords the Dex-TA conjugate through the formation of carbamate groups. For the intended application as an injectable hydrogel using the enzymatic crosslinking by HRP and hydrogen peroxide of phenolic groups, the number of tyramine groups on the dextran backbone needs to be within certain values. Low degrees of substitution (DS $<$ 10) do give slow gelation applying a conjugate concentration of 10 wt%. The upper limit in the DS of tyramine groups in Dex-TA with an average molecular weight of 16,000 is approximately 17. At higher degrees of substitution, the conjugate will become insoluble in PBS. Within the range of DS values given previously, the gelation time can be tuned to approximately 20 s by using appropriate HRP and H_2O_2 concentrations. Such gelation times are highly suitable for the conjugate to be applied as an injectable hydrogel.

In a recent paper, we described the synthesis of a HA-TA conjugate using the well-known method of carboxylic acid activation by ethyl diaminopropyl carbodiimide and NHS and subsequent reaction with tyramine. In this one pot synthesis using an excess of activation reagents and tyramine, a DS of 2 to 3 was obtained. The DS of the HA-TA was determined both by ^{1}H NMR (D_2O) (Fig. 2) and ultraviolet–visible (UV–Vis) at 275 nm using a calibration curve of tyramine in Milli-Q water. The values obtained by NMR (DS = 2.0) and UV–Vis (DS = 2.2) are similar. This means that approximately 5%

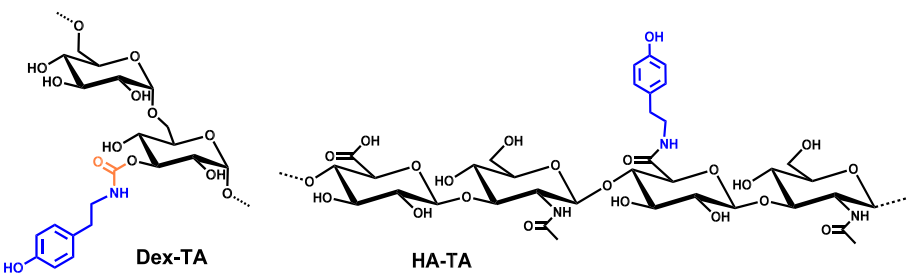

Figure 1. Chemical structure of the dextran–tyramine conjugate (Dex-TA) and hyaluronic acid–tyramine conjugate (HA-TA). This figure is available in colour online at wileyonlinelibrary.com/journal/pat

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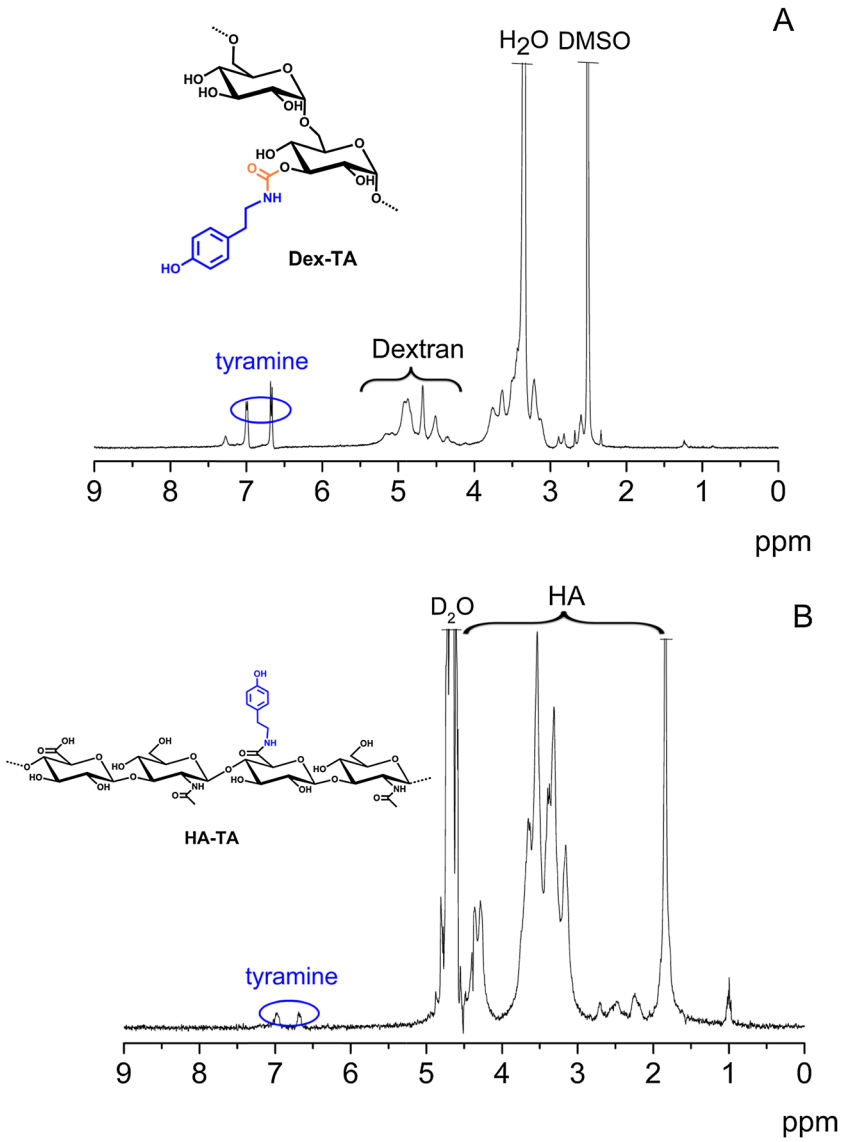

Figure 2. ^{1}H NMR spectra (400 MHz) of (A) dextran–tyramine conjugate (Dex-TA) (DMSO-d^{6}) and (B) hyaluronic acid–tyramine conjugate (HA-TA) (D_2O). This figure is available in colour online at wileyonlinelibrary.com/journal/pat

of the carboxylic acid groups have been substituted with a tyramine group. Despite this apparent low DS, fast gelation within 20 s using HRP and H_2O_2 concentrations similar as given previously takes place.

An important issue in the application of an *in situ* forming hydrogel to fill cartilage defects is the integration and adhesion of the gel to the surrounding tissue. In previous research, we showed that using a double syringe as an injection system homogeneous enzymatically crosslinked Dex-TA gels could be applied to fill defects made in fresh bovine explants.[15] SEM and Raman analysis showed that the gel even at irregular sites filled the defects. The adhesion of the Dex-TA gels was explained by the co-crosslinking of collagen tyrosine groups with the tyramine groups on the polysaccharide backbone.

In this study, we used a home-built tensile tester to quantify the adhesion of the gels to cartilage. Fresh bovine knees were dissected and the synovial fluid drained. Using a Trukor sleeve, cylindrical specimens (ø = 8 mm) were taken from the cartilage up to the subchondral bone. The tissue was kept moist by regularly rinsing with PBS. The tester consists of a 9 N load cell and two holders of which the lower one can be pushed downwards. Two cylindrical specimens (ø = 8 mm) were taken from bovine knees and mounted in the holders (Fig. 3). The hydrogel precursor solutions were injected in between the two specimens (gap size of 3 mm) using a double syringe. The gel was formed almost instantaneously, and after 5 min curing, a tensile test experiment was performed at a measured rate of 100 μm/s. From the measured forces corresponding to a certain position, a stress–strain curve was determined.

However, occasionally, it could be observed that the shrinkage of the Dex-TA gel upon crosslinking leads to detachment of the gel from the tissue. Apparently, no integration or insufficient integration of the Dex-TA with the cartilage takes place, and generally, this could clearly be observed visually. It thus appears that a proper balance is needed between gelation time and the time needed for the precursor solutions to integrate with the tissue. Detachment of a commercial fibrin gel (Tissucol Duo 500, Baxter) was also observed by applying this injectable gel in between nontreated cartilage surfaces. In this case, the mixing of fibrinogen and thrombin forms a gel.

In the following experiments, we therefore pretreated the cartilage surface with a 10 wt% Dex-TA solution, and after

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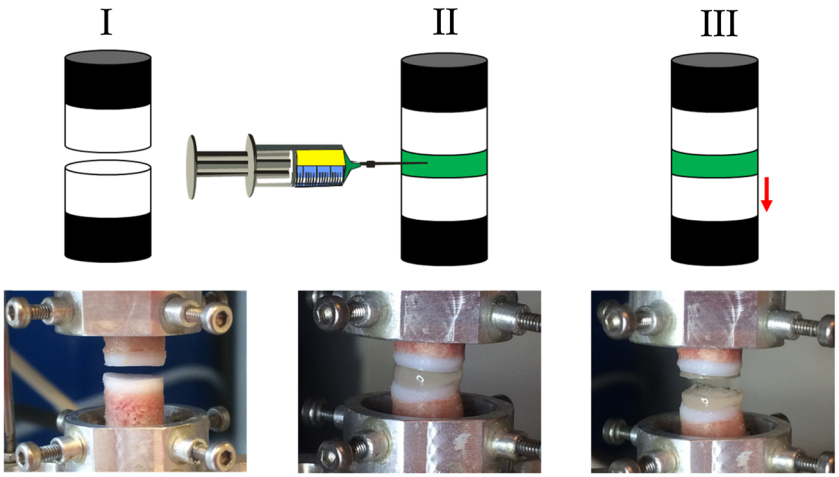

Figure 3. Schematic representation of the tensile tests performed. (I) Cylindrical cartilage specimens placed in the holders of the tensile tester, (II) hydrogel after injection, and (III) disrupted hydrogel after loading. This figure is available in colour online at http:/wileyonlinelibrary.com/journal/pat

5 min, the hydrogel precursor solutions were injection in the gap. The pretreatment ensured in all cases tensile tests could be performed revealing integration of the gel with the cartilage.

Several experiments using fresh bovine cartilage cylindrical specimens afforded similar stress–strain curves, and a typical example is presented in Fig. 4A. In this graph, the stress is given in excited force (kPa) as a function of the corresponding tensile strain (%).

The stress–strain diagram visualizes the mechanical properties of the hydrogel. In all cases, failure takes place in the Dex-TA hydrogel and not at the interface (for a typical experiment, a video is provided in the supplementary material). As can be seen in Fig. 4A, the stress increases almost linearly with the strain applied. No clear yield point is observed and an E-modulus of 3.7 kPa was calculated in between 0.2% and 1.0% strain. An average ultimate stress of approximately 17 kPa and ultimate strain of 8% were found (Table 1).

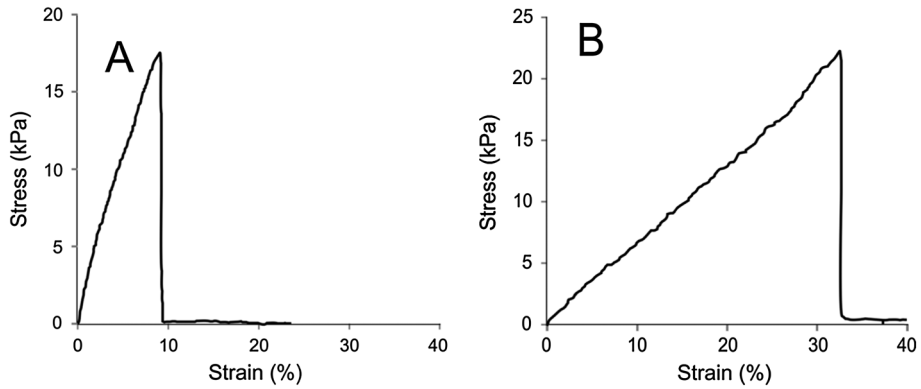

Figure 4. Stress–strain curves of hydrogels injected in between two cartilage specimen. (A) A 10 wt% Dex-TA gel and (B) a 10 wt% HA-TA gel. The cartilage surfaces were pretreated with a 10 wt% Dex-TA solution for 5 min.

Table 1. Mechanical properties of injected Dex-TA, HA-TA, and Dex-TA/HA-TA (50/50) hydrogels in between cartilage surfaces[a]

	E-modulus (kPa)	Ultimate stress (kPa)	Ultimate strain (%)	Storage modulus (kPa)	Loss modulus (kPa)	Damping factor
Dex-TA[b]	3.7 ± 0.6	17.6 ± 0.5	7.6 ± 2.1	12.2 ± 0.04	0.9 ± 0.03	0.07
HA-TA[b]	0.7 ± 0.1	20.8 ± 1.7	34.0 ± 1.8	3.3 ± 0.04	0.005 ± 0.01	0.002
Dex-TA/HA-TA[b] (1/1)	3.8 ± 1.2	37.3 ± 11.0	30.8 ± 6.0	11.3 ± 0.5	0.3 ± 0.02	0.03
Dex-TA/HA-TA[c] (1/1)	3.4 ± 0.6	18.2 ± 0.4	10.4 ± 3.5			
Fibrin[c]	0	—	—	0.5 ± 0.02	0.007 ± 0.01	0.02

[a]Standard deviations were determined from at least three independent measurements.
[b]The cartilage was pretreated with a 10 wt% Dex-TA polymer solution for 5 min.
[c]The cartilage was nonpretreated.

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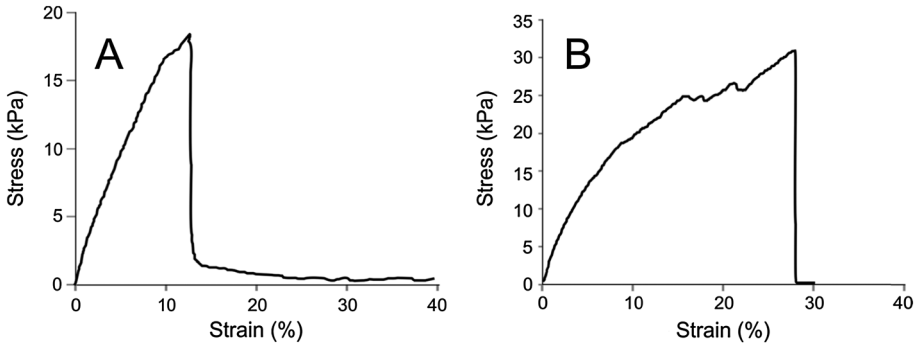

Figure 5. Stress–strain curves of adhesion forces to cartilage obtained from (A) a 10 wt% Dex-TA/HA-TA (50/50) gel at a nonpretreated cartilage surface and (B) a 10 wt% Dex-TA/HA-TA (50/50) gel at a pretreated surface. The cartilage surface was pretreated with a 10 wt% solution of Dex-TA for 5 min.

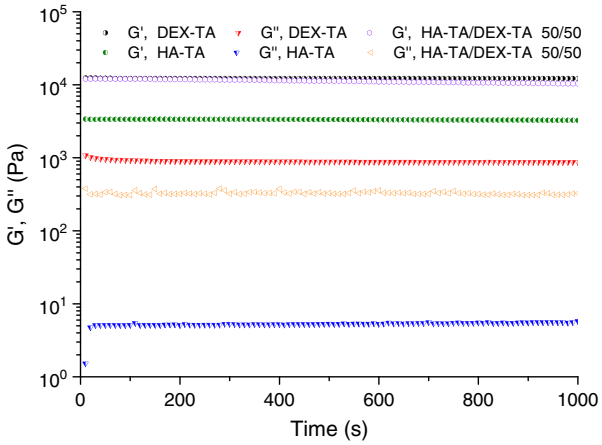

Figure 6. Storage and loss moduli of a 10% Dex-TA, HA-TA, and a 1 : 1 mixture (wt%) of Dex-TA:HA-TA hydrogels as measured with oscillatory rheology. This figure is available in colour online at http:/wileyonlinelibrary.com/journal/pat

A typical stress–strain curve of an injected 10 wt% HA-TA gel, applying the same concentrations of HRP and H_2O_2, is presented in Fig. 4B. Also in this case, the cartilage surface was pretreated with a 10 wt% Dex-TA solution for 5 min. Failure occurs in the gel and not at the interface. Compared with the Dex-TA gel, the HA-TA gel shows a somewhat lower E-modulus, similar ultimate stress but a higher strain at break.

Previously, we have shown that Dex-TA gels very slowly degrade, which may hamper cartilage tissue regeneration. Combining Dex-TA with HA-TA at a 50/50 weight ratio resulted in a hydrogel, which degraded in approximately 50 days.[14] It was also shown that the ultimate stress and ultimate strain, as determined by rheology, even exceed that of a Dex-TA hydrogel at a similar concentration. On the basis of these data, we decided to test the tissue adhesive properties of this hydrogel. Both nonpretreated and pretreated cartilage surfaces (10 wt% Dex-TA solution for 5 min) were used, and the results are presented in Fig. 5. Applying a nonpretreated surface, failure was observed in 40% of cases partly in the gel and 60% of the cases at the interface. A typical stress–strain curve of a successful experiment is shown in Fig. 5A. The ultimate stress and strain measured resemble that of a Dex-TA gel. When the Dex-TA/ HA-TA conjugates were applied on a Dex-TA pretreated surface, the stress–stain curve as presented in Fig. 5B shows a more similar behavior as found for the HA-TA gel. In all cases, failure occurs in the gel and not at the interface, and a high ultimate strength of 37 kPa and a strain at break of 31% were found. Although a clear yield point cannot be indicated, the curve shows a clear nonlinear increase in stress revealing the viscoelastic properties of the gel. The E-modulus was determined at low strain and is comparable with the Dex-TA gel.

Using oscillatory rheology, we next determined the storage and loss moduli of the 10 wt% Dex-TA, HA-TA, and Dex-TA/HA-TA (50/50) enzymatically crosslinked hydrogels, and the results are presented in Fig. 6. Storage and E-moduli cannot be directly compared because the E-moduli are difficult to measure at very low strains as applied in the rheological experiments. Although the storage moduli are a factor 3 higher than the E-moduli, in general, the moduli show a similar trend for the different hydrogels. Importantly, the data shows that combining Dex-TA and HA-TA in a hydrogel yields a viscoelastic injectable hydrogel with a high ultimate stress and high strain. Combined with the degradation properties, such co-crosslinked hydrogels may well serve to fill defects intended for regeneration of cartilage.

CONCLUSIONS

In this study, we have developed a method to integrate enzymatically crosslinked hydrogels to cartilage tissue and measure the adhesion properties. The results show that Dex-TA/HA-TA (50/50) hydrogels well adhere to cartilage tissue, particularly when the cartilage surface is pretreated with a polysaccharide precursor solution. The integration leads to failure within the gels and not at the interface of the gel and the tissue. The rheological properties of a 10% Dex-TA/HA-TA hydrogel and bonding to damaged cartilage is in the order of 1 to 2 logs better than the properties of clinically applied fibrin glue. These results are important regarding the clinical translation of hydrogels for the treatment of arthritic cartilage or cartilage defects.

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REFERENCES

[1] E. B. Hunziker, *Osteoarthr. Cartilage* **2002**, *10*, 432–463.
[2] J. Elisseeff, *Expert Opin. Biol. Ther.* **2004**, *4*, 1849–1859.
[3] Z. Ge, C. Li, B. C. Heng, G. Cao, Z. Yang, *J. Biomed. Mat. Res. Part A* **2012**, *100A*, 2526–2536.

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[4] Y. Li, J. Rodrigues, H. Tomas, *Chem. Soc. Rev.* **2012**, *41*, 2193–2221.
[5] D. J. Overstreet, D. Dutta, S. E. Stabenfeldt, B. L. Vernon, *J. Pol. Sci. Part B-Pol. Phys.* **2012**, *50*, 881–903.
[6] I. M. Khan, S. J. Gilbert, S. K. Singhrao, V. C. Duance, C. W. Archer, *Eur. Cells Mat.* **2008**, *16*, 26–39.
[7] K. L. Spiller, S. A. Maher, A. M. Lowman, *Tissue Eng. Part B-Rev.* **2011**, *17*, 281–299.
[8] D.-A. Wang, S. Varghese, B. Sharma, I. Strehin, S. Fermanian, J. Gorham, D. H. Fairbrother, B. Cascio, J. H. Elisseeff, *Nat. Mater.* **2007**, *6*, 385–392.
[9] B. Sharma, S. Fermanian, M. Gibson, S. Unterman, D. A. Herzka, B. Cascio, J. Coburn, A. Y. Hui, N. Marcus, G. E. Gold, J. H. Elisseeff, *Sci. Transl. Med.* **2013**, *5*, 1–9.
[10] I. E. Erickson, S. R. Kestle, K. H. Zellars, G. R. Dodge, J. A. Burdick, R. L. Mauck, *Biomed. Mat.* **2012**, *7*, 1–10.
[11] S. A. Maher, R. L. Mauck, L. Rackwitz, R. S. Tuan, *J. Tis. Eng. Reg. Med.* **2010**, *4*, 25–29.
[12] B. Balakrishnan, N. Joshi, R. Banerjee, *J. Mat. Chem. B* **2013**, *1*, 5564–5577.
[13] R. Jin, C. Hiemstra, Z. Zhong, J. Feijen, *Biomaterials* **2007**, *28*, 2791–2800.
[14] J. W. H. Wennink, K. Niederer, A. I. Bochyńska, L. S. Moreira Teixeira, M. Karperien, J. Feijen, P. J. Dijkstra, *Macromol. Symp.* **2011**, *309-310*, 213–221.
[15] L. S. Moreira Teixeira, S. Bijl, V. V. Pully, C. Otto, R. Jin, J. Feijen, C. A. van Blitterswijk, P. J. Dijkstra, M. Karperien, *Biomaterials* **2012**, *33*, 3164–3174.
[16] D. Bruneel, E. Schacht, *Polymer* **1993**, *34*, 2633–2637.

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