Determination of the viscoelastic properties of hydrogels based on polyethylene glycol diacrylate (PEG-DA) and human articular cartilage

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Abstract: In this work, a systematic study of the viscoelastic properties of hydrogels based on polyethylene glycol diacrylate (PEG-DA) is presented. In addition to artificial PEG-DA-based hydrogels, natural hydrogels in the form of human articular cartilage were examined. Specimens were (unconfined) compression tested under static and dynamic load. Besides this, instrumented indentation tests with different indenter geometries (cylindrical, spherical) and load ranges (macro- and nano-indentation) were carried out and relaxation tests for the determination of moduli and relaxation time were performed. Tensile tests completed the list of measurement techniques. The measured initial moduli of the evaluated hydrogels range from 10^4–10^7 Pa. Spherical indentation was used in testing human articular cartilage in phosphate buffered saline (PBS). Cartilage samples were measured shortly after explantation, being stored at room temperature. The influence of freezing and shock-freezing was evaluated. It turned out that freezing has a massive impact on sample properties, especially on the stress relaxation time and the ratio of initial to equilibrium modulus.

Keywords: hydrogel; polyethylene glycol; PEG; mechanical properties; viscoelasticity; articular cartilage; degradation.

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1 Introduction

1.1 Natural hydrogels – cartilage

The human cartilage may be termed a 'natural hydrogel' as it contains large amounts of water embedded into a fibre network. In contrast to other types of tissue, cartilage neither contains blood vessels nor nerves or lymph channels to support and nourish its living cells. Cells subsist on dissolved substances in the synovial liquid. The water content of the cartilage is between 65%–85%, two thirds of which is weakly linked to the extracellular matrix and therefore can be pressed out and soaked in (Bader and Lee, 2000). Periodic load cycles on the joints keep the synovial liquid in motion and remove the metabolic products.

Different collagen species with about 2/3 of the solid phase are the major component of cartilage (Eyre, 2002). Proteoglycans (PGs) are another relevant component of cartilage (Kempson et al., 1970; Armstrong and Mow, 1982; Mollenhauer and Aurich, 2003). They can be described as strongly negatively charged cations. Outside cartilage, they would take up five times the volume which they occupy inside cartilage. Due to this highly compressed state, PGs exert a strong swelling pressure in their environment which is a key factor in understanding the viscoelastic deformation of the tissue (Maroudas et al., 1992).

Only few weight percentage of the cartilage is made up by living cells, the chondrocytes (Mollenhauer and Aurich, 2003; Hamerman and Schubert, 1962). Nonetheless, they have significant impact on the physiological and mechanical properties of cartilage. They generate the cartilage components and therefore control the dynamic behaviour of cartilage. Mechanical loads induce a hydrostatic pressure gradient within the cartilage, which leads to several electrokinetic effects, alters ion concentrations as well as the osmolarity and the pH-value (Gray et al., 1988).

1.2 Artificial hydrogels

Artificial hydrogels offer several advantages for use in biomedical engineering. Their mechanical properties can be adjusted over several orders of magnitude by changing the network density and the solvent concentration. Their open network is accessible for an outside culture medium and their functional properties can be tuned by the utilised base monomer and crosslinker.

Polyethylene glycol diacrylate (PEG-DA) in a cured state is capable of forming such a hydrogel when immersed in water. The polymer network is relatively stable and can be broken only at the crosslink points, it can therefore be termed partially biodegradable

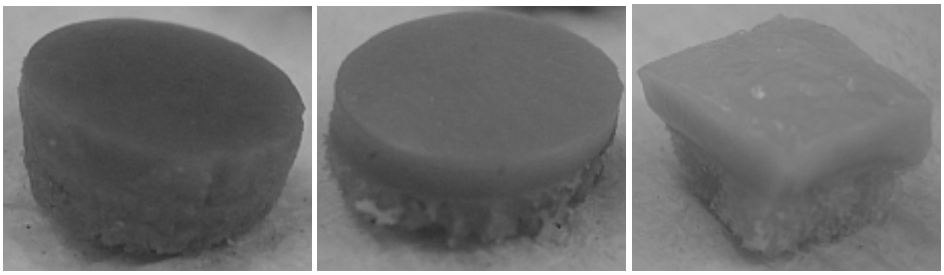
(Merrill and Salzman, 1983; Baudis, 2007). The speed of this hydrolytic degradation process is slow and leads to degradation products with fairly low toxicity (poly acrylic acid and linear PEG chains). Bryant et al. (2004, 2005) showed the suitability of PEG-DA hydrogels as cartilage replacement material, which to a large extent can be attributed to their ability to incorporate vital chondrocytes. Chondrocytes inside PEG-DA were even found to produce extracellular matrix (i.e., PGs, collagens) similar to cartilage components in the natural environment.

2 Materials and methods

2.1 Human pelvic cartilage (hpc)

The pelvic cartilage used in this work was obtained from patients receiving artificial hip implants after approval by the institutional ethical review board. Specimens were extracted from regions that showed no visual signs of degeneration or damage. Initial mechanical tests were performed in the fresh state, with the presence of vital cells inside the tissue. Later on, the same samples were temporally frozen and re-tested after unfreezing. The age of the donors was between 70 and 80 years and the thickness of the tested cartilage was fairly constant on each sample. Between different samples, the thickness varied between 1 mm and 1.6 mm and they were almost flat as the curvature of the surface was estimated to be around 0.02 mm^{-1}. Samples were obtained in form of cylindrical discs with 10 mm diameter. The cartilage was still attached to the underlying bone, as can be seen from Figure 1.

Figure 1 Human articular cartilage: from pelvis (hpc, left and middle) and from the femoral condyle (cfc, right)



The sample geometry enables the characterisation of the tissue by macro indentation as well as by compression testing. Due to the slight non-flatness of the samples, compression tests are estimated to give results with lower accuracy than indentation tests. The specimens were stored at 35 ± 2°C until the first measurement was finished. Samples were kept in phosphate buffered saline (PBS), since it is used as liquid medium by numerous research groups (Bryant et al., 2004; Revzin et al., 2001; Korhonen et al., 2003; Jin and Lewis, 2004; Pfister et al., 2007). After storing the samples for more than seven weeks at room temperature, two samples were slowly frozen to –18°C and another one was shock frozen in liquid nitrogen. After thawing the original tests were repeated.

2.2 Human cartilage from femoral condyle (cfc)

The samples taken from the knee joint (femoral condyle) were of rectangular shape of about 1 cm edge length (compare Figure 1 on the right). The thickness of the cartilage was between 2 mm–4 mm, but the variations over the sample area were substantial. Many of the 13 specimens that came from different persons were strongly curved. The explants have been frozen slowly to –18°C after being wrapped in gauze and soaked with PBS several times in accordance with the procedure recommended by Niederauer et al. (2004). The time for which the explants were stored frozen after the operational removal from the joint lay between few months and several years. Samples were tested a first time shortly after thawing. A second loop of measurements followed a few days later to control the reproducibility of cartilages characteristics.

2.3 PEG-DA

PEG-DA specimens were obtained by thermally polymerising PEG-DA monomer (M_N approx. 258 g/mol) in oligomeric polyethylene glycol {$H(OCH_2CH_2)_nOH$} with an average molar mass of 400 g/mol (PEG400) initiated by 5% of azo-bis-isobutyronitrile (AIBN – $C_8H_{12}N_4$). The chemicals were obtained from Sigma-Aldrich. In order to dissolve the initiator, the suspension was put into an ultrasonic bath for about 20 minutes prior to curing, which was achieved by heating up the samples to 90°C and keeping this temperature for half an hour.

After the polymerisation, samples were put into water to drive the organic solvent out of the network. The samples were then stored at room temperature in water. The solid phase of the PEG-DA samples ranged from about 8 ma% to 51 ma%. PEG-DA samples were cylinders of about 8 mm height and 11 mm diameter for all the methods but shearing and tensile tests (Gaebler, 2008). Tensile specimens were manufactured according to the standardised form 5A (DIN e.V., 1996).

2.4 Measuring methods

Macroscopic indentation and compression tests as well as the tensile tests were performed uniaxially on a Zwick Z050 Figure 2 universal testing device with a 20N-load cell of 1 mN-resolution. The spherical indenter had a diameter of 3 mm, while the cylindrical indenter (flat punch) was of 2 mm diameter. Indenter size was not changed. Nanoindentation used a ‘NANO indenter XP’ system with resolutions of 50 nN in force and of 0.02 μm in indenter travel. The diameter of the flat punch was about 54 μm. Dynamic compression tests were performed on a ‘2980 dynamic mechanical analyser’ from TA Instruments. The dynamic compression in this work means a static preforce that deformed the sample to a specific degree plus an oscillation of 10 μm amplitude in different frequencies. Hydrogels were kept in water at room temperature during all the measurements but tensile testing. Moduli for the indentations were calculated according to the widely used equation, according to Hayes et al. (1972). κ is a dimensionless scaling factor, F is the applied force in Newton, ν is the Poisson’s ratio, r_{act} is the actually indenting radius of the indenter in mm, G is the shear modulus in N/mm² and s is the penetration depth of the indenter. After reorganising equation (1) we obtain a relation for the shear modulus as in equations (2).

$$\kappa = \frac{F(1-\nu)}{4r_{act} \cdot G \cdot s} \tag{1}$$

$$G = \frac{F(1-\nu)}{4r_{act} \cdot s \cdot \kappa} \tag{2}$$

$$G = \frac{E}{(2+2\nu)} \tag{3}$$

By considering the relation between E and G from equation (3), we obtain a relation for the elastic (or Young's) modulus E (in N/mm²) in equation (4).

$$E = \frac{F(1-\nu^2)}{2r_{act} s \kappa} \tag{4}$$

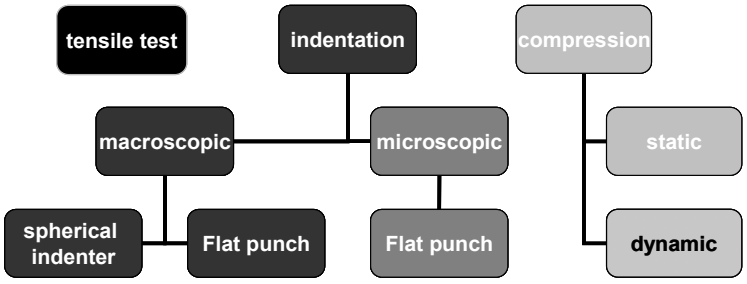
In this work, the indentation depth for all PEG-DA samples was 500 µm. The compression of the cartilage samples was 15%, which is higher than the usually recommended 10% of Buckle (1973). The benefit of the higher compression is a reduction of surface effects which occur during the first tens of microns of compression. Elastic moduli from compression tests were calculated with the help of equation (5).

$$E = \frac{4h}{\pi \cdot d^2} \bullet \frac{\Delta F}{\Delta s_{abs}} \tag{5}$$

where h is the sample height in mm, d is the sample diameter in mm and s_{abs} is the absolute crosshead position also in mm. In contrast to equation (4), the Poisson's ratio is not required, although a modulus value can only be calculated if there is an ascent in force level between two neighbouring measured values. A valid modulus therefore cannot be calculated directly if force or compression is kept constant which is the case in relaxation measurements. Poisson's ratio for cartilage was assumed to be 0.4 according to literature values (Jin and Lewis, 2004; Jurvelin et al., 1986; Lyyra et al., 1995; Korhonen et al., 2002a; Lu et al., 2007), where values between 0.35–0.5 have been reported. Poisson's ratio for PEG-DA was assumed to be 0.5 because of the elastomeric character of its network.

In the case of tensile tests, the strain range between 0.25%–0.5% was taken into account for obtaining the elastic modulus. Cartilage samples were only tested with spherical indenter and as far as allowed by the shape, with unconfined compression.

Figure 2 Evaluated measuring methods for the determination of mechanical properties



Young's modulus is defined as a time independent material property and is therefore not suitable to be used for materials with varying stiffness. For such viscoelastic materials two parameters step in: Firstly the initial elastic modulus (E_{ini}), which describes the initial stiffness upon loading and secondly, the equilibrium modulus (E_{eq}) which describes the time independent share of the stiffness. In the case of viscoelastic materials, E (being a function of time) is generally termed 'modulus'. The ratio between E_{ini} and E_{eq} gives a measure of the viscoelastic character of the material. If E_{ini}/E_{eq} is close to one, the material is completely elastic. If E_{ini}/E_{eq} is greater than two, the material is strongly viscoelastic. As could be seen in the performed experiments, small E_{ini}/E_{eq} values in the case of cartilage indicate a vital material. Small E_{ini}/E_{eq}-values help to better withstand static loading (carrying heavy loads for instance) as well as fast and demanding load cycles (e.g., from jumping or running).

The relaxation time (τ) under load is defined as shown in equation (6).

$$\tau = t\left(\frac{E_I}{\exp(1)}\right) - t(E_I) \tag{6}$$

$t(E_{ini})$ is the time at which the modulus value is called initial modulus and $t[E_{ini}/\exp(1)]$ is the time after which the modulus value decreases to approx. 36.8%. Relaxation times can only be calculated for rather viscoelastic materials with a E_{ini}/E_{eq} of more than 2.7. For the evaluation of τ, equilibrium moduli E_{eq} were measured after static loading for 30 minutes. In several cases, τ could not be measured in a reliable fashion since the modulus drop was not pronounced enough (e.g., in case of PEG-DA).

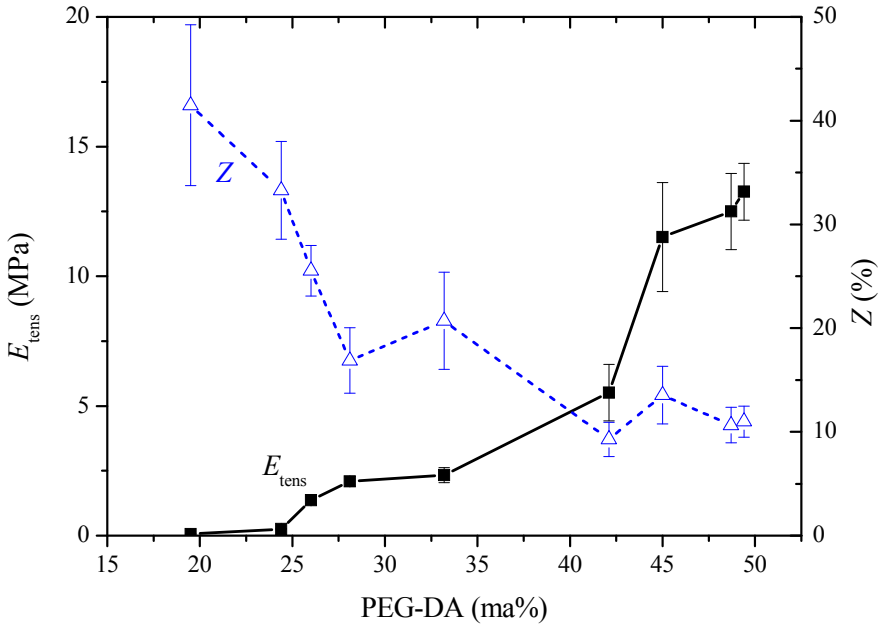
3 Results and discussion

3.1 Comparison of PEG-DA modulus values

The elastic moduli measured in tensile tests and the corresponding strains at break of PEG-DA-hydrogel samples with varying PEG-DA content are shown in Figure 3. The elastic modulus at 45 ma% PEG-DA (55 ma% water) is about 12 MPa. Strain at break (Z) decreases from about 40% down to 10% for high PEG-DA contents.

When comparing the elastic modulus and the strain at break (in tension) of artificial hydrogels based on PEG-DA and cyanoethylacrylate/PEG-DA to values observed in human and bovine cartilage (Bader and Lee, 2000; Williamson et al., 2003; Guo et al., 2007), it can be seen, that PEG-DA reaches elastic moduli comparable to cartilage, while the strain at break, which is related to the deformability of the material, is smaller than cartilage's. PEG-DA-CEA mixtures show higher strain at break, but the elastic modulus is far lower than in typical cartilage samples. This indicates that the complex structure of cartilage is necessary to achieve high toughness at the required stiffness.

Figure 3 Tensile test of PEG-DA hydrogels: elastic modulus vs. strain at break for different PEG-DA contents (see online version for colours)

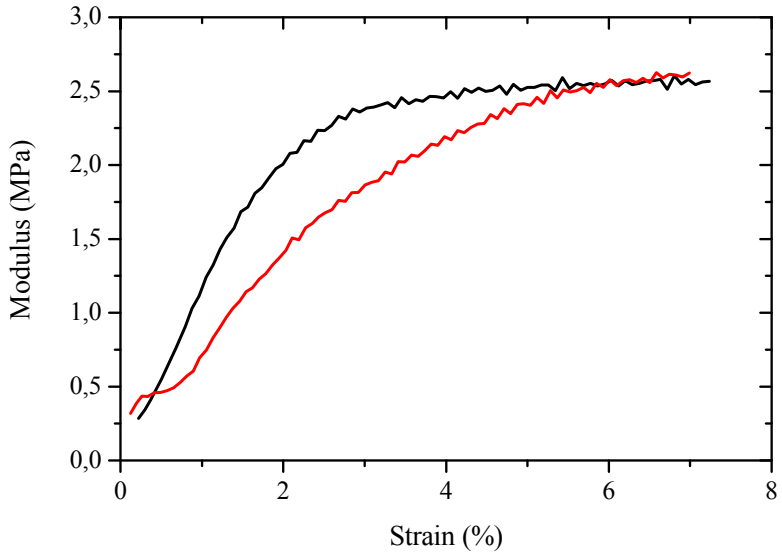


Dynamical mechanical analysis in compression showed that the modulus increases slightly with increasing frequencies. The utilised frequency band ranged from 0.1 Hz–30 Hz. For compression values of 7%, the modulus changes were 2%–5% in the investigated frequency range. The DMA measurements showed no significant temperature dependence when varying the measurement temperature between 23°C–37°C.

For the interpretation of nano-indentation measurements it has to be considered that the contact areas are relatively small compared to typical loading scenarios in a natural environment. The network density of the investigated hydrogels can vary locally and water can evaporate at the interface between sample and the atmosphere inside the chamber. Indentation methods are therefore more prone to surface effects. Due to such effects, the nano-indentation results of PEG-DA samples differ significantly from macroscopic techniques. Depending on the network density, the measured elastic moduli ranged from 1.8 MPa for 13 ma% PEG-DA to only 3.3 MPa for 50 ma% of PEG-DA.

The moduli of PEG-DA samples measured by static compression are highly depending on the applied strain. The modulus typically increases with increasing compression and reaches a plateau between 2% and 7% compressive strain. As can be seen from Figure 4, the plateau-strain varies between different samples. The main factor influencing this behaviour is the planarity of the samples. If the top and bottom-surface of the cylindrical sample are not perfectly parallel, the plateau in the modulus-compression-curve is reached at higher strains due to the inhomogeneous loading of the sample.

Figure 4 Different progressions of the modulus in compression for two PEG-DA specimens with identical water content (~67 ma%) due to geometrical differences between the samples (see online version for colours)



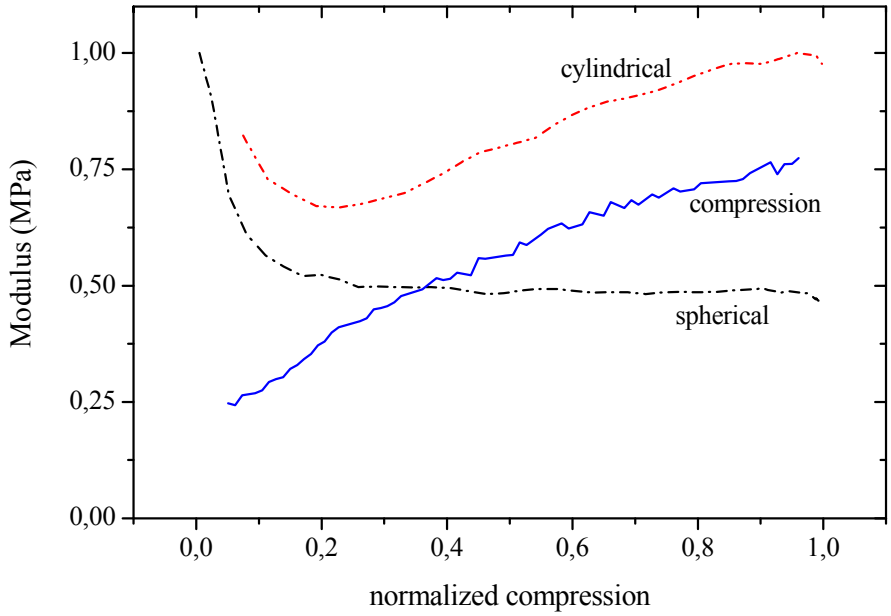
Due to the low strain at break of PEG-DA, the maximum achievable deformation is 10% and most measurements were carried out up to deformations of 7%–8%, in order to avoid damaging the sample. An indication of damages inside the sample is a modulus-compression curve where the plateau is not reached within 7% compressive strain. In the case of undamaged samples, moduli with small standard deviation could be measured. By taking into account the limitations given by the material, compression tests on PEG-DA hydrogel samples yield reproducible results and show a good correlation between PEG-DA content and elastic moduli.

While compression tests automatically give a macroscopic modulus representing the average properties of the investigated volume, indentation experiments lead to more localised results. Additionally, the deformation is not homogeneous, since the strains close to the indenter surface are higher than far away from it. In many investigations, these influences are not taken into account for the determination of the elastic modulus. As far as viscoelasticity is considered at all, initial moduli are taken after a specific time [0.6 or 2s for instance (Bader and Lee, 2000)] or when half of the load is put up onto the sample (Raesaenen and Messner, 1996).

From Figure 5, it can be seen, that indentation methods and macroscopic compression can lead to very different moduli, especially for small deformations. In case of indentation experiments, the modulus decreases significantly with progressing deformation whereas in the case of macroscopic compression, an opposite trend is observed (Gaebler, 2008). For moderate deformations (0.2–0.3) indentation and compression lead to comparable results. The shape of the indenter has to be specified. In general, spherical indenters should be preferred since, at least in the case of PEG-DA-hydrogels, there is a pronounced minimum in the modulus-deformation-curve which can be used to easily determine a well defined material property. Additionally, non-planar surfaces are less problematic for spherical indenters due to the much smaller

indentation area compared to a cylindrical indenter. Indentation measurements allow the determination of relaxation times and equilibrium moduli in one step in contrast to compression testing. The indentation data (equilibrium and initial moduli as well as τ-values) are given in Table 1.

Figure 5 Schematic representation of the measured moduli upon continuous deformation up to 7.5% of the sample thickness (either compression or penetration) (see online version for colours)



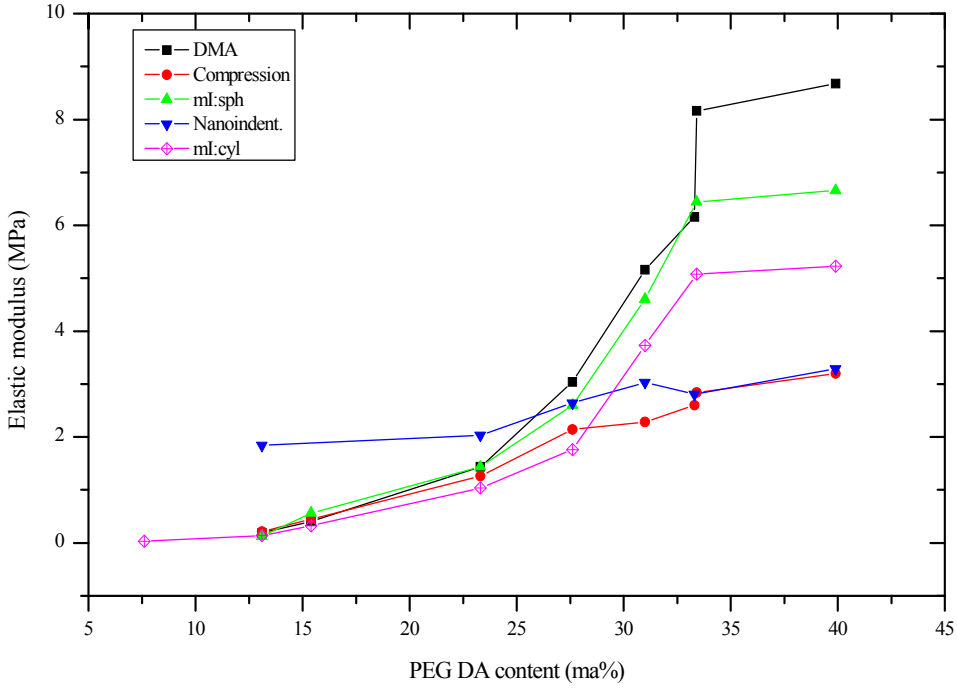
As the equilibrium moduli are only 20%–40% lower than the inital moduli, no relaxation time can be specified. This behaviour implies that the spontaneous elastic character outweighs the viscoelastic behaviour; as can be seen from Table 1, the fluctuations for E_{ini}-moduli are between 15%–30% while E_{eq}-moduli only vary between 10%–15%. The smallest variations are measured for specimens with the broadest plateau in compression. This further proves the assumption that geometrically accurate samples exhibit a pronounced plateau in the deformation-modulus curve.

In Figure 6, the initial moduli in dependence of the PEG-DA content are plotted for comparison. The depicted measuring methods include compressive test with static loading and dynamic loading (DMA) as well as indentation tests with spherical and cylindrical indenters. The numbers plotted in Figure 6 are also explicitly given in Table 2.

Table 1 Macro-indentation (spherical, indentation depth 0.5 mm) on cylindrical PEG-DA samples with different water contents

Sample	*PEG-DA (ma%)*	E_{ini} *(MPa)*	E_{eq} *(MPa)*	E_{ini}/E_{eq}	τ *(min)*
35-1	12.6	0.126–0.139	0.09–0.1	1.4	>>30
43-3	14.9	0.41–0.52	0.27–0.31	1.5	>>30
44-3	16.8	1.02–1.43	0.68–0.79	1.5	>>30
45-2	27.1	2.23–2.52	1.5–1.73	1.5	>>30
48-1	30.5	4.56–4.62	3.73	1.2	>>5
39-1	32.8	2.58–4.62	1.54–2.36	1.7	>>30
38-1	32.4	3.69–4.77	2.71–3.09	1.4	>>30
47-2	32.9	5.4–6.3	3.6–3.83	1.5	>>30
46-1	39.5	4.82–6.66	3.97–4.33	1.2	>>30

Figure 6 Experimentally determined elastic moduli (PEG-DA hydrogels) obtained by different measuring methods (mI:cyl = macro indentation with cylindrical indenter; mI:sph = … with spherical indenter) (see online version for colours)



It can be seen that the correlation between the different methods is best for materials with medium stiffness and elastic moduli around 1 MPa. Both for very soft as well as very stiff materials the relative deviations between the different methods are getting larger.

DMA showed the highest values in this comparison. This may be explained by the need for planar and perfectly parallel sample surfaces In contrast to static compression tests, the utilised DMA setup does not allow for in-situ alignment of the clamping plates, which leads to enhanced problems with non-parallel specimen surfaces. Additionally, the

measurements on the DMA systems were conducted force controlled, which leads to creeping of the samples and in consequence to higher measured moduli due to the higher imposed strains (see Figure 4).

Table 2 Initial moduli of PEG-DA hydrogels as measured with different measuring methods

Sample	*PEG-DA [ma%]*	*Nanoindent [MPa]*	*mI:cyl [MPa]*	*mI:sph [MPa]*	*Compression (MPa)*	*DMA (MPa)*
46-2	39.4	3.29 ± 0.17	5.23 ± 0.24	6.66 ± 0.92	3.19 ± 016	8.68
47-2	32.9	^|–|^	5.08 ± 0.07	6.44 ± 0.098	2.84 ± 0.006	8.16
49-4	32.8	2.81 ± 0.29	3.55 ± 0.02	3.35 ± 0.152	2.60 ± 0.016	6.16
48-1	30.5	3.03 ± 0.38	3.73 ± 0.10	4.60 ± 0.032	2.28 ± 0.009	5.16
45-1	27.1	2.65 ± 0.07	1.76 ± 0.012	2.6 ± 0.055	2.15 ± 0.004	3.04
44-1	22.8	2.04 ± 0.04	1.04 ± 0.008	1.44 ± 0.007	1.26 ± 0.014	1.43
43-3	14.9	^|–|^	0.32 ± 0.003	0.57 ± 0.006	0.44 ± 0.005	0.41
35-4	12.6	1.84 ± 0.06	0.14 ± 0.003	0.12 ± 0.016	0.22 ± 0.004	0.19
36-3	7.1	^|–|^	0.03 ± 0.001	^|–|^	0.05 ± 0.002	0.04

For the determination of the required preforce-level preliminary static compression measurements had to be carried out.

Both macro-indentation curves correlate quite well. Spherical indentation provides slightly higher moduli than cylindrical indentation. This discrepancy may be due to the differences in indenting area and stress fields, leading to different strains in the indented volume.

3.2 Cartilage properties

Cartilage moduli were highly time dependent as well as dependent on the sample history and treatment. Therefore, the viscoelastic properties are given in terms of E_{ini}- and E_{eq}-moduli as well as in relaxation times. Furthermore, these values are compared between fresh and aged, shock-frozen and slowly frozen states, as given in Table 3.

The influence of storing the samples at room temperature in PBS can be seen from the comparison between hpc#4P (53 days old) with #1P and #2P (fresh). The values for E_{eq}, E_{ini} and τ are all within the standard deviation and no change in the values due to storage can be observed.

For the hpc-samples minimal E_{ini}-moduli range from 5 MPa–7.5 MPa. The maximum E_{ini}-moduli ranged from 10 MPa–16 MPa. That fits quite well to the statement of Bader and Lee (2000) who found the short term elastic modulus of cartilage to be approximately 10 MPa. While the contact stress inside the joint is estimated to be around 5 MPa for normal load conditions, it was only between 1.8 MPa–2.8 MPa in the measurements performed for this work.

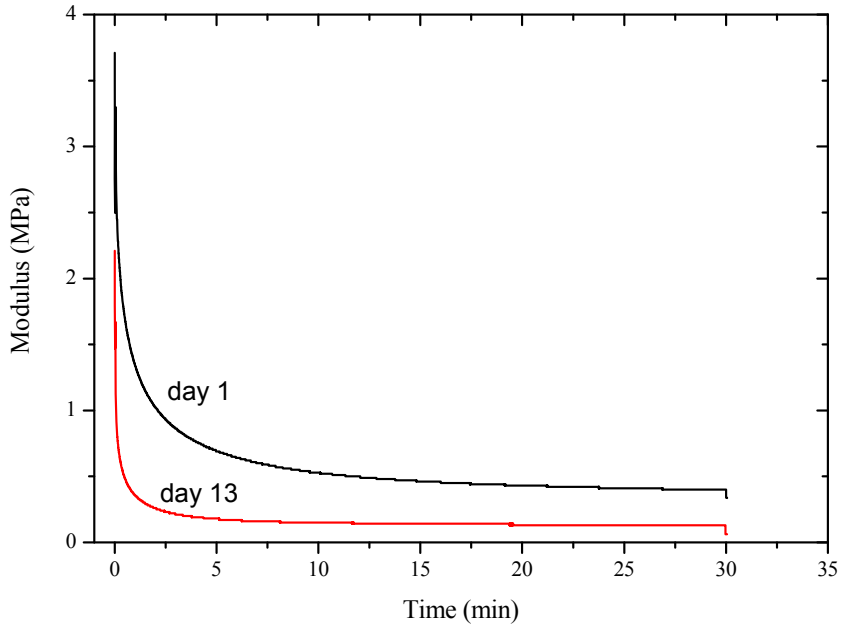
In contrast to the E_{ini}-moduli, the E_{eq}-moduli decreased massively for the frozen specimens. This decrease is larger for hpc#1P and #2P, which have been frozen slowly than for hpc#4P, which was shock frozen in liquid nitrogen. The cfc-samples were all stored at room temperature but have been frozen slowly several times before the measurement. As can be seen, E_{ini}, E_{eq} and τ are decreasing continuously even from their

low level and especially the relaxation times become extremely short. The changes are depicted in Figure 7.

Table 3 mI:sph: time dependency of the specific viscoelastic values of hpc and cfc; 't' refers to the days passed after delivery (hpc) or after thawing (cfc)

			1st measurement		
hpc (#)	*t (d)*	E_{ini} *(MPa)*	E_{eq} *(MPa)*	τ *(s)*	*M/L*
2P	0	5.18	1.34	135	3.9
1P	1	6.75	2.12	45	3.2
4P	53	7.32	1.95	122	3.8
cfc					
19	4	1.20	0.045	1.8	27
15	1	1.48	0.126	16	12
11	1	8.21	0.530	21	15
7	0	1.77	0.315	48	6
13	0	6.62	0.248	7.3	27
			2nd measurement		
hpc (#)	*t (d)*	E_{ini} *(MPa)*	E_{eq} *(MPa)*	τ *(s)*	*M/L*
2P	53	9.27	0.78	10	11.9
1P	53	7.77	0.59	14	13.2
4P	55	6.31	1.52	48	4.2
cfc					
19	12	1.48	0.033	0.8	46
15	12	1.25	0.071	11	18
11	38	1.82	0.073	2.1	25
7	39	1.07	0.035	3.1	30
13	39	1.75	0.088	0.7	20
			Difference		
hpc (#)	*t (d)*	E_{ini} *(%)*	E_{eq} *(%)*	τ *(%)*	*M/L*
2P	53	79	–42	–93	2.1
1P	52	15	–72	–69	3.1
4P	2	–14	–22	–61	0.1
cfc					
19	8	23	–27	–57	0.7
15	11	–16	–44	–32	0.5
11	37	–78	–86	–90	0.6
7	39	–40	–89	–93	4.4
13	39	–74	–64	–90	–0.3

Figure 7 mI:sph on hpc#15 different times after thawing indicating degradation in the cartilage relaxation curve (see online version for colours)



The degeneration of the mechanical behaviour can be explained by the reduction of the PGs between the measurements of the cfc samples. The half-life period was exceeded several times, leading to the significant reduction of the PG concentration. Additionally, the chondrocytes were also no longer vital at the time of the second measurement leading to further degradation of the material.

The above shown measurements reveal that freezing has a massive influence on initial and equilibrium characteristics. Especially equilibrium moduli and relaxation times show a high sensitivity and decrease down to 1%–10% within few days after thawing, while samples kept at ambient temperature showed virtually no changes. Slow freezing is worse than shock freezing and degeneration of the tissue is generally accelerated by both of them, after thawing.

Table 4 Unconfined static compression of hpc: E_{ini} moduli and relaxation times

hpc #	*t (d)*	*s (µm)*	$E_{ini,\ comp}$ *(MPa)*	τ *(min)*
1M	7	225	0.69–0.72	>>30
1P	7	153	0.51–0.56	~11
2P	7	176	0.27–0.55	~20
3P	7	153	0.66–0.91	>>30

Notes: s = absolute compression

τ = relaxation time from extrapolated relaxation curves

The unconfined static compression measurements (Table 4) lead to very low values for the initial moduli in the range of 5%–10% of the indentation values. This can be explained by the curvature of the specimens, since uneven surfaces prohibit the full contact between specimen and clamp, even at higher loads. Other research groups (Korhonen et al., 2002b), who used smaller sample diameters and/or cut off the cartilage from the subchondral bone before measuring, obtained higher values, but still only 30%–60% of the values obtained by indentation. Relaxation times with unconfined compression could be obtained from extrapolation of the force-time curves. So relaxation time may also depend on the viewed volume and stress field. Cartilage might withstand aerial induced loads longer than sharp stitches.

4 Conclusions

Several methods for the characterisation of viscoelastic properties of natural and artificial hydrogels have been investigated. The utilised methods include indentation with various indenters, tensile and compressive tests under static loading and dynamic mechanical analysis. Indentation with spherical indenters and static compression proved to be especially suitable for the investigation of these hydrogels. Both techniques can be performed on a standard universal testing device and allow fast and uncomplicated measurements of the stiffness over several orders of magnitude. The most convenient sample shape for both tests, a compact cylinder, can be produced easily (especially in the case of artificial hydrogels) and allows the handling of relatively soft materials. For hpc-samples, unconfined compression did not produce valid results because of the sample curvature.

It has to be emphasised that viscoelastic constants are not independent from the measurement technique, as has been reported by Jurvelin et al. (1997) and Toeyraes et al. (1999). It is essential always to report the exact details of the utilised measurement technique. Korhonen et al. (2002b) report that the elastic modulus from indentation is also dependent on indenter size, which was not considered in this paper.

It could be shown that PEG-DA samples with an elastic response similar to human articular cartilage could be prepared. But compared to cartilage, the toughness of PEG-DA is relatively low. It was also shown that the characterisation of the classic elastic moduli is insufficient for the assessment of cartilage materials. The creeping behaviour of these materials must be addressed too. For adequate characterisation of cartilage, the knowledge of the storage history of the material is of importance. Freezing has significant influence on the mechanical properties of articular cartilage. If the measurements can be completed within few weeks, it should not be frozen at all. If for some reason freezing is required, shock frosting is to be preferred.

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