Poly(ethylene glycol)-Based Hydrogels as Cartilage Substitutes: Synthesis and Mechanical Characteristics

A. Rakovsky, D. Marbach, N. Lotan, Y. Lanir

Faculty of Biomedical Engineering, Technion, Israel Institute of Technology, Haifa 32000, Israel

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ABSTRACT: Cartilage substitutes are needed to replace cartilage tissue, damaged in accidents or by pathologies (e.g., osteoarthritis). Treatment by total hip replacement has disadvantages, particularly due to immunological reaction to the implant's wear debris. One promising alternative is to replace damaged cartilage with substitutes based on hydrogel-type material, designed to mimic the structure and properties of cartilage. The development of such a substitute must consider a wide spectrum of requirements. In this study, we addressed one aspect of this development namely the preparation and investigation of hydrogels exhibiting the required mechanical characteristics. To this aim, poly(ethylene glycol) (PEG) hydrogels and amphiphilic interpenetrating polymer networks (IPNs) of PEG with poly(methyl methacrylate) (PMMA) were prepared and characterized for their mechanical and swelling properties. Twenty-seven types of hydrogels were synthesized, differing in their composition: PEG molecular weight, crosslink density, and PMMA volume fraction. The properties measured were water content, compression modulus, strength, fatigue durability, and poroelastic properties (hydraulic permeability and equilibrium modulus). All were investigated as functions of hydrogel's composition. Results show that lower PEG M_w, higher crosslink densities and higher PMMA fraction, all lead to higher modulus and lower water content, and that these properties can be controlled independently by proper choice of ingredients. Introduction of IPN greatly improved the hydrogels' strength. No reduction in the compression modulus resulting from fatigue damage was evident. Poroelastic properties varied nonmonotonously with structural characteristics. Seven types of the hydrogels were found to fit cartilage in their water content, modulus, and poroelastic properties. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 112: 390–401, 2009

Key words: cartilage substitutes; hydrogel; interpenetrating polymer networks (IPN); amphiphilic; poroelasticity; mechanical properties

INTRODUCTION

Dysfunctional or damaged cartilage is a major health concern in many developed countries. In the United States alone, nearly 20 million people suffer from osteoarthritis, a condition which results in significant impact on quality of life.[1] With population aging in these countries over the next decades, these numbers are expected to rise even more because of the relatively high frequency of osteoarthritis among older people.

Joint replacement is one of the most abundant treatments in case of severe cartilage degeneration.[2] In this procedure an ultra high molecular weight polyethylene (UHMWPE) implant serves as artificial cartilage, thus providing lubrication at the joint. Two major disadvantages may restrict application of this treatment modality: first, the shock absorbing features of natural cartilage are absent in this stiff plastic implant, which may lead to damage to the underlying bones and to the implant itself; and second, there are biocompatibility problems with the wear debris of the implant.[3,4]

There is thus a growing need for cartilage substitutes which provide the required features of natural cartilage, and do so for long periods of time. In seeking for such substitutes, the first goal is to develop materials which mimic the cartilage global function (e.g., compressive load bearing, low friction, and biocompatibility). Focusing on the global cartilage properties emphasizes that similar to other organ substitutes, it is the overall function of the cartilage that one intends to mimic, rather then its detailed micro-structure. The latter is one way of achieving the desired properties but not necessarily the only one.

Hydrogels are a unique class of materials consisting of highly swollen, hydrophilic, polymeric networks.[5] Because of their high water content, hydrogels exhibit mass transfer properties close to those of pure liquids, whereas their mechanical properties may approach those of solids. These features resemble the properties of many biological tissues, making hydrogels suitable candidates for a variety of biologically oriented applications, including

Correspondence to: Y. Lanir (yoram@bm.technion.ac.il).
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cartilage replacement. Numerous methods are available for synthesis of hydrogels, resulting in diverse chemical, mechanical, and swelling properties.[6,7] Some of these methods were used in various attempts to synthesize and evaluate hydrogels as cartilage substitutes.[8–12] These attempts were, however, mostly focused on friction and wear properties of these materials. Little or no attention was given to other important features of hydrogels in this area.

Hydrogel-based cartilage substitutes have to be mechanically comparable with the natural cartilage. The latter is a specialized connective tissue. It consists of cells and extracellular components, but unlike other tissues, does not contain blood vessels or nerves. For this reason cartilage does not repair itself effectively. Articular cartilage has a complex, three-dimensional fibrous-fluid structure which provides the ability to bear and distribute load and slide under very low friction. The extracellular matrix is the main component of this tissue and is responsible for most of its properties, in particular mechanical, swelling, and lubrication. The composition of the matrix is dominated by water (65–80%), collagen type II fibers (15–22%), and charged proteoglycans (4–7%).[13,14]

The physical mechanism of swelling phenomena of neutral hydrogels is different from that of cartilage tissue. Although in cartilage the swelling pressure arises mainly from the Donnan effect which stems from the presence of fixed, negatively charged groups of the proteoglycans, it's origin in neutral hydrogels lies in hydrophilic interactions between the solvent (water) and the polymer itself. Nevertheless, both cartilage and hydrogels are poroelastic materials—upon external pressure they undergo a biphasic creep process during which the fluid phase slowly exudates from the tissue/gel until equilibrium is reached at the point when the external pressure is equal to the sum of pressure exerted by the elastic forces of the solid phase and the swelling (osmotic) pressure of the fluid phase in the tissue/gel. The main mechanical properties of these poroelastic materials under compression originate in their water content, $W(\%)$, (which determines the polymer concentration and resulting osmotic forces), their hydraulic permeability, k, (which determines the rate of the creep process), and the intrinsic rigidity (i.e., modulus, E_s) of their solid phase (which, together with the water content, determines the magnitude of creep at equilibrium). For natural cartilage, water content, permeability and modulus vary with location, age, and gender. Their pertinent values reported[14] are within the ranges of 65–80% for $W(\%)$, 1.2–6.2 $\times$ 10^{-16} $m^2/(Pa \cdot s)$ for k, and 0.41–0.85 MPa for E_s, respectively. These properties are key features which allow cartilage to serve as the load-bearing material of diarthrodial joints and to provide absorption of mechanical shocks and even distribution of the joint loads across the underlying bony structures, with excellent lubrication and wear characteristics.

Cartilage is a biphasic material with poroelastic behavior. As such, its deformation (and, hence, the associated modulus) depends on time and on the rate of loading. *In vivo* cartilage is subjected to many years of loading cycles at various rates. It has been demonstrated that the compressive modulus determined at 20 ms after loading is 32–75% higher than that determined after 2 s.[15,16] The same is true for the compressive strength of the tissue: Ottani et al.[17] indicates a value as low as 1 MPa, Obeid et al.'s[18] results are in range of 14.5 MPa for cartilage loaded at 1 mm/s rate (for $\sim$ 3 mm thickness samples), and Kerin et al.[19] reported mean values of 35.7 MPa for 1 s duration experiments. Hence in addition to its poroelastic features, a substitute must be similar to cartilage in other mechanical properties, such as the instantaneous compressive modulus and fatigue characteristics. The latter have so far not been reported for cartilage substitutes. The "instantaneous" elastic modulus of cartilage, E, (which characterizes its response at much shorter times than the equilibrium modulus, E_s) is always higher then E_s since, prior to equilibrium, a portion of the applied pressure is supported by fluid which did not exudate out of the specimen.

Mechanical and swelling properties of hydrogels can be related to their chemical composition and structure using analytical models based on statistical and classical thermodynamics, particularly on rubber elasticity theory. A most useful model was developed by Peppas for hydrogels prepared in presence of solvent.[20] It establishes the relationship between stress σ and elongation α for a nonionic hydrogel obtained by crosslinking previously made linear polymer and by subsequently swelling it in water. The pertinent relationship is:

$$\sigma = \frac{\rho RT}{\overline{M}_c}\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} v_{2,r}\left(1-\frac{2\overline{M}_c}{\overline{M}_n}\right)\left(\alpha-\frac{1}{\alpha^2}\right) \qquad (1)$$

where ρ is the bulk density of the polymer, T and R are the absolute temperature and gas constant, $\overline{M}_c$ is the number average molecular weight between crosslinks, $\overline{M}_n$ is the number average molecular weight of the polymer before crosslinking, $v_{2,s}$ is the polymer volume fraction in the hydrogel at equilibrium swelling, and $v_{2,r}$ is the polymer volume fraction immediately after preparation. This expression works well for neutral hydrogels under small deformations.[20]

Equation (1) suggests two possible ways to control mechanical properties of a hydrogel: by changing the average molecular weight between crosslinks ($\overline{M}_c$), or by changing the polymer volume fraction in the gel immediately after preparation ($v_{2,r}$).

The first parameter may be controlled during the synthesis stage by altering the monomer/polymer to crosslinker molar ratio or altering the polymer molecular weight. Short polymer molecules between crosslinks or high crosslinker concentrations will lead to low $\overline{M}_c$. It is also influenced by the presence of solvent during the synthesis of the network. The Peppas-Merill equation[20] establishes a widely used method for determination of $\overline{M}_c$ from swelling properties of a hydrogel:

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\overline{V}/V_1\left(\ln(1-v_{2,s}) + v_{2,s} + \chi v_{2,s}^2\right)}{v_{2,r}\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{2}{f}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]} \quad (2)$$

where $\overline{V}$ is the specific volume of the polymer, V_1 is the molar volume of water, χ is the polymer-water interaction parameter, and f is the crosslinker functionality.

The second parameter, $v_{2,r}$, may be controlled by altering the concentration of monomer/polymer in the crosslinking reaction. Thus, hydrogels prepared from more concentrated solutions will have higher $v_{2,r}$ whereas those prepared in bulk (such as PEG or HEMA[21] based hydrogels), will have $v_{2,r} = 1$, and higher elastic modulus as a consequence.

A completely different approach for affecting the mechanical properties of hydrogels is through formation of interpenetrating polymer networks (IPNs) (i.e., by addition of a second, independently crosslinked network which, while being formed, becomes interwoven with the first one), since there are some studies showing significant improvement of these properties in IPNs.[22,23] Of special interest are amphiphilic IPNs, in which one network is hydrophilic and the other one is hydrophobic.

Based on these considerations, we focused on polyether-urethane (PEU) hydrogels and PEU/poly (methyl methacrylate) interpenetrating polymer networks (PEU-PMMA IPNs) prepared under bulk polymerization conditions. PEG is a hydrophilic polymer with good biocompatibility properties and with low melting temperature, thus allowing for bulk polymerization under relatively mild conditions.[24] Glycerol was used as trifunctional crosslinker and isophorone diisocyanate (IPDI) as chain extender, thus leading to formation of urethane links between the network components.[25] PMMA was chosen as hydrophobic component of IPNs due to several reasons: first, free radical polymerization, by which it is synthesized, can run independently and simultaneous with PEU polymerization, meaning that both networks in the IPN can be formed simultaneously; and second, PMMA was long used as bone cement with good stability and biocompatibility performance.[26]

The goals of the present study were, therefore, to synthesize and evaluate hydrogel type materials with mechanical properties close to those of articular cartilage. Although from a polymer perspective it is to be expected that lower M_w of PEG, higher crosslink density, and introduction of a second, more hydrophobic network would lead to a higher modulus and lower water content, our study aimed at something different: reliable quantification of these relationships, which is essential for the design of appropriate cartilage substitute. This, to the best of our knowledge, has not been reported before. Within the aim stated, the following target characteristics were set: water content of at least 60%, compression modulus of at least 1 MPa at low strain rates, while the hydraulic permeability, k, and the equilibrium modulus, E_s, should be within the ranges stated above.[14] Moreover, fatigue durability of at least 10 million cycles (equivalent to roughly 10–15 years of normal daily activity) was also required. It was hypothesized that functional, cartilage-like hydrogels can be synthesized by controlling their structural parameters through suitable combinations of their ingredients. This hypothesis was tested by synthesizing hydrogels of various monomers, crosslink densities, and IPN contents, and by evaluating the products for their water content, immediate and equilibrium compressive stiffness, hydraulic permeability, fatigue resistance, and compressive strength.

TABLE I
PEG Samples Used

Designation	Fluka Cat #	$\overline{M}_n$	PDI
1000	81190	990	1.17
1500	81210	1490	1.24
2000	81221	1987	1.22

$\overline{M}_n$, number average molecular weight; PDI, polydispersity index, as provided by the manufacturer.

MATERIALS AND METHODS

Materials

Poly(ethylene glycol) (PEG) of molecular weight of 1000, 1500, and 2000 was purchased from Fluka (Buchs, Switzerland). Details of number average molecular weight ($\overline{M}_n$) and polydispersity index (PDI) are indicated in Table I. Glycerol (Baker 7044, 99.5%, Phillipsburg, NJ), isophorone diisocyanate (IPDI, Aldrich 317624, 98%, Munich, Germany), and di(ethylene glycol) divinyl ether (DEGDVE, BASF 53203452, min 99.5%, Ludwigshafen, Germany) were used as received. Methyl methacrylate (MMA, Fluka 64200, $\geq$99.0%) was freed from stabilizer (hydroquinone) by passing through an inhibitor remover column (Aldrich, 306312). Dibenzoyl peroxide, (BPO, Fluka 33581, $\geq$97.0%, dry based) is supplied wetted

TABLE II
Parameters Controlled During the Synthesis of Hydrogels and Some Examples of Specimen Designations Used

PEG M_w	CLD	% IPN	Designation
1000	0.5	0	PEU-1000-0.5
1500	0.75	10	PEU-1500-0.75-PMMA-10
2000	1	20	PEU-2000-1-PMMA-20
XXX	YY	Z	PEU-XXX-YY-PMMA-Z

CLD, cross-link density; % IPN, volume fraction of MMA in reaction feed.

with water and was dried by the following procedure[27]: small amount of BPO was placed in a beaker with 100% ethanol, mixed and left for 15 min. It was then filtered under vacuum and thoroughly dried.

Synthesis

As part of this study, two classes of materials were synthesized: (a) single-system hydrogels (PEU hydrogels) and (b) two-system, interpenetrating polymer networks (PEU/PMMA IPNs). The single-system hydrogels were obtained following the basic approach reported previously,[28–30] but modified as required. In the present study, PEG, IPDI, and glycerol were used as the macromere, chain extender and crosslinker, respectively. The two-system IPN-type materials were obtained by a procedure described elsewhere,[31] modified as required. PEG, IPDI, and glycerol were used to form the primary, hydrophilic polymer network as described above, whereas MMA, DEGDVE, and BPO were the monomer, crosslinker, and the initiator, respectively, forming the secondary, hydrophobic network.

Various hydrogels and IPNs were synthesized by changing three major structural parameters: PEG molecular weight (PEG M_w), the glycerol-to-PEG molar ratio (the crosslink density, CLD) and MMA volume fraction in the reaction feed (% IPN). Accordingly, 27 different types of materials were prepared. Table II lists a few examples of samples used in the present research, their parameters and designations.

A major obstacle in obtaining good quality, homogeneous, gel specimens was the extensive formation of bubbles during the polymerization process. This results from the reaction between IPDI and traces of water present and from MMA evaporation due to highly exothermic nature of PEU formation during IPN preparation. To overcome this bubbles problem, the following procedures were developed: molds were designed on one hand to maximize heat loss and, on the other hand, to minimize bubble entrapment. The following procedures proved to provide reasonable results.

Synthesis of PEU hydrogels

Desired amounts of PEG and glycerol were weighted into a round bottom flask provided with a Dean-Stark apparatus and azeotropically distilled with toluene to remove the traces of water present. The solvent was then evaporated on a rotary evaporator at 60°C to keep PEG melted. Subsequently, an amount of IPDI, calculated to achieve a 1 : 1 -OH/-NCO molar ratio was added and thoroughly mixed. Air trapped during mixing was removed by holding the mixture for 1 min under vacuum. The melt was poured into preheated molds and placed in an oven kept at 70°C. After 20 h, the gels thus obtained were removed from the molds, cooled and placed into DI water for swelling.

Synthesis of interpenetrating polymer networks

MMA was crosslinked with DEGDVE at 5% molar concentration. BPO was used as thermal initiator at 0.5% w/v concentration (relative to the MMA/DEGDVE mixture). Required amounts of MMA, DEGVE, and BPO were mixed together and kept for at least 1 h under dry nitrogen bubbling. Then, desired amount of this mixture was added into the PEG melt just after addition of IPDI (as described above), mixed, degassed for 1 min, poured into a preheated mold, and placed in oven at 70°C. After 20 h, the gels thus obtained were removed from the molds, cooled, and placed into DI water for swelling.

Specimen preparation

Two geometries of specimens were used: mechanical testing required cylindrical sample with diameter-to-thickness ratio of at least 2 : 1 (to prevent buckling under compression) and thickness of at least 4 mm (to guarantee acceptable accuracy of strain measurement); on the other hand, biphasic creep tests required thin disks with radius-to-thickness ratio of at least 10 : 1 (to guarantee compatibility with assumed one dimensional response). Therefore, molds for sheets of the required thicknesses were made of two glass plates coated with PTFE adhesive tape (for easy release of the moldings) and silicone rubber spacer of 4 or 0.5 mm width, respectively.

After polymerization, gels were released from the mold, weighted, allowed to swell in DI water for at least 48 h and weighted again. Specimens were then cut from the gel sheets using a cork borer, and 12.5 mm radius cylinders (for mechanical testing) and 19.3 mm radius thin disks (for poroelastic creep

testing) were thus obtained. The samples' swollen thickness varied among the hydrogels depending on their composition, and was measured before testing.

Swelling and water content

The water content of a hydrogel, $W(\%)$, was determined gravimetrically using the following expression:

$$W(\%) = \frac{M_s - M_d}{M_s} \times 100\% \quad (3)$$

where M_s is the weight of hydrogel at equilibrium swelling and M_d is its dry weight measured after preparation and before swelling.

Compression modulus and strength

Unconfined compression modulus and compressive strength of the hydrogel samples were calculated from the stress-strain relationships as measured using Instron Single Column Testing System (Instron). The testing chamber consisted of two horizontal positioned Perspex plates above and below the sample. The lower was placed on the machine base and the upper one was attached to a moving console via a load cell. The plates were lubricated with silicone grease to allow free lateral expansion of the specimen during loading. Reference (load-free) dimensions (diameter and height) of each specimen were measured by caliber. The experimental protocol consisted of one compression cycle with displacement up to 10% of reference height, at compression rate of 0.5%/s. Data thus measured were plotted as stress-strain relationship. The compression modulus was calculated from the linear regression of the stress-strain data. Compression strength tests were performed under a similar protocol and carried out up to failure. Failure stress and strain were recorded.

Fatigue testing

Fatigue durability of the samples was assessed using a setup presented schematically in Figure 1. Samples were placed in the testing chamber between two solid porous filters. The upper filter was attached to a Perspex lid placed on a vertical shaft. Cyclic strain was applied to the samples through balanced horizontal arm attached at one end to an eccentric motor shaft as seen in the figure. Displacement amplitude was detected by an LVDT sensor and adjusted to the desired value by changing radius of the eccentric connector. The forces developed during the load were measured and recorded by a force transducer placed on the arm.

Gels were subjected to 10^6 cycles of fatigue testing at $\sim$ 1.5 Hz frequency and at 10% strain. Compression moduli were measured and compared with reference value (prior to fatigue testing). In later experiments, extended fatigue evaluation was performed on PEG-2000-0.75-PMMA-10 gels. Samples were subjected to 12 million fatigue cycles with compression modulus measured every 10^6 cycles. Time control (uncompressed) samples from the same batch were time-lapsed tested simultaneously with the fatigue-tested samples, to examine the effect of time independently from that of fatigue.

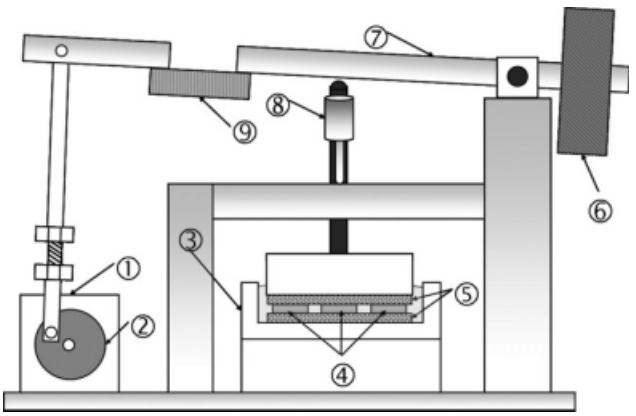

Figure 1 Fatigue testing setup: (1) DC motor; (2) eccentric connector; (3) testing chamber; (4) specimens; (5) porous filters; (6) counter balance; (7) balanced horizontal arm; (8) LVDT displacement sensor; (9) force transducer.

Hydraulic permeability and equilibrium modulus

Upon exposure to external pressure, poroelastic materials like cartilage or hydrogels, undergo a creep process, due to slow water exudation out of the porous solid phase. The rate of this process is controlled by the hydraulic permeability (k), usually expressed in units of $m^2/(Pa \cdot s)$. The equilibrium creep deformation is determined in the uncharged hydrogels by the intrinsic Young modulus E_s. The dependence of creep rate on k is rather complex, so both parameters were estimated by curve fitting of a mathematical model[14] to the experimental creep data. The model was developed based on bi-component mixture theory. To simplify the mathematical and experimental treatment, it is desirable to consider a one dimensional case. This can be achieved with specimens with high radius to thickness ratio (R/H_i), as described in Figure 2.

If such a specimen is pressed between two solid but permeable surfaces, both the solid deformation and fluid flow will be one dimensional. The present model assumed quasi-static conditions, small deformations, constant k and E_s, homogenic, free of voids material, and incompressible solid and fluid phases. Then, by using Hooke's law for small elastic deformation, Darcy's law for fluid filtration through the porous solid, and mass and force balance equations, the time (t) and position (Z)-dependent strain of the

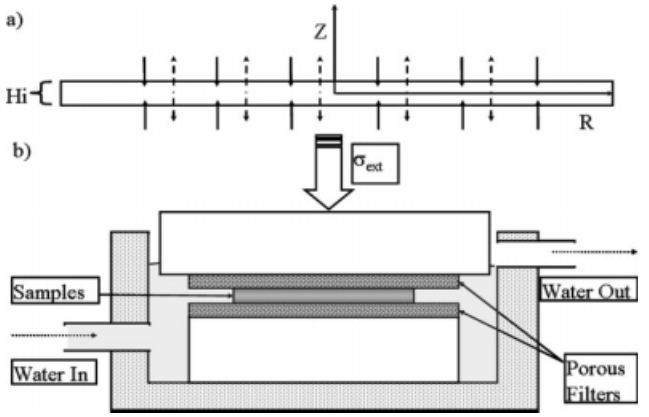

Figure 2 Creep testing setup: (a) Geometry of a specimen for one dimensional creep tests. Solid arrows represent solid phase creep and dashed arrows represent fluid phase exudation; (b) testing setup.

sample $\varepsilon(t,Z)$ is governed by the following differential[32–34]:

$$\frac{\partial \varepsilon}{\partial t} = kE_s \frac{\partial^2 \varepsilon}{\partial Z^2} \tag{4}$$

Equation (4) is similar to the equation of heat conduction and to Fick's second law of diffusion, and can be solved if boundary and initial conditions are known. In the present case, these conditions are: (1) at infinite time the system reaches equilibrium; (2) the solution must be symmetric with respect to the mid-plane at $Z = 0$; (3) at the surface of the specimen the hydrostatic and osmotic pressures are mutually equal at all times after loading. Under these conditions, the final solution of eq. (4) becomes:

$$\Delta\varepsilon(Z,t) = \frac{4\sigma_{ext}}{E_s\pi}\sum_{n=0}^{\infty}\left[\frac{(-1)^{n+1}}{(2n+1)} \times \cos\left(\frac{2n+1}{H_i}\pi Z\right) e - \left(\frac{2n+1}{H_i}\right)^2 \pi^2 E_s k t\right] + \frac{\sigma_{ext}}{E_s} \tag{5}$$

where $\Delta\varepsilon$ is the strain relative to the initial (swollen) configuration, H_i is the specimens' initial thickness, k is the hydraulic permeability, σ_{ext} is the external loading pressure (negative under compression), and E_s is the equilibrium modulus of the gel.

The thickness of the specimen as function of time, $H(t)$, can now be developed by integrating eq. (5) over the whole range of Z from $-H_i/2$ to $H_i/2$:

$$H(t) = H_i + \frac{H_i\sigma_{ext}}{E_s} - \frac{8H_i\sigma_{ext}}{E_s\pi^2}\sum_{n=0}^{\infty}\left[\frac{1}{(2n+1)^2} e - \left(\frac{2n+1}{H_i}\right)^2 \pi^2 E_s k t\right] \tag{6}$$

This equation may be used for fitting experimental data and calculating both the permeability and the equilibrium modulus of the gel. Thin hydrogel disks 0.55–0.85 mm thick (depending on the swelling degree of each particular sample) and 19.3 mm in diameter, were subjected to a constant pressure between two porous filters. The pressure was chosen so that, on the one hand, the total creep will be no more than 10% of the thickness and, on the other hand—to allow for sufficient measuring accuracy. Hence, depending on the modulus of the gel, the pressures were set between 0.037 and 0.114 MPa. The system is schematically presented in Figure 2. Time dependent poroelastic creep was measured by a dial gauge with continuous monitoring by a web-cam. Since due to uncontrolled end effects there is an immediate deformation of the specimen after pressure application (this is not taken into account by the model), the actual initial thickness H_i is lower than measured before pressure application and was therefore considered as a parameter to be estimated. Hence the hydraulic permeability, equilibrium modulus, and the initial thickness of the gel were estimated from data of displacement versus time using a curve fitting software program. Example of data measured and nonlinear curve fit is presented in Figure 3.

RESULTS AND DISCUSSION

Results are presented and discussed in terms of the dependence of the samples' mechanical properties (swelling, compression modulus, strength, morphology, fatigue durability, hydraulic permeability, and equilibrium modulus) on the PEG molecular weight, crosslink density, and IPN volume fraction of the gel.

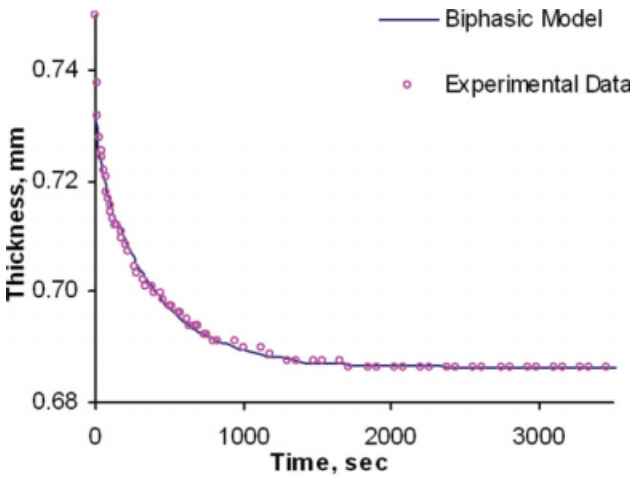

Figure 3 Representative creep data and the curve fitting by a bi-component creep model [eq. (6)]. The sample used was PEG-1500-0.5. The parameters estimates were: $H_i = 0.733$ mm, $k = 2.4 \times 10^{-16}$ m^2/Pa · s, $E_s = 0.57$ MPa. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

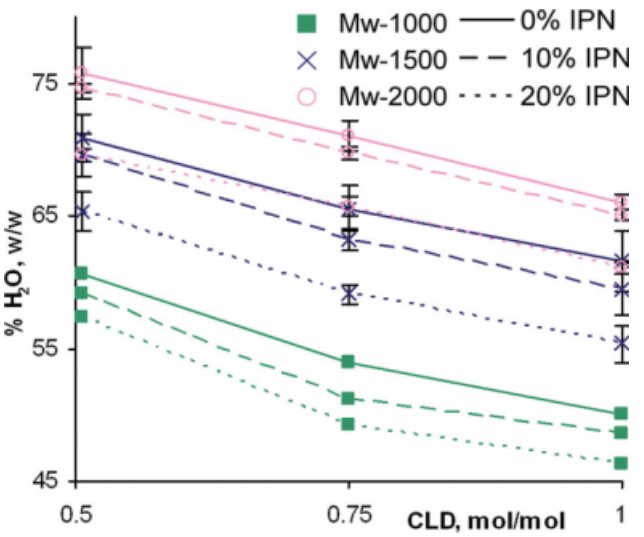

Figure 4 Water content of PEU hydrogels and PEU-PMMA IPNs as function of PEG M_w, crosslink density (CLD) of PEU network and IPN's PMMA fraction. The symbols (■), (X), and (○) are indicative for samples with PEG of molecular weight of 1000, 1500, and 2000, respectively. The lines (—), (– – –), and (---), are indicative for gels containing 0, 10, and 20% IPN, respectively. The number of samples $n = 1$ for M_w-1000 gels, $n = 4$ for M_w-1500 and M_w-2000 gels. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Swelling and water content

In Figure 4, results of water content of PEU hydrogels and PEU-PMMA IPNs obtained at various crosslink densities, MMA feed volume fractions, and PEG molecular weights, are presented. It can be seen that the amount of water depends on these factors in the following manner: increasing crosslink density and PMMA content decrease the extent of swelling, whereas higher M_w of PEG increases it. It is interesting to analyze the quantitative aspect of the relationships between these parameters: when the PEU network has high molecular weight between crosslinks $\overline{M}_c$ (i.e., long PEG molecules or low crosslink density or both) the reduction in water content resulting from addition of 10% PMMA is insignificant, as may be seen by comparing between the curves of PEU-2000-YY (circles, solid line) and PEU-2000-Y-PMMA-10 (circles, dashed line). When the amount of the hydrophobic component is high (20%), a corresponding and significant drop in water content is always present (circles, dotted line). However, as the PEU network becomes more compact (shorter PEG molecules or higher CLD as in specimen based on PEG-1000), there is a decrease in water content in all cases (squares). An explanation for these differences between high and low $\overline{M}_c$ may stem from the fact that these gels have different morphologies (results presented below). Interestingly, PEU-2000-YY-PMMA-20 gels (circles, dotted line) have almost the same water content as PEU-1500-YY gels (X's, solid line) for all crosslink densities.

In terms of the application considered, several of the gels prepared in the present study have the desired water content (between 60 and 80%) to be considered as cartilage substitutes. But, as can be seen in Figure 4, only a fraction of the gels with PEG-1000 have reached the required 60% water content. Based on this finding, all gels of PEG-1000 were excluded from further testing.

Compression modulus

Low PEG molecular weight, high crosslink density and high PMMA content of the gels were all found to lead to high values of the compression modulus (Fig. 5), as expected from the rubber elasticity and equilibrium swelling theories [eq. (1)]. All gels prepared have compression moduli of about 1 MPa or higher, as required for cartilage substitutes (see research goals). This is consistent with data from other sources for PEU gels of similar composition.[28]

The compression moduli of the gels in which $\overline{M}_c$ of the PEU network is high, changes very little as a result of 10% addition of PMMA, as indicated by comparing the curves of PEU-2000-YY (circles, solid line) and PEU-2000-YY-PMMA-10 (circles, dashed line) in Figure 5. However, when M_c is low (as in

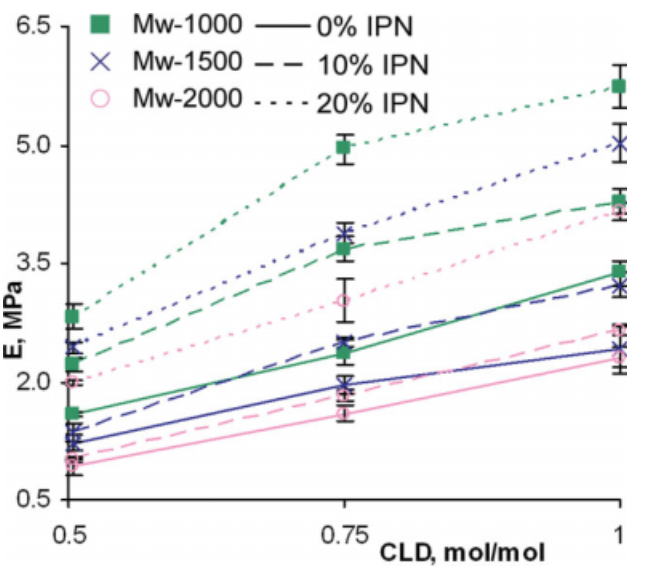

Figure 5 Instantaneous compression modulus, E, of PEU hydrogels and PEU-PMMA IPNs as function of PEG M_w, crosslink density (CLD) of PEU network and IPN's PMMA fraction. The symbols (■), (X), and (○) are indicative for samples with PEG of molecular weight of 1000, 1500, and 2000, respectively. The lines (—), (– – –), and (---), are indicative for gels containing 0, 10, and 20% IPN, respectively. The number of samples $n = 8$ for samples M_w-1000 gels, $n = 8 \times 4$ (number of batches) for M_w-1500 and M_w-2000 gels. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

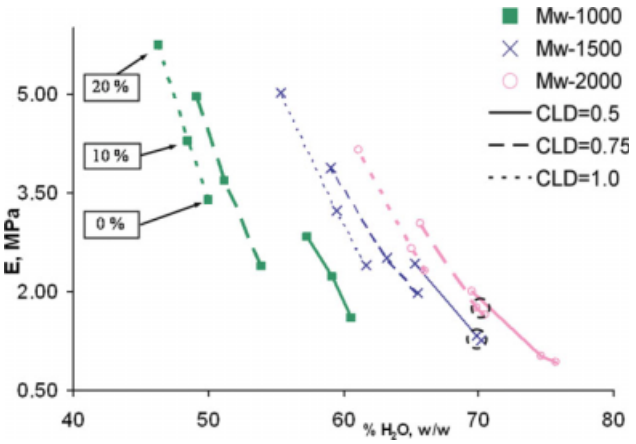

Figure 6 Relationships between instantaneous compression modulus, E, and water content, $\%H_2O$, of PEU hydrogels and PEU-PMMA IPNs. The symbols (■), (X), and (○) are indicative for samples with PEG of molecular weight of 1000, 1500, and 2000, respectively. The lines (—), (– – –), and (---), are indicative for gels with CLD of 0.5, 0.75, and 1.0. Within each line, the lowest point represents samples with 0% IPN, the point in the middle with 10% IPN, and the highest with 20% IPN, as shown by boxes at the left side of the figure. PEU-1500-0.5 and PEU-2000-0.75 gels and their 10% IPN derivatives are encircled to demonstrate mutually close mechanical and swelling properties within each group. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

case of gels based on PEG-1000, squares in Fig. 5) or when 20% PMMA is added (all dotted lines in Fig. 5), the modulus increases significantly. Again, different morphologies of the gels may explain these differences.

Relationships between compression modulus and water content

Equation (1) suggests that lowering the water content of the gel will result in higher elastic modulus. However, Figure 6 clearly shows that several specimens have mutually similar water contents, yet vary in their compression moduli. To explain this fact, it should be recalled that eq. (1) was developed for ideal, homogenic materials and is not necessarily valid for interpenetrating networks. Another way to look at these results is that by varying structural parameters (PEG M_w, CLD and % IPN), gels with the same modulus but different water content (up to 10% difference) may be obtained.

Immediate compressive strength

Both water content and compression modulus of loosely crosslinked gels (high PEG M_w and low CLD) change very little as a result of 10% IPN addition (Figs. 4 and 5). This phenomenon was especially pronounced in PEU-1500-0.5 and PEU-2000-0.75 gels and their 10% IPN derivatives, (Fig. 6, circled data symbols). In the same time, the results presented in Figure 7 show that addition of the second (PMMA) network has a significant effect on the compressive strength: in both PEU-1500-0.5 and PEU-2000-0.75 gels, the failure stress in IPNs is about two fold higher than in pure gels and the failure strain is about 15% higher. Single network specimens failed at 1.5 ± 0.5 MPa for PEU-1500-0.5 and at 1.6 ± 0.15 MPa for PEU-2000-0.75, with no significant difference between the two groups ($P > 0.05$). Gels with 10% IPNs failed at significantly higher stresses: 3.5 ± 1.1 MPa for PEU-1500-0.5-PMMA-10 and 3.2 ± 0.5 MPa for PEU-2000-0.75-PMMA-10, again, with no significant difference between the groups ($P > 0.05$). Both of these values are still lower then those reported for cartilage.[17–19] However, it should be recalled that the deformation rate here is much slower compared with those used with cartilage in the related experiments. The strength at higher

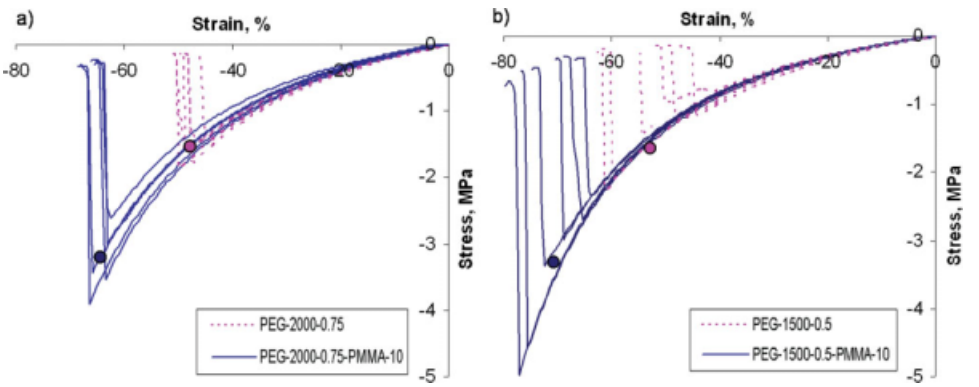

Figure 7 The effect of adding 10% PMMA on compressive strength of weakly crosslinked IPN hydrogels at 0.5% strain rate. The strength of 10% IPN samples (solid lines) is nearly double ($P < 0.05$) of that of single network hydrogels (dashed lines), although the stiffness is about the same. (a) Hydrogels of PEG-2000-0.75 and PEG-2000-0.75-PMMA-10; (b) hydrogels of PEG-1500-0.5 and PEG-1500-0.5-PMMA-10. Each line represents a different specimen, $n = 6$. The dots (●) represent sample-average failure stress and strain. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

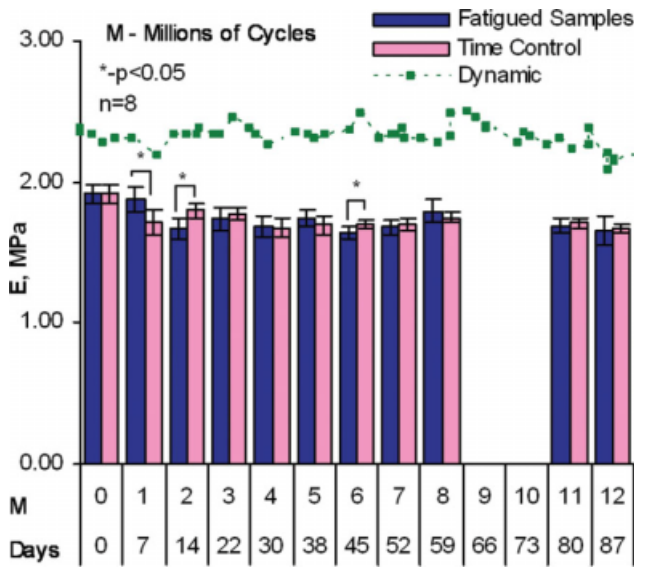

Figure 8 Fatigue durability of PEU-PMMA IPNs. Bars: instantaneous compression modulus of PEG-2000-0.75-PMMA-10 IPN as function of number of fatigue cycles and time. Squares (■): dynamic modulus calculated from LVDT and force transducer readings during the fatigue cyclic test. Significant differences between fatigued and time control samples are marked with asterisk. Following initial (14 days) slight reduction of the moduli in both loaded and time controls samples, the modulus stabilized thereafter. For a more detailed statistical analysis, see text. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

deformation rates is expected to be higher by virtue of the gels poroelasticity (see below).

Fatigue testing

Six types of gels were subjected to one million fatigue cycles. All samples endured the testing without developing any visual evidence of deterioration. Generally, there was a small decrease in compression modulus in all types of gels. Only two gel types showed a significant decrease in their modulus of about 10% (ANOVA single factor, $P = 0.05$).

The results of the extended 12 million cycles fatigue tests are presented in Figure 8. After the first million cycles (1 week of cycling), there was a slight decrease in the modulus of both fatigued and time control samples, when compared with the pertinent initial value. However, only in time control samples this difference is statistically significant ($P < 0.05$). After two million cycles (14 days of testing), the modulus became significantly and persistently lower by about 10% compared with the respective initial value in both types of samples. Starting from three millions cycles (22 days), the moduli of both loaded and time-control samples stabilized (as shown by ANOVA analysis), and there was no significant difference between them. This fact may indicate that the modulus dropped initially as a result of some unreacted components leaching out of the gel during the first weeks following preparation, and that this drop was not related to weakening due to fatigue.

The dynamic modulus (as measured during the test) was always higher than the static compressive modulus (Fig. 8). The possible reason for this difference is that during the fatigue cycling, the specimen were compressed between two porous filters which had slightly rough surfaces which may have hindered lateral expansion of the sample. This is in contrast to unconfined compression tests in which the plates were smooth and lubricated, thus allowing for lateral slip. As a result, barreling took place when using the filters, so that the apparent modulus is expected to be higher.[35] There were no measurable changes of the dynamic modulus during the test.

Hydraulic permeability and equilibrium modulus

The present results show that both the equilibrium modulus, E_s, and the hydraulic permeability, k, of the hydrogels considered depend on both the characteristics of their components (e.g., M_w of PEG) and the structural features (e.g., CLD) of the material produced. The data are presented in Figures 9 and 10, and summarized in Table III.

In extenso, the permeability rises with molecular weight of PEG and decreases as the crosslink density is increased. The dependence on IPN volume fraction is more complex—the permeability of gels with 10% IPN is slightly higher than of pure hydrogels

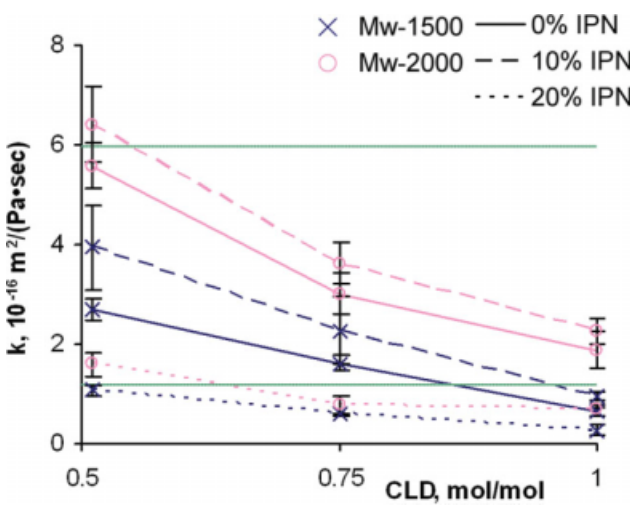

Figure 9 Hydraulic permeability, k, of PEU hydrogels and IPNs as function of PEG M_w, CLD, and % IPN. The symbols (X) and (○) are indicative for samples with PEG of molecular weight of 1500 and 2000, respectively. The lines (—), (– – –), and (---), are indicative for gels containing 0, 10, and 20% IPN, respectively. The two horizontal lines are the limits of desired properties for cartilage substitutes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

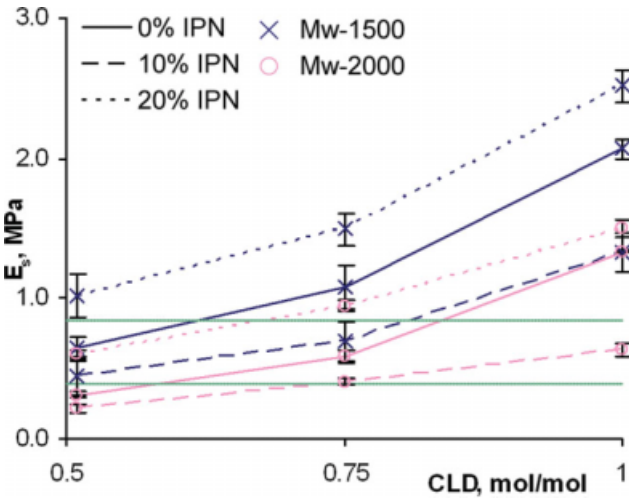

Figure 10 Equilibrium modulus, E_s of PEU hydrogels and IPNs as function of PEG M_w, CLD, and % IPN. The symbols (X) and (○) are indicative for samples with PEG of molecular weight of 1500 and 2000, respectively. The lines (—), (– – –), and (---), are indicative for gels containing 0, 10, and 20% IPN, respectively. The two horizontal lines are the limits of desired properties for cartilage substitutes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

but drops for IPNs with 20% PMMA. On the other hand, the equilibrium modulus exhibits an opposite trend to that of the hydraulic permeability. The modulus rises when PEG chains are shorter and CLD is higher (see Fig. 10); the modulus of 10% IPNs is lower than of pure gels but becomes higher in 20% IPNs. The higher permeability and lower modulus of 10% IPNs may be accounted for by incomplete chemical reaction during preparation of these specimens in a very thin (0.5 mm) mold.

The interrelationship between E_s and k, as deduced from the data in Figures 9 and 10, is depicted in Figure 11. Interestingly, within the experimental errors involved, samples prepared on different routes may exhibit similar E_s, k pairs of functional characteristics. This finding is most valuable, as it allows one a certain range of variability in designing the preparation mode to be followed when seeking materials of predefined features.

The above E_s-to-k relationship, it was next applied towards realizing the second main aim of this research, namely to delineate materials that are suitable candidates for cartilage substitutes. The relationships between the equilibrium modulus and hydraulic permeability (Fig. 11) show that there are seven types of gels having both hydraulic permeability and equilibrium modulus within the required range (1.2–6.2 $\times$ 10^{-16} $m^2/(Pa \cdot s)$ and 0.41–0.85 MPa, respectively). Chemical composition, values of water content (%H_2O), instantaneous compression modulus (E) and poroelastic parameters (k and E_s) of these gels are listed in Table III.

Among those gels, there are two specimen of single-network PEU hydrogels, four specimen of 10% PEU-PMMA IPNs and one of 20% PEU-PMMA IPNs. In most cases, the values of the standard deviations are within the desired range too, and the entire range of determined values of E_s is covered by these seven gels. It is important to point out that there is a high scattering of values of both k and E_s obtained from different measurement, as can be seen from the values of standard deviations (up to ±20%). The reason for this scatter may be related to the indirect estimates of these parameters from creep data, and due to the considerable covariance between them in the nonlinear model [eq. (6)].

Limitations of the study

The present study is part of a more encompassing program aimed at developing a cartilage substitute. The investigations reported herein addressed one aspect: the search for materials exhibiting the mechanical, swelling and poro-elastic characteristics similar to those of cartilage.

The present study, although highly significant in relation to its aims, nevertheless, has several

TABLE III
Composition and Properties of Hydrogels that Fulfill All the Criteria Stated in This Research for an Artificial Cartilage Substitute

Designation	E, MPa	H_2O, %	k, 10^{-16} $m^2/(Pa \cdot s)$	E_s, MPa
PEG-1500-0.5	1.22 ± 0.10	70.8 ± 1.8	2.7 ± 0.2	0.65 ± 0.07
PEG-1500-0.5-PMMA-10	1.36 ± 0.12	69.6 ± 1.5	3.9 ± 0.8	0.45 ± 0.14
PEG-1500-0.75-PMMA-10	2.50 ± 0.05	63.3 ± 0.9	2.3 ± 0.7	0.70 ± 0.14
PEG-2000-0.5-PMMA-20	1.99 ± 0.03	69.5 ± 0.5	1.6 ± 0.2	0.60 ± 0.04
PEG-2000-0.75	1.59 ± 0.10	71.0 ± 1.2	3.0 ± 0.4	0.59 ± 0.05
PEG-2000-0.75-PMMA-10	1.82 ± 0.07	69.8 ± 0.5	3.6 ± 0.4	0.41 ± 0.02
PEG-2000-1-PMMA-10	2.66 ± 0.06	65.1 ± 0.4	2.3 ± 0.3	0.63 ± 0.05

See Table II for designation explanation. E, instantaneous compression modulus; H_2O %, water content, (w/w); k, hydraulic permeability; E_s, equilibrium compression modulus.

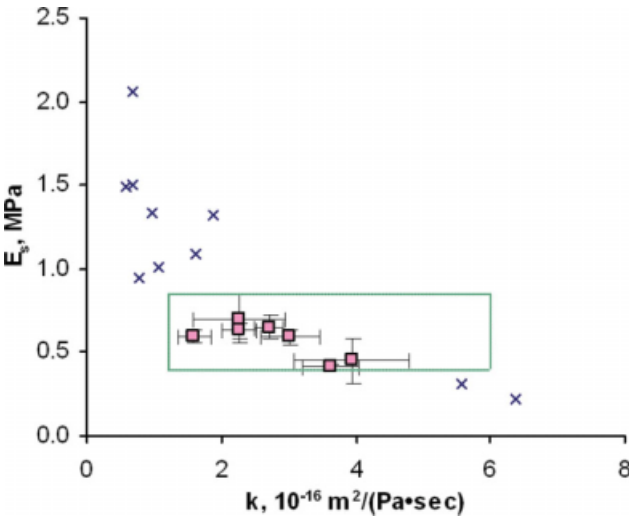

Figure 11 Relationships between hydraulic permeability (k) and equilibrium modulus (E_s) of PEU hydrogels and IPNs. The desired limits of these parameters are boxed. Gels having parameters within desired range are presented by squares (■, means) together with their standard deviation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

limitations. These are mainly related to the manner by which poroelastic properties were determined, and for several reasons. The first lies in the model itself [eq. (6)]: it assumes that under small deformations the hydraulic permeability is constant. However, it is known that the permeability is a function of strain.[36] Therefore, the value obtained using eq. (6) is an average permeability of the gel throughout the course of the experiment. On the other hand, the relationships between equilibrium stress and equilibrium strain are linear up to $\sim$ 22%,[36] so that the constant modulus assumption of eq. (6) is valid.

The second limitation lies in the fact that the methodology used for determination of poroelastic properties was not validated against a "gold standard," such as confined compression. Furthermore, the properties of samples prepared as thin slabs (for poroelastic determination) may differ from those prepared as thick cylinders in other type of molds (used for mechanical and swelling characterization). This may explain, in part, the fact than the poroelastic properties varied nonmonotonically with structural parameters (see Figs. 9 and 10). Until these issues are cleared by future studies, the above developed method should be regarded as a fast screening technique for estimation of poroelastic properties of hydrogels, rather than an accurate analytical route.

Still another limitation lies in the fact that all the required properties were tested soon after preparation of the gel while any proper cartilage substitute should maintain its features for several decades. Although it was shown that hydrogels prepared are not susceptible to degradation by fatigue, they may do so by other mechanisms such as chemical or biological degradation. Hence, the long-term stability of these materials is a topic for additional research.

Indeed, the compressive modulus, poroelastic properties, and fatigue durability are critical characteristics. Yet, there are other mechanical aspects such as the tensile and tribological (friction and wear) properties and the long-term degradation that have not been considered here and are left for future studies.

CONCLUSIONS

The main goal of this study was to prepare and characterize materials with mechanical properties close to those of articular cartilage. Hydrogel-based materials, possessing mechanical and swelling characteristics close to those of articular cartilage, were developed. These materials retain the required amount of water, and exhibit good fatigue durability and the required range of mechanical and poroelastic properties of cartilage. They are thus good candidates for further evaluation of properties important for cartilage substitute such as biocompatibility, stability, lubricity, and wear.

Some important observations concerning amphiphilic IPNs were made. First, it was found, that by inserting a hydrophobic component (PMMA in the present case) into a hydrophilic network, it is possible to ensure high water content in a hydrogel, without impairing its compressive modulus. This may be an important finding since many essential properties of a hydrogels, such as diffusion of ions and lubrication, depend on its water content. An additional finding is that in the case of weakly crosslinked PEU network, the addition of small volume fraction of PMMA, although not affecting the water content and compressive modulus, has a significant effect on the gel's strength.

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