Polymer Journal, Vol. 23, No. 8, pp 983—990 (1991)

Mechanical Properties of Copoly(*N*-hydroxyethyl L-glutamine-*co*-*N*-hydroxypentyl L-glutamine) Hydrogels

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(Received October 5, 1990)

ABSTRACT: Random copolymers, poly(*N*-hydroxyethyl L-glutamine-*co*-*N*-hydroxypentyl L-glutamine) (PHPeEG) as well as the corresponding homopolymers, poly(*N*-hydroxyethyl L-glutamine) (PHEG) and poly(*N*-hydroxypentyl L-glutamine) (PHPeG), were prepared by aminolysis with a mixture of 2-amino-1-ethanol (EA) and 5-amino-1-pentanol (PeA) in the presence of crosslinking agent, octamethylenediamine (OMDA), on membranes of poly(γ-benzyl L-glutamate). The properties of the resultant membranes, such as the degree of swelling Q_w, conformational properties and mechanical properties were studied in the pseudo-extracellular fluid (PECF). Q_w and helix content are dependent on the composition of the random copolymers. The mechanical strength of PHEG hydrogel was improved largely by introducing HPeG residues, while low Young's modulus, high swelling and low helix content were found to remain unchanged. The transition from the elastomer-type mechanical behavior to the skin-type mechanical behavior was observed at 82—90% of helix content and 110—160% of Q_w for the copolymers from tensile tests. The difference in mechanical behavior was investigated in detail by creep, stress relaxation and hysteresis experiments.

KEY WORDS Poly(*N*-hydroxyethyl L-glutamine-*co*-*N*-hydroxypentyl L-glutamine) Hydrogel / The Degree of Swelling / Helix Content / Tensile Property / Skin-type / Elastomer-type / Creep Test / Stress Relaxation / Hysteresis /

Hydrogels of poly(α-aminoacid)s are considered useful for biodegradable medical applications such as temporary artificial skin substitutes, temporary barriers to prevent adhesion and so on.[1] Several medical applications of hydrogels require not only a suitable degree of swelling, but also favourable elastic and mechanical properties in the swollen state.[2,3] The molecular design of useful hydrogel which possesses both high water content and moderate mechanical strength is not so easy. As the molecular conformation of polyaminoacids seems to affect the physical properties,[4] the above requirements may be fulfilled by use of polyaminoacid hydrogels which can be used to control molecular conformation by the hydrophobicity of side chains.

Membrane properties, such as water vapor permeability, enzymatic degradation and tensile property, of homopoly(*N*-hydroxyalkyl L-glutamine) hydrogels with alkyl side chains of different length were investigated to develop new biodegradable materials in connection with the degree of swelling by Hayashi *et al.*[4-6] The stress-strain curve of human skin shows characteristic concave behavior with low modulus at low strain and high mechanical strength at high strain.[7] Poly(*N*-hydroxyethyl L-glutamine) (PHEG) hydrogel shows tensile

behavior similar to that of human skin but it is too brittle to be used as a medical material. On the other hand, poly(*N*-hydroxypentyl L-glutamine) (PHPeG) hydrogel shows typical elastomeric behavior, exhibiting an inflection point in the low strain region, although it has high mechanical strength.

In this study, random copolymer hydrogels consisting of HEG and HPeG residues were prepared and characterized. The aim is (a) to improve the mechanical strength of PHEG hydrogel by the introduction of more hydrophobic HPeG residue while skin-type tensile behavior of PHEG is maintained and (b) elucidate the origin of differences between skin-type tensile behavior and elastomeric tensile behavior.

EXPERIMENTAL

Materials

Synthesis of Mother Polymer. Poly(γ-benzyl L-glutamate) (PBLG) was synthesized by the *N*-carboxyanhydride (NCA) method. Trichloromethyl-chloroformate (phosgene dimer) was supplied by Hodogaya Chemical Co. The BLG-NCA was prepared according to the method reported in previous paper,[8] and purified by recrystallization from ethyl acetate solution with petroleum ether. Recrystallization was repeated more than three times. Polymerization was initiated with triethylamine (TEA) at an NCA-to-TEA molar ratio of 50 in a 1 : 1 (v/v) mixture of dioxane and dichloromethane. The starting polymer was purified and fractionated as described in a previous paper.[9] The molecular weight of PBLG was determined as 335,000 by viscosity measurement[10] in dichloroacetic acid (DCA). All solvents used were distilled twice.

Preparation of Hydrophilic Copolymer Membranes. Hydrophilic copolymer membranes, PHPeEG, were prepared by aminolysis[11] of PBLG membranes cast from chloroform solution. PBLG membranes of *ca.* 60 μm and 1 μm in thickness were immersed in a mixture at various compositions of 2-amino-1-ethanol (EA) and 5-amino-1-pentanol (PeA) with 3 mol% of the crosslinking agent, 1,8-octamethylenediamine (OMDA), at 58$\pm$0.2°C. Aminolysis was completed after 7 to 14 days. Debenzylation of γ-BLG was confirmed by the disappearance of absorption at 250—260 nm in UV spectra and absorption at 700 and 750 cm^{-1} in IR spectra. The absence of benzyl ester group was confirmed by the disappearance of absorption due to ester groups at 1730 cm^{-1} in IR spectra. The copolymer membranes were washed exhaustively with distilled water and ethanol, then stored in ethanol. Compositions of all the copolymers as listed in Table I were determined by elementary analysis. It was suggested that the reactivity of PeA with γ-BLG residues is lower than that of EA.

Table I. Composition of PHPeEG Membranes

Sample code	Feed (mol%)		PeG (mol%)	OMDA (mol%)
	EA	PeA		
PHEG	100	0	0	3
PHPeEG-10	75	25	10	3
PHPeEG-32	50	50	32	3
PHPeEG-61	25	75	61	3
PHPeEG-75	20	80	75	3
PHPeEG-80	15	85	80	3
PHPeEG-82	10	90	82	3
PHPeEG-90	5	95	92	3
PHPeG	0	100	100	3

Measurements

The Degree of Swelling Q_w (%) of PHPeEG Hydrogels. The Q_w (%) in pseudo-extracellular fluid (PECF)[12] (115 mM NaCl, 30 mM $NaHCO_3$, 3 mM KCl and 2 mM K_2HPO_4) at pH 7.4 was determined by equilibrating the membrane in PECF solution at 37.0°C. The membrane was then treated with a blotter to remove surface PECF and weighed until constant weight. The membrane was then dried

in a vacuum oven. Q_w (%) was defined as the ratio of the amount of PECF contained in the hydrogel to the weight of xerogel.

Molecular Conformation of PHPeEG Hydrogels. The molecular conformation of linear PHPeEG copolymers in PECF solution was examined by circular dichroism CD (Jasco J-40 CD/ORD Spectropolarimeter) measurement. Helix content (X_H) was evaluated from mean residue ellipticity (θ) at 222 nm. The conformation of membranes in xerogel and in hydrogel was examined by IR (Hitachi Model 285) spectra. The Amide V band[13] in IR spectrum was used for identification of the conformation in hydrogel.

Mechanical Properties of PHPeEG Hydrogels. The tensile properties of hydrogels were measured in PECF at 25°C using a Tensilon UTM-4LH (Orientec Co.) equipped with a 1 kg loadcell. A wrapping film was used at chuck parts to prevent hydrogels from breaking or from slippage at the chuck. All hydrogels were tested at a strain rate of 40% per minute. Mechanical parameters such as Young's modulus (E), tensile strength (σ_B) and strain at break point (ε_B) were estimated from stress–strain curve. Stress relaxation was measured by Tensilon for 1000 s with strain up to 100%. Creep was measured for 1000 s with a load in the range of 5—100 g by a creep testing machine designed by us. Permanent strain was measured by a hysteresis curve after stretching the samples at various strains.

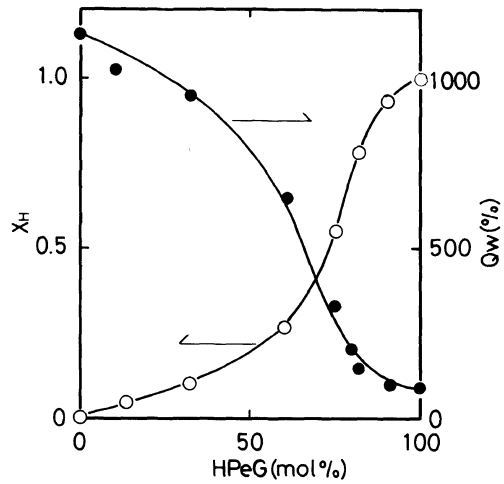

Figure 1. Degree of swelling Q_w (●) and helix content X_H (○) of PHPeEG copolymers as a function of PeG content (mol%) in PECF solution at 25°C.

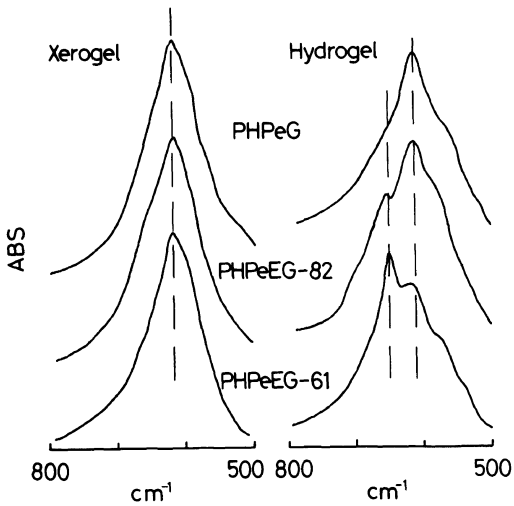

Figure 2. Amide V band in IR absorption spectra of PHPeEG membranes in the dry state and swollen state.

RESULTS AND DISCUSSION

Relation of the Degree of Swelling to Molecular Conformation of PHPeEG Hydrogels

Linear poly(*N*-hydroxyalkyl L-glutamine)s without crosslinking are nonionic water soluble polymers whose conformations depend on the length of the side chain. Helix content increases with increasing length of hydrocarbon of the side chain in aqueous solution, and PHEG exists in the random coil conformation, while PHPeG, completely in the α-helix conformation.[14,15] Helix content X_H of linear PHPeEG copolymers in PECF solution at 25°C is shown as a function of mole fraction of HPeG in Figure 1. PHPeEG copolymers are shown to exist in the interrupted α-helix conformation, but X_H values of PHPeEG copolymers are lower than those expected from the copolymer composition. It is suggested that the α-Helix conformation of PHPeG is

disrupted by the introduction of HEG units.

Molecular conformations of crosslinked membranes are identified by IR absorption spectra and some spectra in the Amide V region are shown in Figure 2. Although all membranes consist of α-helix molecules in xerogel, the absorbance of 650 cm^{-1} which corresponds to the random coil conformation increases with decreasing HPeG content in hydrogel. Thus, the molecular conformations of linear copolymers in PECF solution are considered to reflect those of crosslinked hydrogels. The results from CD measurements and wide-angle X-ray diffraction patterns of PHPeEG hydrogels also support this viewpoint.[16] The molecular conformations of the hydrogels are shown to be hardly affected by crosslinking at the crosslinking agent concentration used in this study. Estimation of effective crosslink density and effects of crosslinking on membrane properties for homo-poly(*N*-hydroxyalkyl L-glutamine) hydrogels having different alkyl side chains will be discussed elsewhere.[17]

The degree of swelling in a solvent is determined by the interaction energy between solvent molecules and polymer segments. The degree of swelling Q_w of crosslinked membranes in PECF solution is shown in Figure 1. Q_w of PHPeEG copolymers decreases with increasing HPeG content, which can be explained by change in hydrophobicity due to side chains with copolymer composition. Moreover, at a mole fraction of HPeG less than 60%, PHPeEG copolymers show high Q_w and low X_H, suggesting that the change in Q_w value corresponds to that in X_H. It is indicated that Q_w is also affected by the molecular conformation of the PHPeEG membrane.

Mechanical Properties of PHPeEG Hydrogels

The tensile properties of hydrophilic membranes are highly dependent on the degree of swelling. Further, hydrogels with low modulus are highly suited for biomedical applications, such as membranes for artificial organs, reconstructive prosthesis and cosmesis. Stress–strain (S–S) curves of PHPeEG hydrogels in PECF at 25°C are shown in Figure 3 in comparison with that of a human skin of the lower abdomen in longitudinal direction.[7] The mechanical parameters obtained from S–S

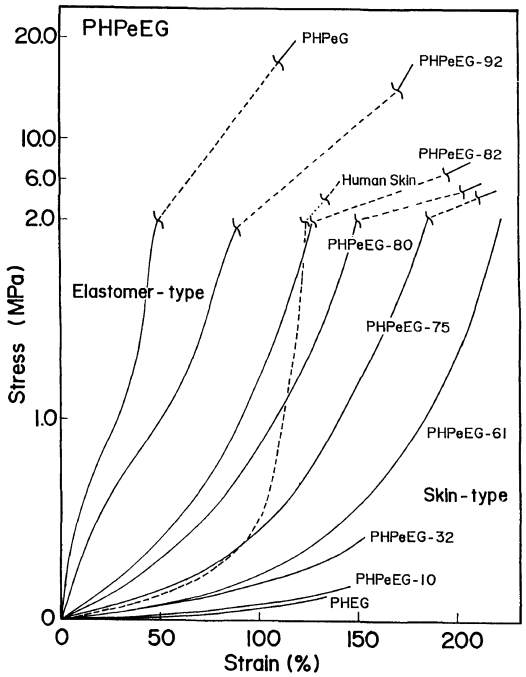

Figure 3. Stress-strain curves of PHPeEG membranes in PECF solution at 25°C. The dotted line denotes the S–S curve of human skin from the lower abdomen in a longitudinal direction.

Table II. Mechanical Parameters obtained from S–S Curves of PHPeEG Membranes in PECF Solution at 25°C

Sample code	E	σ_B	ε_B
	MPa	MPa	%
PHEG	0.039	0.12	130
PHPeEG-10	0.042	0.16	150
PHPeEG-32	0.045	0.45	150
PHPeEG-61	0.055	2.1	220
PHPeEG-75	0.11	3.9	220
PHPeEG-80	0.17	5.8	210
PHPeEG-82	0.29	8.0	210
PHPeEG-92	6.8	18	180
PHPeG	38	21	120

curves, such as Young's modules E, the tensile strength σ_B and strain at the break point ε_B are listed in Table II. The S–S curve of human skin shows characteristic behavior with low modulus at low strain and high mechanical strength at high strain. We define this kind of tensile behavior as the skin-type. For hydrogels to be used as skin substitutes, it is necessary to have skin-type behavior such as will prevent exfoliation from wound sites. The PHEG hydrogel with high Q_w and random coil conformation, shows a skin-type behavior with values of lower E and σ_B than those of human skin. While, PHPeG hydrogel with lower Q_w and helix conformation shows typical elastomeric behavior, exhibiting a inflection point in the low strain region, with high E and σ_B values. We define this kind of tensile behavior as the elastometer-type. In PHPeEG copolymers σ_B and ε_B increase with increasing HPeG content while low Young's modulus remains unchanged up to 82 mol% of HPeG content. It was found that the mechanical strength of PHEG hydrogel has been improved largely by introducing HPeG residues, without sacrificing a skin-type behavior and high Q_w. Mechanical properties similar to that of human skin are shown by some copolymers.

Transition from the skin-type to the elastomer-type in the S–S curve is found in the region between 82 and 92 mole fraction of HPeG, corresponding to 78—92% of X_H and 110—160% of Q_w. However, the shape and order of the S–S curves of PHPeEG hydrogels remained unchanged after removal of the solvent, *i.e.*, the S–S curves obtained using the stress calculated from cross section in xerogel were similar in shape and order to those calculated from cross section in hydrogel. Thus, the shape of the S–S curve seems to be mainly governed by X_H. Also, the transition from the skin-type to the elastomer-type in tensile behavior can be related to change in intrinsic viscosity with the helix content reported in isopropanol-water mixture for PHEG.[18] It is indicated that a small content of random coil residues affords considerable flexibility to the whole chain segments by interrupting the helix section.

Comparison between Skin-Type and Elastomer-Type on S–S Curves

Polyaminoacid fiber is well known to exhibit conformational transition from α-helix to β-sheet by stretching.[19] As tensile properties of PHPeEG hydrogels are considered affected by molecular conformation, it is important to study conformational change by stretching. Changes in molecular conformation of copolymer hydrogels during stretching were monitored by the position of IR Amide V band (600—700 cm^{-1}). The IR spectra of elastomer-

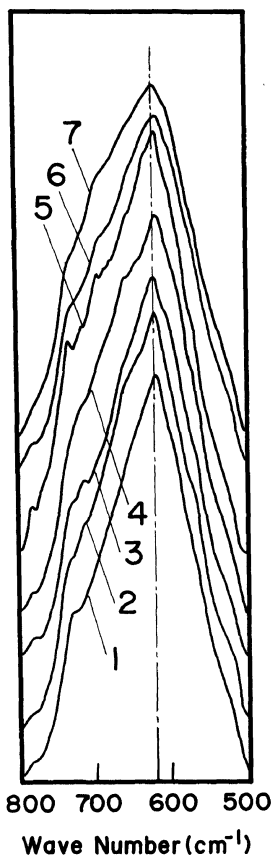

Figure 4. Amide V band in IR spectra at various strains of swollen PHPeG membranes at 25°C, 1, 0%; 2, 12%; 3, 28%; 4, 40%; 5, 55%; 6, 72%; and 7, 88%.

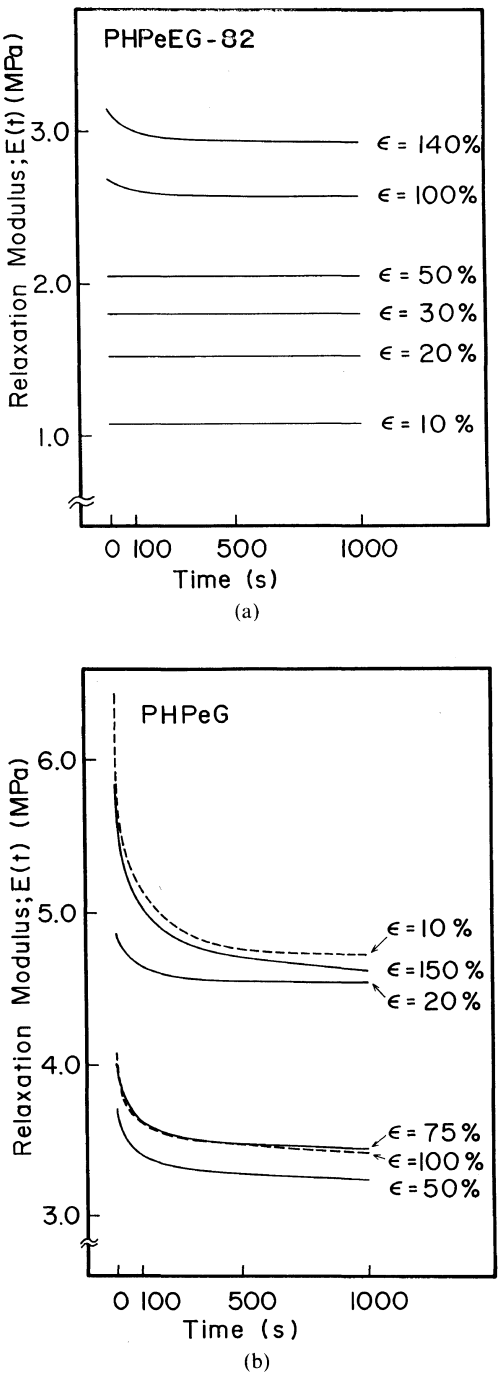

Figure 5. Change in relaxation modulus $E_{(t)}$ at various strains of (a) skin-type PHPeEG-82 membrane and of (b) elastomer-type PHPeG membrane in PECF solution at 25°C.

type PHPeG are shown in Figure 4. Qualitatively, the helix peak near 610 cm^{-1} remained unchanged on stretching up to 100% strain. Above 100% strain, IR measurement could not be made because of difficulty in preparing thin film. The molecular conformation in the skin-type membrane also seems to be maintained on stretching, though this is not clear because the absorption of water affects the spectra. In the case of crosslinked polyaminoacid hydrogels, water molecules are considered to act effectively as a plasticizer, so that change in molecular conformation on stretching is not observed.

Transition from the skin-type to the elastomer-type in tensile behavior is indicated by stress relaxation, creeping and the hysteresis curve. The relaxation moduli of copolymer hydrogels obtained from stress relaxation test through 1000 s are shown in Figure 5. No relaxation is observed for skin-type copolymers up to 100% strain, while relaxation is observed even in a low strain region for the elastomer-type copolymers, suggesting that random coil segments play a important role in relaxation. For elastomer-type copolymers, the major stress relaxation in low strain region can be regarded as disentanglement among helix molecules. The stress relaxation above 100% strain for both tensile types may be due to reduction of the amount of free water in hydrogel by high stretching, such as conformational change and molecular orientation. The creep strain hydrogels is also measured through 1000 s. For elastomer-type copolymers, creep strain is constant with time, just like that of skin-type copolymers, but the order and interval fluctuate with different loadings. The network structures in hydrogels seem to be constructed completely judging from the fact that relaxation modulus and creep strain immediately attained their equilibrium values. The hysteresis behavior of both skin- and elastomer-type copolymers was studied. The behavior of skin-type copolymers is typical of entropy elasticity without heat loss (ideal

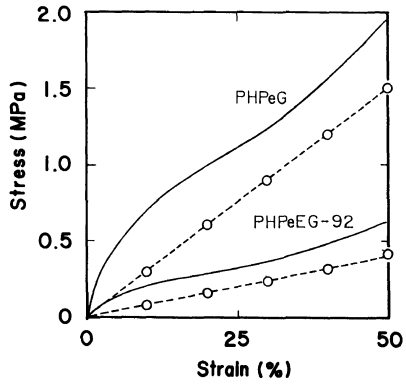

Figure 6. Equilibrium stress σ_e at various strains in stress relaxation test for elastomer-type copolymers in PECF solution. Sigmoidal curves show nominal stress-strain curves.

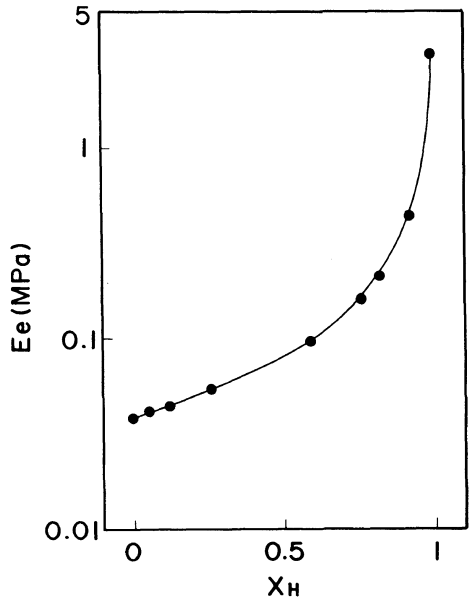

Figure 7. Relationship between equilibrium relaxation Modulus E_e and helix content X_H of PHPeEG membranes in PECF solution. The solid curve is obtained from the equation in this paper.

rubber) by the finding that the recovering curve coincided completely with the stretching curve. Elastomer-type copolymers show substantial hysteresis between the stretching curve and recovering curve but without permanent set. The area surrounded by both curves is due to heat loss which may be caused by friction among helix molecules. Elastomer-type behavior is considered to include the contribution of energy elasticity. Thus, the difference in mechanical properties between the skin-type and elastomer-type is shown clearly and can be explained based on molecular conformation.

The equilibrium stress values in stress relaxation experiments are compared with observed S–S curves in Figure 6. The equilibrium stress is considered to correspond to the mechanical strength of the network structure. This stress varies linearly with strain for elastomer-type copolymers. As mechanical strength of the network structure formed by peptide chain segments and crosslinking agents is considered to depend on the helix content X_H, Young's modulus E_e obtained from the equilibrium stress-strain relation is shown as a function of X_H in Figure 7. As can be seen, copolymers containing random coil conformation have low E_e due to flexibility of the random coil region. Here, we propose the following approximated equation for the relationship between E_e and X_H.

$$E_e(X_H) = \{X_H/E_{PeG} + (1 - X_H)/(E_{EG}\}^{-1}$$

where E_{PeG} and E_{EG} denote Young's modulus of PHPeG and PHEG, respectively. As obvious in Figure 7, the values calculated from the equation are in fair agreement with the experimental values. Thus, the mechanical strength of the network structure is related to X_H by the above equation. It is pointed out that the mechanical strength of PHPeEG hydrogels can be predicted by X_H. Simulation of the whole S–S curve of PHPeEG hydrogels is now under investigation with attention to deviation at initial and high strain regions.

The major conclusions from this study are: (1) By introducing more hydrophobic HPeG residues in PHEG, mechanical parameters increase without sacrificing skin-type behavior and the degree of swelling, and tensile property similar to that of human skin is obtained for some copolymers. (2) The mechanical strength

of the network structure is related to molecular conformations of hydrogels and tensile behavior should be governed by helix content in hydrogels.

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