Preparation and Characterization of Hyaluronate–Hydroxyethyl Acrylate Blend Hydrogel for Controlled Release Device

Masao Inukai, a Yu Jin, a Chikako Yomota, b and Masakatsu Yones e a

Faculty of Pharmaceutical Sciences, Nagoya City University, 3–1 Tanabe-dori, Mizuho-ku Nagoya 467–8603, Japan and National Institute of Health Sciences, Osaka Branch, 1–1–43 Hoenzaka, Chuo-ku, Osaka 540–0006, Japan.

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Hyaluronate–hydroxyethyl acrylate blend hydrogels were investigated as matrices for controlled release devices. Glycidyl methacrylate (GMA) derivatized HA (GMA–HA) was synthesized by coupling of GMA to HA in the presence of a suitable catalyst. These hydrogels were prepared by a free radical copolymerization of GMA–HA and hydroxyethyl acrylate. The water content of these hydrogels at equilibrium swelling in water (Ww) was 0.97±0.0073 (n=18); however, these hydrogels was mechanically tough and could be used as disk shape. The hydrogels swelling were found to depend on ionic strength and pH. The dried hydrogels quickly regained their original condition in water, and they swelled to more than 90% of its initial water contents after 30 min. This swelling–deswelling behavior was reproducible. The release of chlorpromazine HCl as a model cationic drug from the gels was suppressed significantly in water. The release increased with increasing the ionic strength and decreasing pH of bulk solutions.

Key words hyaluronic acid; hydroxyethyl acrylate; hydrogel; swelling; drug release

Hydrogels are formed from hydrophilic synthetic polymers and many natural polymers such as proteins and polysaccharides, and their crosslinking mechanisms depend on their properties. Due to three-dimensional hydrophilic polymer networks, hydrogels are insoluble in water and hold much water in them under keeping equal the chemical potentials in the both phases i.e., the gel and the bulk phases. They swell or deswell depending on various ambient conditions such as pH, ionic strength, solvent composition, temperature and pressure. Especially, hydrogels prepared from natural polymers are more suitable adequate than synthetic polymers because of their biocompatibility and biodegradability. Because of these advantages, natural polymers have been widely applied to pharmaceutical fields such as controlled delivery devices of various drugs.

Hyaluronate (HA) is a natural polysaccharide, and is an important component of connective tissues such as the intercellular matrix of skin, muscle and cartilage cells. It is a linear block copolymer of repeating units composed of N-acetyl-D-glucosamine and D-glucuronic acid connected by alternate β(1→3) and β(1→4) glucosidic bonds. The important properties of HA solutions are high viscoelasticity resulting from the very high molecular weights and high preserving ability of water. Furthermore, as HA is highly transparent, it is used as a surgical tool in eye surgery and as a remedy for arthritis.

HA gels are reported to maintain such properties as HA solutions. In this paper, the novel blend hydrogel obtained from the copolymerization of a methacrylate derivative of HA was prepared and the characteristics of the HA gel were investigated. The effects of ionic strength on the swelling, cationic drug releases and the reversibility of the swelling were elucidated.

Materials and Methods

Sodium HA (NaHA, M w 207000 g mol−1) from Streptococcus zooepidemicus was purchased from Kibun Food Chemift Co., (Tokyo, Japan). Glycidyl methacrylate (GMA, 95%), ammonium peroxodisulfate (APS), 2-hydroxyethyl acrylate (HEA, 95%) and chlorpromazine hydrochloride (CPHC) were purchased from Wako Pure Chemical Co. (Osaka, Japan).

Other chemicals were used of analytical grades. Distilled and deionized water was used for the preparation of aqueous solutions.

Preparation of Glycidyl Methacrylated HA Glycidyl methacrylated HA (GMA–HA) was prepared as follows: 1) 2.0 g of NaHA was dissolved in 40 cm3 of 0.05 mol·dm−3 carbonate buffer (pH 11). 2) GMA (1.0 cm3, 7.6 mol·dm−3) was added to the NaHA solution and the mixture was gently stirred at room temperature. 3) After 7 d, the solution was adjusted to pH 7 with HCl. 4) The solution was purified by dialyzing against distilled and deionized water for 7 d. 5) After filtering to get rid of insoluble substances, the GMA–HA was freeze dried and stored at −20°C. The chemical reaction of NaHA with GMA and the chemical formula of GMA–HA are shown in Fig. 1.

Preparation of HA-polyhydroxyethyl acrylate (PHEA) hydrogels GMA–HA (100 mg) and HEA (0.92 cm3, 100 mg) were dissolved in 0.8 cm3 of water, and APS solution (200 µmol·dm−3, 0.1 cm3) was added. After the reaction mixture was vigorously mixed and degraded, the samples solutions in a petri dish (diameter 3 cm) were allowed to react for 30 min at 60°C and the blend gels composed of HA and PHEA were prepared. In the process of formation of HA–PHEA hydrogels, PHEA should be formed due to the polymerization of HEA. Due to the reactions of PHEA with GMA groups of GMA–HA, HA molecules are crosslinked by PHEA and simultaneously PHEA extended furthermore. Finally, HA and PHEA are crosslinked at the GMA groups of GMA–HA. Thus, the blend gels composed of HA and PHEA are formed as shown in Fig. 2.

After polymerization, the HA–PHEA hydrogels were removed from the mould. After washing in distilled water, they were dialyzed in distilled water for 3 d. The swollen hydrogel was cut into disks (diameter: 1.5 cm, thickness: 0.3 cm). The hydrogels were placed in vials contains 0.2% w/v sodium azide solution to prevent bacterial contamination. The samples were equilibrated in water for 3 d. The water contents (W w ) of these hydrogels were measured as the ratios of the mass changes due to drying of the swollen gel to dry one,

\[ W_w = \frac{(W_{w1} - W_{w0})}{W_{w0}} \]

(1)

where W w0 and W w1 are the weights of swollen gel and dried one.

Ionic Strength and pH-Responsive Swelling of HA–PHEA Hydrogels

The equilibrium swellings of the HA–PHEA hydrogel were studied in NaCl and Na 2SO 4 solutions. Several samples were immersed in the solutions in the range of ionic strengths J=0.001–2.0 mol·dm−3 for 3 d at 25°C. The effects of pH were measured in JP XIII 1st fluid (pH: 1.2, J=0.105 mol·dm−3) and JP XIII 2nd fluid (pH: 6.8, J=0.074 mol·dm−3). Relative swellings are shown as the percentages of the weights of the equilibrated hydrogel in the solutions to the initial equilibrated weights in water.

Receives of Swelling and Drying States of HA–PHEA Hydrogels

The HA–PHEA gels equilibrated in water were dried in a desiccator for 1 d at room temperature. The dried samples were immersed in vials containing water, JP XIII 1st and 2nd fluid. For the first 1 h at fixed time intervals and

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after 1 d gel samples were taken out, wiped dry using filter paper, and weighed.
Swelling reversibility of hydrogel was determined using the same samples swelling in water and desiccated in a desiccator for 1 d at room temperature, repeating 3 times. Relative swelling is the ratio of the swelling/dewelling of the hydrogel to the initial swelling of the hydrogel calculated as a percentage.

**Measurements of Creep and the Analysis**
The creep of HA–PHEA gels measured using a creep meter (RE-33005, Yamaden Co., Tokyo). The front of the probe was a circular plate of diameter 0.8 cm and the creep can be measured with 0.001 cm accuracy. The results can be analyzed by Voigt models and the viscoelastic parameters are estimated by curve fitting methods. In the simplest 2 element model composed of a spring and a dashpot, a strain $\gamma$ under a stress $\sigma$ is expressed by Eq. 2.

$$\gamma = \sigma/G_e (1 - \exp(-t/G_e/\eta)) = \sigma/G_v (1 - \exp(-t/\tau))$$

where $G_e$ and $\eta$ are an elastic modulus and viscosity coefficient, and $\tau$ is a delay time ($= \eta/G_v$).

**Measurement and Determination of Drug Release of HA–PHEA Hydrogels**
CPhCl was used as a cationic model drug. The disks of the HA–PHEA gel were equilibrated in 0.01 mol·dm$^{-3}$ CPhCl solution for 24 h at room temperature under shielding light as CPhCl is decomposed under light. Drug content of the HA–PHEA hydrogels $C_0$ was determined from absorbance before and after loading CPhCl using a spectro multi channel photo detector (MCPD-2000: Otsuka Electronics Co.) at 245 nm.

Measurements of CPhCl release were measured at 25 °C under shielding light using a diffusion cell (50 cm$^2$) made of a glass. The HA–PHEA gel was fixed in the manner of the releases from one surface of the disk. The releases of CPhCl were measured in water, NaCl solution or HCl solution. The bulk solutions were stirred continuously at 350 rpm by a magnetic stirrer unit. Sample solutions were withdrawn at regular intervals of 20 min and replaced with equal volumes of the media. The absorbance of CPhCl in the sample solutions was determined using a spectro (MCPD-2000 Otsuka Electronics Co.) at 245 nm.

Total amount of CPhCl released till $i$th sampling time $Q_i$ is obtained by Eq. 3

$$Q_i = C_i V_i + \sum C_j V_j$$

where $C_i$ is the concentration of CPhCl at $i$th sampling, $V$ is the volume of the diffusion cell and $V_j$ is the volume of the sample solution.

**Results and Discussion**

**Characteristics of HA and HA–PHEA Hydrogels**
HA–PHEA hydrogels prepared from the polymerizations of HEA and GMA–HA were stable and transparent as shown in Fig. 3. The water contents ($W_w$) of the hydrogels were

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**Fig. 1.** Reaction of HA with GMA

**Fig. 2.** Schematic Representation of the Formation of HA–PHEA Hydrogel
Fig. 3. Photograph of HA–PHEA Hydrogel
Arrow shows the transparent HA–PHEA gel.

Fig. 4. Effect of Ionic Strength on Swelling of HA–PHEA Hydrogels
- NaCl, ○ Na₂SO₄: 1st fluid and ▲: 2nd fluid.

$0.978 \pm 0.0073 \ (n = 18).$ In spite of such high water contents, these hydrogels were mechanically tough and could be used as disk shape. However, HA hydrogels prepared from GMA–HA alone had much higher water contents ($W_w = 0.996$) and were so brittle that they could not be used as gel samples. The HA–PHEA hydrogels were found to be useful for basic research of transparent hydrogels and applications to drug delivery system.

The viscoelastic properties of HA–PHEA hydrogel were measured in water using a creep meter at 25 °C. The creeps of the HA–PHEA hydrogels showed long relaxations and the results under the load of 50 g could be analyzed using the simplest 2 element Voigt model. Their viscoelastic parameters in Eq. 2 were $G_i = 6.63 \times 10^4 N \cdot m^2$, $\eta_i = 2.10 \times 10^5 N \cdot m^2 \cdot s$ and $\tau = 3.165 \times 10^7 s$. From these values, the HA–PHEA hydrogels were found to possess relatively high viscoelastic properties which were good enough for delivery experiments.

Effects of Added Salts on Swellings of HA–PHEA Hydrogels
Effects of added salts on the swells of the HA–PHEA hydrogels were studied in NaCl and Na₂SO₄ solutions at 25 °C. Their swells were found to attain to the equilibrated states after 24 h. The relative weight percentages to the weight of the HA–PHEA gels equilibrated in water are defined as relative swells. The relative equilibrated swells after 24 h are shown as a function of ionic strengths ($\lambda$) in Fig. 4. The relative swells were found not to depend on the kind of electrolyte but depend significantly on $\lambda$. The relative swells decreased with increasing $\lambda$ as expected from the properties of charged gels and at $\lambda = 2$ mol·dm$^{-3}$ decreased to 20%. The decrease of the relative swells resulted from the decreases of the electrostatic repulsion between HA due to shielding effects of added salts on the carboxylic groups of HA. The two characteristic swelling behaviors of the HA–PHEA gels are as follows: 1) In the diluted added salt solutions ($\lambda < 0.01$ mol·dm$^{-3}$), the relative swells were beyond 100% and showed 115% at $\lambda = 0.001$ mol·dm$^{-3}$. 2) In very high added salt solutions ($\lambda = 2$ mol·dm$^{-3}$), the relative swelling was 20%, i.e., the HA–PHEA gels shrunk significantly in high ionic strength solutions.

To examine the effects of pH of bulk solutions, the relative swells were measured in 1st and 2nd fluid. As shown in Fig. 4, in the 2nd fluid the relative swelling was in accordance with the results of NaCl and Na₂SO₄ solutions. However, in the 1st fluid being strong acid the relative swelling was found to be smaller than the strong electrolyte solutions. As the HA–PHEA gels possess carboxylic groups, in the strong acidic region the carboxylic groups are not dissociated and the swells became smaller than in other strong electrolyte solutions. The HA–PHEA gels equilibrated in the 1st fluid became brittle. HA is known to decompose in the strong acidic region. Thus, the brittleness is considered to result from the decomposition of HA.

Recycles of Swelling and Drying States of HA–PHEA Hydrogels
The HA–PHEA gels equilibrated in water at 25 °C were dried at 80 °C for 24 h. The dried HA–PHEA gels were reswelled at 25 °C and the time courses of the reswelling processes in water, the 1st and the 2nd fluid are shown in Fig. 5. The ordinate shows the relative swelling on the basis of the original HA–PHEA gels equilibrated in water. The relative swells were found to depend on pH and ionic strengths. The dried gels reswelled in the same solutions attained to almost 90% during 60 min and recovered to the original states after 24 h, and the relative swells were found to depend on pH and $\lambda$. The relative swelling in water was found to recover to the original one. However, the results of the 1st and the 2nd fluids were much smaller than 100% and the former was found to be smaller than the latter.

The HA–PHEA gels equilibrated in water were dried at room temperature and reswelled to the equilibrium state. The cycles of dry and swell states were repeated. The relative swells to the original gel equilibrated in water are shown in Fig. 6 which shows 4 cycles. The cycles were found to be re-
Fig. 6. Reversibility of Swelling–Drying Cycles of HA–PHEA Hydrogel
In: initial condition, D_i; nth drying condition and S_i; nth swelling condition.

Fig. 7. Effect of Added NaCl on Release of CPHCI from HA–PHEA Hydrogels
- water, ○: 0.01 mol·dm⁻³ and ▲: 0.1 mol·dm⁻³.

Fig. 8. Effect of Added HCl on Release of CPHCI from HA–PHEA Hydrogels
- water, ●: 0.01 mol·dm⁻³ and ▲: 0.1 mol·dm⁻³.

Fig. 9. Effect of Drying on Release of CPHCI from HA–PHEA Hydrogels
- water, ●: dried gel (NaCl and HCl) and ○, ◀: original swelling gel (NaCl and HCl).

Reversible processes and the HA–PHEA gels were found to recover completely to the original swelling. In general, natural polymer gels such as an alginate and a gelatin do not recover to the original swelling states after drying and their relative swelling decreases with increasing the number of the recycles. The reversible recycles of HA–PHEA gels are considered to result from the high affinity of water for HA. The HA–PHEA gels were found to be recycling gels.

**Characteristic Releases of CPHCI from HA–PHEA Hydrogels due to Added Ions**
As a cationic drug, CPHCI was used. The HA–PHEA gels equilibrated in CPHCI solution (0.01 mol·dm⁻³) shrank significantly and became opaque. CPHCI is known to associate due to hydrophobic interactions. Thus, CPHCI does not interact only electrostatically with the carboxylic groups of HA but also hydrophobically. Furthermore, CPHCI molecules bound with the HA–PHEA matrixes interact with each others and form intraassociated aggregations. Thus, the shrink of the HA–PHEA gels due to CPHCI is considered to result from electrostatic shielding and the formation of the intraassociation.

Releases of CPHCI from the HA–PHEA gels were measured in NaCl and HCl solutions at 25°C and the time courses of the results are shown in Figs. 7 and 8. The ordinate is the relative released amounts to the original CPHCI Q/Q₀. In water, the releases were found to be very small and after 24 h only 10% was released. In NaCl solutions (C_{NaCl} = 0.01 and 0.1 mol·dm⁻³), the values of Q/Q₀ after 24 h increased with increasing the concentration C_{NaCl} as shown in Fig. 7. Even in higher NaCl concentration C_{NaCl} = 0.1 mol·dm⁻³, all CPHCI could not be released and after 24 h the value of Q/Q₀ was found to reach only to 0.8. In lower concentration of NaCl C_{NaCl} = 0.01 mol·dm⁻³, a lower amount (Q/Q₀ = 0.6) was released.

The releases in HCl solutions are shown in Fig. 8. The release rates and Q/Q₀ after 24 h were more than those of the results in NaCl solutions. From these results, CPHCI bound electrostatically with carboxylic groups of HA is suggested to release due to the counterion exchanges and the dissociation equilibrium.

**Effects of Drying on Releases of CPHCI from HA–PHEA Hydrogels**
The HA–PHEA gels equilibrated in CPHCI solution (0.01 mol·dm⁻³) were dried in a desiccator for 1 d at room temperature. The weight ratio of dried weights was 0.055. The dried gels containing CPHCI showed also reversible swelling–drying processes in the same manner as in water. The releases of CPHCI from the dried HA–PHEA gels were studied in water, 0.1 mol·dm⁻³ NaCl and 0.1 mol·dm⁻³ HCl solutions at 25°C. As shown in Fig. 9, the slight releases were found in water and by adding electrolytes the releases increased. The results in HCl solution were more than those in NaCl in the same manner as those from swollen gels. It should be noticed that the rates of releases were almost equal to the results from the swollen gels i.e., in the releases, the HA–PHEA gels were recyclable.

Other natural polymer gels such as gelatin and alginate do not recover to their original states after a drying process. Recycling uses of these gels are difficult. Thus, the HA–PHEA gels are considered to be useful not only in pharmaceutical fields but in other fields.
Conclusion

1) The interpenetrating HA–PHEA gels composed of HA and PHEA were prepared from the copolymerization of GMA–HA and HEA.

2) The HA–PHEA gels were negative charged hydrogels of high water content and transparent.

3) The swells of the HA–PHEA gels responded to ionic strengths and pH as expected from the properties of charged gels.

4) The HA–PHEA gels were recyclable and reversible in the many swelling–drying cycles.

5) Releases of CPHCl as a model cationic drug from the HA–PHEA gels were suppressed significantly in water. The releases increased with increasing the ionic strength and decreasing pH of bulk solutions. However, all CPHCl in the gel could not be released. From these results, not only electrostatic interactions but also intraassociations were found to contribute to the releases. Even from the dried HA–PHEA gels, the rates of releases were almost equal to the results from the swollen gels.

References


