Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

SYNTHESIS OF A NOVEL POLYSACCHARIDE HYDROGEL

Kimberly A. Smeds; Anne Pfister-Serres; Diane L. Hatchell[a]; Mark W. Grinstaff
[a] Departments of Chemistry, Ophthalmology and Cell Biology, Duke University and VA Medical Center, Durham, NC

To cite this Article Smeds, Kimberly A. , Pfister-Serres, Anne , Hatchell, Diane L. and Grinstaff, Mark W.(1999) 'SYNTHESIS OF A NOVEL POLYSACCHARIDE HYDROGEL', Journal of Macromolecular Science, Part A, 36: 7, 981 — 989
To link to this Article: DOI: 10.1080/10601329908951194
URL: http://dx.doi.org/10.1080/10601329908951194

J.M.S.—PURE APPL. CHEM., A36(7&8), pp. 981–989 (1999)

SYNTHESIS OF A NOVEL POLYSACCHARIDE HYDROGEL

KIMBERLY A. SMEDS, ANNE PFISTER-SERRES, DIANE L. HATCHELL,† and MARK W. GRINSTAFF*

Departments of Chemistry, Ophthalmology†
and Cell Biology †
Duke University and
VA Medical Center †
Durham, NC 27708
http://www.chem.duke.edu/~mwg

Key Words: Hydrogel, Hyaluronic Acid, Photocrosslinking, Cell Encapsulation

ABSTRACT

A modified hyaluronic acid (HA) biopolymer was synthesized that can be photocrosslinked to form a stable hydrogel. The chemical and physical properties including the amount of modification of the polymer with methacrylate anhydride, the viscosity of the modified biopolymer, and the solute diffusion characteristics of the polymer have been determined.

INTRODUCTION

Hydrogels are polymeric materials which exhibit the ability to swell in water and to retain a significant fraction of water within the structure without dissolving [1-3]. The physical properties exhibited by hydrogels such as high water content, sensitivity to environmental conditions (e.g., pH, temperature, solvent,

* Author to whom correspondence should be addressed.

stress) and rubbery consistency are favorable for biomedical and biotechnological applications. Indeed, hydrogels may be used as coatings (e.g., biosensors, catheters, and sutures), as "homogeneous" materials (e.g., contact lenses, burn dressings, and dentures), and as devices (e.g., artificial organs and drug delivery systems. [4, 5]. Hydrogel matrices for the entrapment of cells as artificial organs have been explored for more than fifteen years, and microencapsulation is a promising approach for a number of disease states including Parkinson's disease (L-dopamine cells), liver disease (hepatocyte cells), and diabetes (islets of Langerhans) [6-8]. For example, in 1980 Lim and Sun encapsulated islets of Langerhans (the insulin producing cells of the pancreas) in an ionically crosslinked alginate (a natural hydrogel) microcapsule with a poly-L-lysine coating, and successfully reduced blood sugar levels in diabetic mice following transplantation [9].

We have developed a novel covalently photocrosslinked hydrogel, based on the carbohydrate polymer hyaluronic acid, that has potential applications for drug delivery and cell encapsulation. Hyaluronic acid (HA), a natural polysaccharide hydrogel, is comprised of β (1-4) linked 2-acetamide-2-deoxy-D-glucose and β (1-3) linked D-glucuronic acid, and is known to be non-antigenic, non-inflammatory and generally non-tissue reactive [10]. Modification of hya-luronic acid with methacrylate functional groups allows for covalent cross-linking in the presence of a radical initiating system. Herein, we report the synthesis, characterization, photocrosslinking, and initial cell encapsulation studies with this methacrylate-modified hyaluronic acid hydrogel.

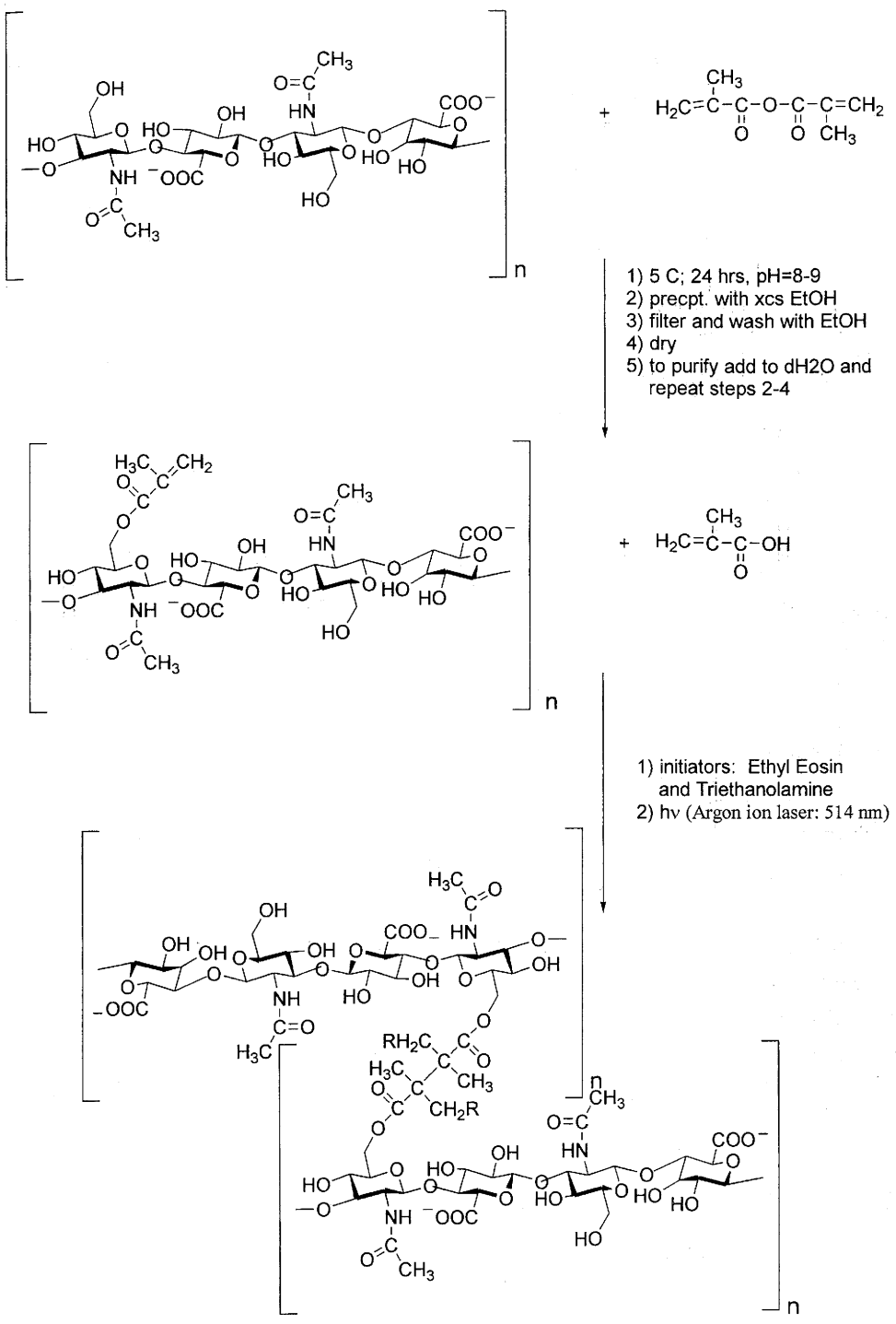
EXPERIMENTAL

Modification of Hyaluronic Acid with Methacrylic Anhydride

A 2% w/v solution of hyaluronic acid (ACROS) was adjusted to a pH of 8 and an excess of methacrylic anhydride was added (Scheme 1). After 24 hours at 5°C, the methacrylated modified hyaluronic acid (HA-MA) was precipitated and washed with ethanol to remove remaining methacrylic acid and methacrylic anhydride. ^{1}H-NMR spectra were recorded on a Varian Invoa 400 spectrometer.

Measurement of Viscosity

The viscosity of the modified and unmodified HA biopolymers was measured on a Wells-Brookfield Cone-Plate Rheometer (LDV-III).



Scheme 1. Synthesis of HA-MA and photocrosslinking.

Synthesis of Photocrosslinked HA-MA Hydrogels

A photocrosslinked polysaccharide hydrogel was synthesized by irradiating HA-MA in the presence of ethyl eosin (5 μL of 0.5% w/v in 1-vinyl-2-pyrrolidinone per ml of polymer) and triethanolamine (50 μL of 5M in 0.1 M HEPES buffer, pH = 7.4, per ml of polymer) using an argon ion laser (Scheme 1; 30 seconds; emission max 514 nm; 2.4 W) [11, 12]. Blocks and films of 2% w/v HA-MA were also made in an analogous manner.

Diffusion of Dextrans

Dextrans of three molecular weights (MW = 4400 , 71000, and 167000 g/mol) labeled with fluorescein isothiocyanate (FITC) were dissolved in HA-MA at concentrations of 4.5 mg/ml, 4.5 mg/ml and 3 mg/ml respectively. One ml of the polymer/dextran solution was mixed with 5 μl of 0.5% ethyl eosin in 1-vinyl pyrrolidinone and 50 μl 5M TEA, spread on a Teflon watch glass and exposed to an argon ion laser (30 seconds; emission max 514 nm; 2.4 W). The resulting photocrosslinked hydrogel was washed with 10 ml 0.1M HEPES buffer (pH = 7.4) twice and incubated in 10 ml of HEPES buffer. The solution was changed at regular time periods and analyzed for FITC-dextran concentration (HP 8452 UV-vis, monitoring at 495 nm).

Synthesis of Photocrosslinked Microcapsules

Capsules were easily made by dropping varying ratios of HA-MA/LVG (Pronova; low viscosity high guluronic acid content alginate) into 0.2 M $CaCl_2$. Microcapsules for these studies were synthesized using a 2% w/v solution of a mixture of 3:1 HA-MA:LVG, with ethyl eosin (5 μL of 0.5% w/v in 1-vinyl-2-pyrrolidinone per ml of polymer) and triethanolamine (50 μL of 5M in 0.1 M HEPES per ml of polymer) using an argon ion laser (Scheme 1; 30 seconds; emission max 514 nm; 2.4 W) [11, 12]. Microcapsules of 500 to 800 microns in diameter were synthesized using a coaxial jet-head generator. The microcapsules were then exposed to an argon ion laser for 90 seconds to covalently cross-link the modified HA.

Disintegration of Microcapsules by Sodium Citrate or EDTA

Microcapsules composed of 2% LVG, 2% LVM (Pronova; alginate high in mannuronic acid) and 2% HA-MA/LVG (3:1) were incubated in 55 mM sodium citrate or EDTA to examine capsule disintegration by calcium chelation. Capsule size was measured over time using a microscope.

Degradation of Microcapsules by Hyaluronidase

Two samples of 0.7 ml HA-MA containing 5 mg/ml blue dextran (MW = 2 x 10^6) were photocrosslinked in polystyrene cuvettes. This created a solid gel block in the bottom of the cuvette. The samples were washed twice with 2 ml 0.1 M HEPES buffer (pH = 7.4). Two ml of HEPES buffer (0.1 M; pH = 7.4) with 180 U/ml hyaluronidase (HAase) was added to one sample while the second sample contained only 2 ml of buffer. The absorbance of the supernatant was measured every ten minutes for 48 hours to determine the rate of blue dextran release (UV-vis, HP 8450, 620 nm). The polymer block was below the UV-vis beam, to ensure that the hydrogel would not interfere with the measurements.

RESULTS AND DISCUSSION

Methacrylate-modified hyaluronic acid (HA-MA) was synthesized as shown in Scheme 1. A ^{1}H-NMR spectrum of the acid-degraded HA-MA showed the acrylate peaks at 5.6 and 6.0 ppm and the methyl peak at 1.8 ppm (Figure 1). The amount of modification per possible sites was determined by integrating the methacrylate and HA peaks, and the polymer was found to be approximately 14% modified. This photocrosslinked hydrogel polymer could be used to form well defined polymeric hydrogel structures such as microcapsules, blocks, or films (Figure 2).

The rheological properties of the modified hyaluronic acid (HA-MA) were next determined and compared to unmodified hyaluronic acid. Chemical modification did not dramatically alter the viscosity of the HA-MA compared to HA (1.5 %w/v; 17,000 cP and 16,000 cP, respectively at 0.1 s^{-1} shear rate). We also observed that HA-MA was a non-Newtonian polymer-like HA (the viscosity changes with shear rate). Specifically, both HA and HA-MA were pseudoplastic (a phenomenon in which the viscosity decreases with increasing shear rate). HA-MA exposed to high sheer rates did not alter the viscosity of the polymer suggesting that HA-MA should withstand large shear forces without significant biopolymer degradation. This is important since large shear forces are present during microcapsule synthesis when using a coaxial jet-head microcapsule generator.

The diffusion properties of the photocrosslinked hydrogels were next determined. Small rectangular blocks of photocrosslinked HA-MA containing different molecular weight FITC-dextrans (MW = 4400, 71000, and 167000 g/mol) were monitored using a spectrophotometer over time for release of the

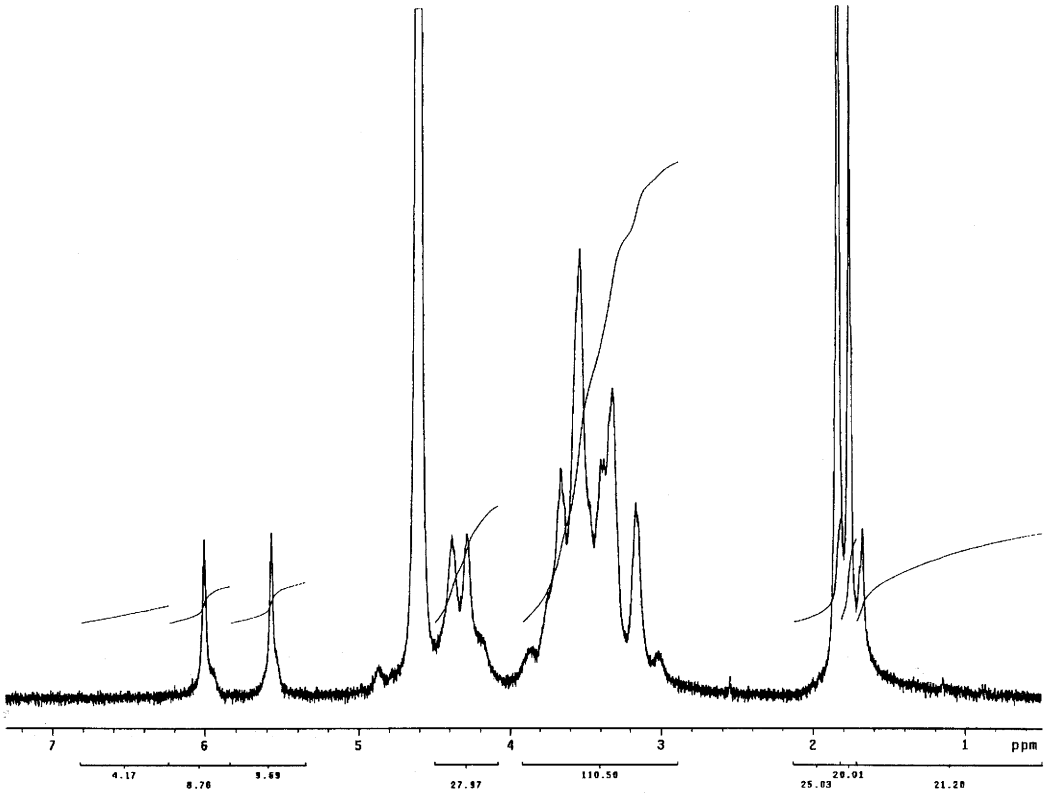


Figure 1. ^{1}H NMR spectrum of HA-MA.

FITC dextran. Facile diffusion of small molecular weight dextrans was observed compared to large molecular weight dextrans (Figure 3) . This molecular weight dependent process suggests that HA-MA may possess favorable diffusion and permeability properties for *in vivo* studies. Large molecular weight species such as antibodies (MW = 150000) would have difficulty penetrating the hydrogel to recognize the encapsulated biologic.

Photocrosslinked microcapsules were synthesized using a standard capsule generator in combination with an argon ion laser for photocrosslinking. In order to create microcapsules of modified hyaluronic acid, a small amount of alginate was used to form sufficient temporary ionic cross-linking with calcium. Without the alginate present, the HA-MA forms discs when used in the capsule generator. This interpenetrating network of alginate and hyaluronic acid enables spherical and well-defined microcapsules with varying ratios of HA-MA/LVG from 1:10 to 10:1 to be synthesized. Microcapsules composed of HA-MA/LVG (3:1) are shown in Figure 1.

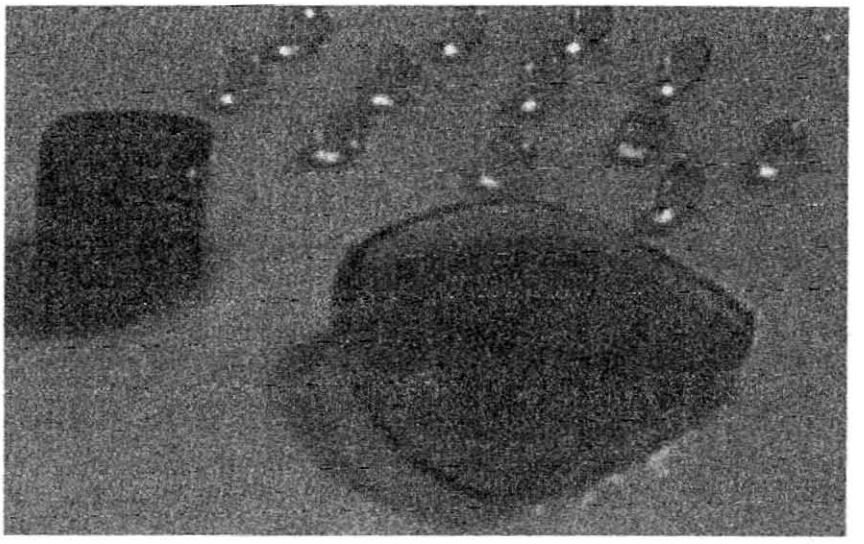


Figure 2. Photocrosslinked gels synthesized with HA-MA.

To ensure sufficient covalent crosslinking had occurred during microcapsule synthesis, the HA-MA/LVG microcapsules were exposed to a solution containing a calcium chelator. Addition of these photocrosslinked microcapsules to solutions of 55 mM Na Citrate or EDTA produced no change in capsule number or diameter during one year. However, HA-MA or alginate microcapsules that were only calcium crosslinked dissolved in less than 30 minutes under the same conditions.

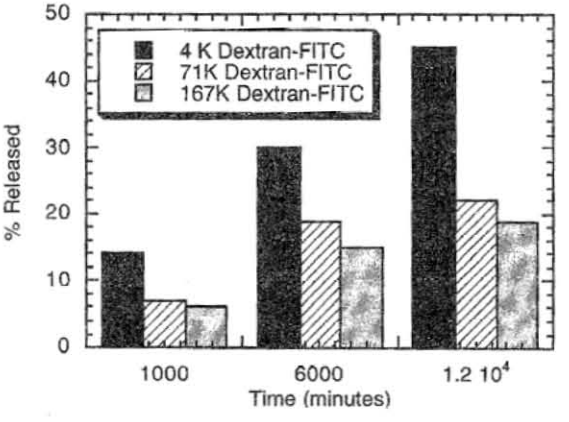


Figure 3. Diffusion profiles of FITC-labeled dextrans in HA-MA.

A kinetic study of the ability of hyaluronidase (HAase) to degrade the photocrosslinked HA-MA was next performed. Hyaluronidase is present in tissue lysozymes and at low levels in blood serum. A photocrosslinked sample of HA, containing blue dextran, was placed in a spectrophometer for 48 hours at 37°C [13]. No release of blue dextran was observed in either HAase or control (no enzyme) samples. After 30+ days the polymer had maintained its block form with no blue dextran release. This resistance to degradation was much longer than what would be predicted by the *in vivo* half-life of HA in the presence of HAase (1 to 3 days) [14]. These results were also consistent with the observation that the ability of HAase to degrade HA is inversely related to the molecular weight of HA. Small molecular weight HA is degraded faster than large molecular weight HA. The photocrosslinked HA-MA has a greater molecular weight than the original polymer since covalent crosslinking has occurred throughout the polymer chains.

A preliminary cell encapsulation study was performed with islets of Langerhans. Islet cells were isolated from Lewis rats following the protocol of Gray, [15] and used for *in vitro* studies. The photocrosslinkable biopolymer (1 ml) was added to a suspension containing approximately 700 islets in 300 μL (HBSS). This mixture was then loaded into the capsule generator and microcapsules were created as previously described. Light microscopy showed the rat islets of Langerhans to be encapsulated within the photocrosslinked microcapsule. Each capsule contained 1-2 islet cells on average. Importantly, the encapsulated cells stained positive for insulin using dithizone. Additional, islet isolation and encapsulation studies are in progress to better evaluate the polymer for cell encapsulation and to asses the function (insulin secreting ability) of the encapsulated islets. Upon successful completion of these studies, we plan initiate an allograft transplantation experiment with encapsulated rat islets transplanted into the intraperitoneal cavity of non-immunosuppressed streptozotocin induced diabetic rats.

CONCLUSION

We have synthesized and characterized a novel photocrosslinkable hyaluronic acid hydrogel. The methacrylate-modified hyaluronic acid possesses favorable chemical and physical properties for biomedical applications such as cell encapsulation.

ACKNOWLEDGEMENT

This work was supported by the North Carolina Biotechnology Center, NIH Training Grant (Biological Chemistry Duke University) and Duke University.

REFERENCES

[1] N. A. Peppas, *Hydrogel in Medicine and Pharmacy*, Vols. I and II, (1987).
[2] O. Wichterle and D. Lim, *Nature, 1851*, 117-118 (1960).
[3] R. M. Ottenbrite, S. J. Huang, and K. Park, *Hydrogels and Biodegradable Polymers for Bioapplications*, Vol. 627 (1994).
[4] N. A. Peppas and R. Langer, *Science, 2631,* 1715-1720 (1994).
[5] B. D. Ratner, *J. Biomed. Mater. Res., 271,* 837 (1993).
[6] R. Langer and J. P. Vacanti, *Science, 2601,* 920-926 (1993).
[7] R. Skalak and C. F. Fox, *Tissue Engineering*, Liss, New York, 1988.
[8] R. M. Nerem, *Ann. Biomed. Eng., 191,* 529 (1991).
[9] F. Lim and A. M. Sun, *Science, 2101,* 908-910 (1980).
[10] S. Takigami, M. Takigami, and G. Phillips, *Carb. Polymers, 221*, 1-8 (1993).
[11] D. C. Neckers, K. S. Raghuveer, and O. Valdes-Aguilera, *Polym. Mater. Sci. Eng., 601*, 15 (1989).
[12] E. Chesneau and J. P. Foussier, *Angew. Makromol. Chem., 1351,* 41 (1985).
[13] T. C. Lauret and E. A. Balazs, *Chemical Modification of Hyaluronic Acid for Drug Delivery, Biomaterials, and Biochemical Probes*, Wenner-Gren Foundation, Stolkholm, 1997.
[14] J. R. E. Fraser, T. C. Laurent, and U. B. G. Laurent, *J. Internal Med. 2421,* 27-33 (1997).
[15] R. Sutton, M. Peters, P. McShane, and D. W. R. Gray, *Transplantation, 181,* 819-820 (1986).