Macromolecules

Volume 39, Number 4 February 21, 2006

Communications to the Editor

Extracellular Matrix-like Cell-Adhesive Hydrogels from RGD-Containing Poly(ethylene glycol) Diacrylate

Junmin Zhu,[†] Jeffrey A. Beamish,[†] Chad Tang,[†] Kandice Kottke-Marchant,[§] and Roger E. Marchant*,[†,‡]

Department of Biomedical Engineering, Case Western Reserve University, Cleveland, Ohio 44106; Department of Macromolecular Science, Case Western Reserve University, Cleveland, Ohio 44106; and Department of Clinical Pathology, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195

Received October 31, 2005
Revised Manuscript Received December 27, 2005

Hydrogels produced by photopolymerization have recently attracted significant interest as scaffolds for repairing and regenerating a wide variety of tissues, such as cartilage, bone, and vasculature.[1] Photopolymerization allows the hydrogel to be generated in vitro or in vivo from a low-viscosity solution of monomers or macromers in a minimally invasive manner.[2] The most important photopolymerizable hydrogels are poly(ethylene glycol) (PEG) hydrogels, which have been extensively investigated as scaffolds for tissue engineering due to their biocompatibility and low toxicity.[3] Several types of PEG macromers, such as PEG diacrylate (PEGDA), PEG dimethacrylate (PEGDM), star PEG multiple acrylate, and related biodegradable macromers like PEG-lactide diacrylate and proteolytically degradable PEG diacrylates, have been used as the precursors to form cross-linked PEG hydrogels in the presence of UV light and a photoinitiator.[4−6] PEG hydrogels have desirable mechanic properties and provide a highly swollen three-dimensional environment similar to soft tissues with a high water content, which allows diffusion of nutrients and cellular waste through the elastic network.[4] Another advantage of photogenerated PEG hydrogels over natural physical gels such as alginate is that their material properties can be more easily adjusted to fit the desired application.[7]

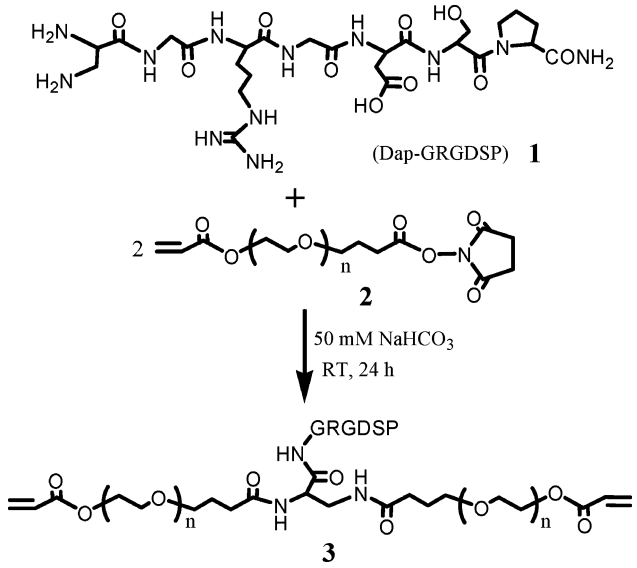
It is well-known that PEG hydrogels do not facilitate the adhesion of cells due to their bioinert and nonadhesive nature.[8] They alone cannot provide an ideal environment for culturing anchorage-dependent cells, such as endothelial cells (ECs), smooth muscle cells (SMCs), fibroblasts, or osteoblasts. To facilitate cell adhesion, Hern and Hubbell reported the use of monoacrylated RGD peptide with and without a PEG spacer, copolymerized with PEGDA to make biospecifice PEG hydrogels.[9] This method has been extensively studied and has been shown to stimulate cell adhesion, spreading, and growth on the otherwise nonadhesive surfaces of PEG hydrogels.[10,11] However, the extent of incorporation of the monoacrylated peptides by copolymerization with PEGDA is limited by concentration of either component and never exceeded that of the PEGDA precursor for the concentrations studied.[9] PEGDA polymerizes better than monoacrylated peptide because PEGDA macromer is acrylated at two sites. As a result, increasing incorporation of monoacrylated peptides affects the mechanic properties and the swelling ratio of the hydrogels.[10]

To address this problem, we report a novel strategy to synthesize a PEGDA macromer with a cell-adhesive peptide ligand. A hexapeptide, GRGDSP, with specific binding to the integrin receptors expressed on the surface of a variety of cell types, is used as the peptide sequence for the bioactive modification of PEGDA. This macromer with RGD peptide attached in the middle of the PEG chain (denoted as RGD-PEGDA, **3**), as shown in Scheme 1, combines the photopolymerization ability of PEGDA and the bioactivity of RGD peptides. In contrast to hydrogels prepared from the copolymerization of PEGDA and the monoacrylated RGD peptides, this RGD-PEGDA macromer can be polymerized or copolymerized with other PEGDA macromers to form ECM-like cell-adhesive PEG hydrogels with better distribution of RGD peptides and higher

[†] Department of Biomedical Engineering, Case Western Reserve University.
[‡] Department of Macromolecular Science, Case Western Reserve University.
[§] Cleveland Clinic Foundation.
* Corresponding author: Tel 216-368-3005; Fax 216-368-4969; e-mail rxm4@case.edu.

10.1021/ma052333s CCC: $33.50
Published on Web 01/24/2006

Scheme 1. Synthesis of RGD-PEGDA



peptide incorporation without affecting the hydrogel physical properties.

Scheme 1 shows the synthetic reactions leading to the formation of RGD-PEGDA (**3**). To attach the RGD peptide in the middle of PEG chain, the RGD-containing hexapeptide, GRGDSP, was capped with diaminopropionic acid (Dap) to generate Dap-GRGDSP (**1**) with two free amine groups at the N-terminus,[12] followed by reacting with acryloyl-PEG-NHS (Acr-PEG-NHS, **2**) (M_w 3400) to generate the final product **3**. Dap-GRGDSP was synthesized using Fmoc chemistry by solid-phase peptide synthesis (SPPS) on a PAL resin, cleaved by Reagent K, and purified by reverse-phase HPLC.[13] Its structure was confirmed by MALDI-TOF MS analysis with a peak at $m/z = 673.65$ for $[M + H]^+$ (calculated mass 673.7) and a peak at $m/z = 695.93$ for $[M + Na]^+$ (calculated mass 695.7) (Figure 1a).

RGD-PEGDA was synthesized by reacting Dap-GRGDSP with a double amount of Acr-PEG-NHS in 50 mM sodium bicarbonate buffer (pH 8.2) for 24 h at room temperature[9,11a] and purified by dialysis through a membrane with M_w cutoff of 5000. The MALDI-TOF MS of RGD-PEGDA (Figure 1b) shows a unique distribution with a repeating M_w difference of 44, which corresponds to one PEG repeating unit. The maximum peak at 7435.27 corresponds to the structure of **3**, which resulted from the conjugation of 2 M Acr-PEG-NHS and 1 M RGD peptide. A control macromer with the same PEGDA backbone as **3** was synthesized by reacting ethylenediamine (EDA) with Acr-PEG-NHS in methylene chloride to produce EDA-containing PEGDA (denoted as EDA-PEGDA).

Hydrogels were fabricated in the form of thin disks (diameter 10 mm, thickness 1 mm) with 20% (w/v) of macromers and 0.05% (w/v) of Irgacure 2959 photoinitiator[14] in PBS under UV irradiation (365 nm, 2−3 mW/cm^2) for 10 min. The swelling

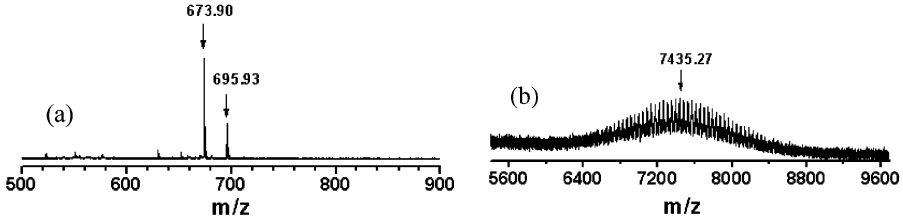


Figure 1. MALDI-TOS MS spectra of (a) Dap-GRGDSP and (b) RGD-PEGDA.

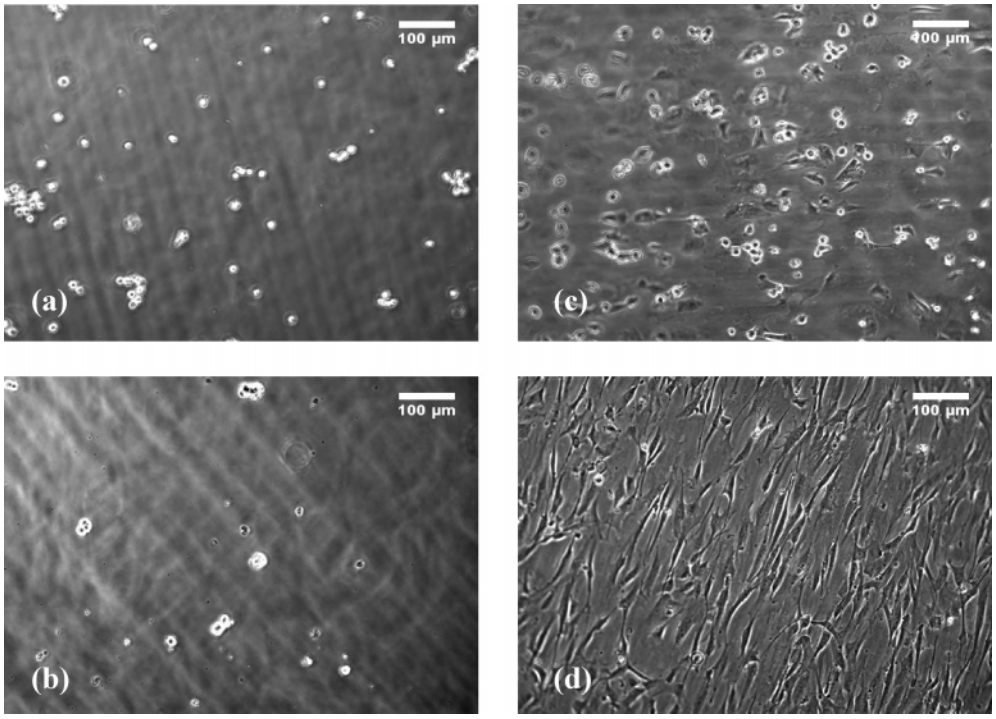


Figure 2. Photomicrographs of SMCs cultured on the surfaces of hydrogels formed from 20% (w/v) macromers in PBS: (a) EDA-PEGDA hydrogels 2 h after seeding SMCs; (b) EDA-PEGDA hydrogels 24 h after seeding SMCs; (c) RGD-PEGDA hydrogels 2 h after seeding SMCs; (d) RGD-PEGDA hydrogels 24 h after seeding SMCs.

Table 1. Properties of Hydrogels Prepared from 20% (w/v) Macromers

hydrogel	M_w	q	M_c (g/mol)	ξ (Å)
RGD-PEGDA	7435	14.7 ± 0.2	2257 ± 30	75.8 ± 0.2
EDA-PEGDA	6883	14.1 ± 0.3	2026 ± 42	70.7 ± 1.3
PEGDA6K	6303	13.0 ± 0.2	1791 ± 26	64.7 ± 0.8

and mesh size results are listed in Table 1. The equilibrium mass swelling ratio (q) was measured based on the ratio of the swollen to the dry mass of the hydrogels. The number-average molecular weight between cross-links (M_c) and average mesh size (ξ) were calculated using the method described by Canal and Peppas.[15] The hydrogel swelling and cross-linking density are particularly important from a tissue-engineering perspective, since they impact transport and overall cell viability and influence cell behavior. Under the same processing conditions, hydrogels made from RGD-PEGDA ($q = 14.7$) exhibited lower cross-linking density with a higher M_c (2257 g/mol) and mesh size (75.8 Å) than the hydrogels made from EDA-PEGDA ($q = 14.1$) due to the RGD attachment. EDA-PEGDA has a similar structure to PEGDA6K,[16] but hydrogels made from EDA-PEGDA had higher M_c and mesh size than the hydrogels made from PEGDA6K ($q = 13.0$) due to its longer PEG chain.

To test the cell response to these materials, human pulmonary artery SMCs were seeded on 20% (w/v) hydrogels made from macromers sterilized with a 0.2 μm syringe filter. Figure 2 shows light micrographs of SMCs on the hydrogel surfaces 2 and 24 h after seeding. SMCs seeded on the EDA-PEGDA hydrogels assumed a rounded morphology with no evidence of spreading at both time points and had a decreased density 24 h after seeding (Figure 2a,b), suggesting that SMCs have only weak, nonspecific interactions with this material. SMCs seeded on the RGD-PEGDA hydrogels (RGD density = 26.3 mM) showed higher initial cell attachment and some cell spreading after 2 h (Figure 2c) and extensive spreading after 24 h (Figure 2d). Thus, the enhanced attachment and spreading on RGD-PEGDA hydrogels were attributed to the specific binding of SMCs to the RGD peptide.

In summary, RGD-containing poly(ethylene glycol) diacrylate (RGD-PEGDA) was synthesized by the conjugation of a Dap-capped RGD peptide with Acr-PEG-NHS. The resulting ECM-like cell-adhesive hydrogels made from RGD-PEGDA facilitated SMC adhesion and spreading on the hydrogel surface. This novel biospecific PEG macromer will be an excellent candidate for tissue engineering applications because they form hydrogels with a better distribution of RGD peptides without affecting the hydrogel physical properties. Further study of its applications is in progress.

Acknowledgment. This project was supported by the National Institutes of Health (Grant EB002067). We gratefully thank Coby Larsen for the assistance with cell culturing and the facilities provided by Center for Cardiovascular Biomaterials.

Supporting Information Available: Details of Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Hoffman, A. S. *Adv. Drug Deliv. Rev.* **2002**, *43*, 3. (b) Halstenberg, S.; Panitch, A.; Rizzi, S.; Hall, H.; Hubbell, J. A. *Biomacromolecules* **2002**, *3*, 710. (c) Bryant, S. J.; Anseth, K. S. *J. Biomed. Mater. Res.* **2003**, *64A*, 70. (d) Mann, B. K.; West, J. L. *Anat. Rec.* **2001**, *263*, 367. (e) Burdick, J. A.; Anseth, K. S. *Biomaterials* **2002**, *23*, 4315.

(2) (a) Lee, K. Y.; Mooney, D. J. *Chem. Rev.* **2001**, *101*, 1869. (b) Nguyen, K. T.; West, J. L. *Biomaterials* **2002**, *23*, 4307. (c) Zheng, Y.; Micic, M.; Mello, S. V.; Mabrouki, M.; Andreopoulos, F. M.; Konka, V.; Pham, S. M.; Leblanc, R. M. *Macromolecules* **2002**, *35*, 5228. (d) Bryant, S. J.; Nuttelman, C.; Anseth, K. S. *J. Biomater. Sci., Polym. Ed.* **2000**, *11*, 439.

(3) Griffith, L. G. *Acta Mater.* **2000**, *48*, 263.

(4) (a) Bryant, S. J.; Anseth, K. S. *Biomaterials* **2001**, *22*, 619. (b) Bryant, S. J.; Anseth, K. S. *J. Biomed. Mater. Res.* **2002**, *59*, 63. (d) Watkins, A. W.; Anseth, K. S. *Macromolecules* **2005**, *38*, 1326. (e) Sawhney, A. S.; Pathak, C.; Hubbell, J. A. *Macromolecules* **1993**, *26*, 581.

(5) (a) Han D. K.; Hubbell, J. A. *Macromolecules* **1996**, 29, 5233. (b) Han D. K.; Hubbell, J. A. *Macromolecules* **1997**, *30*, 6077. (c) Keys, K. B.; Andreopoulos, F. M.; Peppas, N. A. *Macromolecules* **1998**, *31*, 8149.

(6) (a) West, J. L.; Hubbell, J. A. *Macromolecules* **1999**, *32*, 241. (b) Mann, B. K.; Gobin, A. S.; Tsai, A. T.; Schmedlen, R. H.; West, J. L. *Biomaterials* **2001**, *22*, 3045.

(7) Elisseeff, J.; McIntosh, W.; Anseth, K.; Riley, S.; Ragan, P.; Langer, R. *J. Biomed. Mater. Res.* **2000**, *51*, 164.

(8) Hubbell, J. A. *J. Controlled Release* **1996**, *39*, 305.

(9) Hern, D. L.; Hubbell, J. A. *J. Biomed. Mater. Res.* **1998**, *39*, 266.

(10) (a) Hubbell, J. A. *Curr. Opin. Biotechnol.* **1999**, *10*, 123. (b) Gobin, A. S.; West, J. L. *J. Biomed. Mater. Res.* **2003**, *67A*, 255. (c) Mann, B. K.; West, J. L. *J. Biomed. Mater. Res.* **2002**, *60*, 86. (d) Gunn, J. W.; Turner, S. D.; Mann, B. K. *J. Biomed. Mater. Res.* **2005**, *72A*, 91.

(11) (a) Behravesh, E. B.; Zygourakis, K.; Mikos, A. G. *J. Biomed. Mater. Res.* **2003**, *65A*, 260. (b) Yang, F.; Williams, C. G.; Wang, D. A.; Lee, H.; Manson, P. N.; Elisseeff, J. *Biomaterials* **2005**, *26*, 5991.

(12) The consideration to use Dap instead of Lys is that the Dap-capped GRGDSP peptide will be more hydrophilic due to just one methylene group between the Dap amino acid backbone and the side amine group.

(13) Anderson, E. H.; Ruegsegger, M. A.; Murugesan, G.; Kottke-Marchant, K.; Marchant, R. E. *Macromol. Biosci.* **2004**, *4*, 766.

(14) Irgacure 2959 (from Ciba Specialty Chemicals) is used as a water-soluble and cytocompatible photoinitiator. Its chemical name is 2-hydroxy-1-[4-(hydroxyethoxy) phenyl]-2-methyl-1-propanone. For more details, see ref 2d.

(15) (a) Canal, T.; Peppas, N. A. *J. Biomed. Mater. Sci.* **1989**, *23*, 1183. (b) Cruise, G. M.; Scharp, D. S.; Hubbell, J. A. *Biomaterials* **1998**, *19*, 1287.

(16) PEGDA6K was synthesized by reacting PEG (M_w 6000, from Aldrich) with acryloyl chloride in the presence of triethylamine according to the method described in ref 6b.

MA052333S