A Supramolecular Hydrogel Based on Polyglycerol Dendrimer - Specific Amino Group Recognition

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A Supramolecular Hydrogel Based on Polyglycerol Dendrimer-Specific Amino Group Recognition

Ik Sung Cho [a] and Tooru Ooya*[a]

Abstract: Dendrimer-based supramolecular hydrogels have gained attention in biomedical fields. While biocompatible dendrimers were used to prepare hydrogels via physical and/or chemical crosslinking, smart functions such as pH and molecular control remain undeveloped. Here, we present polyglycerol dendrimer-based supramolecular hydrogel formation induced by a specific interaction between the polyglycerol dendrimer and an amino group of glycol chitosan. Gelation was achieved by mixing the two aqueous solutions. Hydrogel formation was controlled by varying the polyglycerol dendrimer generation. The hydrogel showed pH-dependent swelling; strongly acidic conditions induced degradation via dissociation of the specific interaction. It also showed unique L-arginine-responsive degradation capability due to competitive exchange of the amino groups of glycol chitosan and L-arginine. These polyglycerol dendrimer-based supramolecular characteristics allow multimodal application in smart biomaterials.

Hydrogels are chemically or physically crosslinked networks composed of hydrophilic homopolymers or copolymers, which can swell and hold large amounts of water. Many applications of hydrogels have been reported in drug/protein delivery and tissue engineering, due to their promising properties such as high water content, softness, elasticity, and biocompatibility.[1] Recent research has focused on supramolecular interactions such as hydrogen bonding, van der Waals interactions, and charge transfer forces in the formation and deformation of supramolecular hydrogels.[2] Several supramolecules, such as rotaxanes,[3] polyrotaxanes,[4] polypseudorotaxanes,[5] and dendrimers,[6] have been studied as building blocks for hydrogels.

In recent decades, various dendrimer-based hydrogels were developed for biomedical applications. Dendrimers have attracted significant attention related to their wide ranging potential applications, due to their advantageous physical and chemical properties compared to analogous linear polymers, such as low intrinsic viscosities, significantly increased solubility, and a large number of terminal groups.[7] A typical dendritic structure has three distinct components: a central core, branching units covalently bound between a branching point and the central core, and functional surface groups that predominantly determine the physical and chemical properties of the dendrimer.[8] Dendritic macromolecules have a nano-sized, homogeneous, and monodisperse structure consisting of tree-like branches.[9] Because of their highly branched architecture, dendrimer-based systems are potentially useful for many applications including drug delivery,[10] nanocomposites,[11] scaffolds,[12] cell adhesion,[13] and coatings.[14] Recent studies revealed various hydrogels formed from these dendritic macromolecules by physical (hydrogen bonding, ionic association, and host-guest complexation) or chemical crosslinking (photopolymerization and condensation/addition reactions) for various biomedical applications.[15] For example, Wang et al. proposed a strong dendritic self-healing physical hydrogel.[16] This was prepared by mixing dendritic binders functionalized with guanidinium end groups, clay nanosheets, and sodium polyacrylates. The strong physical interaction between the dispersed clay nanosheets and dendritic binders produced the dendritic hydrogels. In another approach, Zhang et al. described degradable hydrogels prepared by photocrosslinking a generation 4 hyperbranched polyester bearing acrylate groups for the formation of a four-cell matrix and sustained drug release.[13] However, the photocrosslinking approach is limited with respect to many clinical applications because it often requires chemical and toxic agents that can generate side products. From this perspective, a reliable and efficient methodology for producing stable hydrogels under physiological conditions is required.

Polyglycerol dendrimers (PGDs) and hyperbranched polyglycerols (HPGs) have been studied for various biomedical applications due to their biocompatibility.[7a] [17] Unlike HPGs, PGDs exhibit well-defined nanostructures with narrow molecular weight distributions, resulting in higher reactivity and loading efficiency compared to other molecules.[18] In our previous study, we found that a generation 3 PGD (PGD-G3) specifically interacted with amino groups of L-arginine in aqueous solution.[18c] Therefore, one can imagine that the amino group of chitosan may interact with PGDs, enabling us to form a PGD-based hydrogel.

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Supporting information for this article is given via a link at the end of the document.

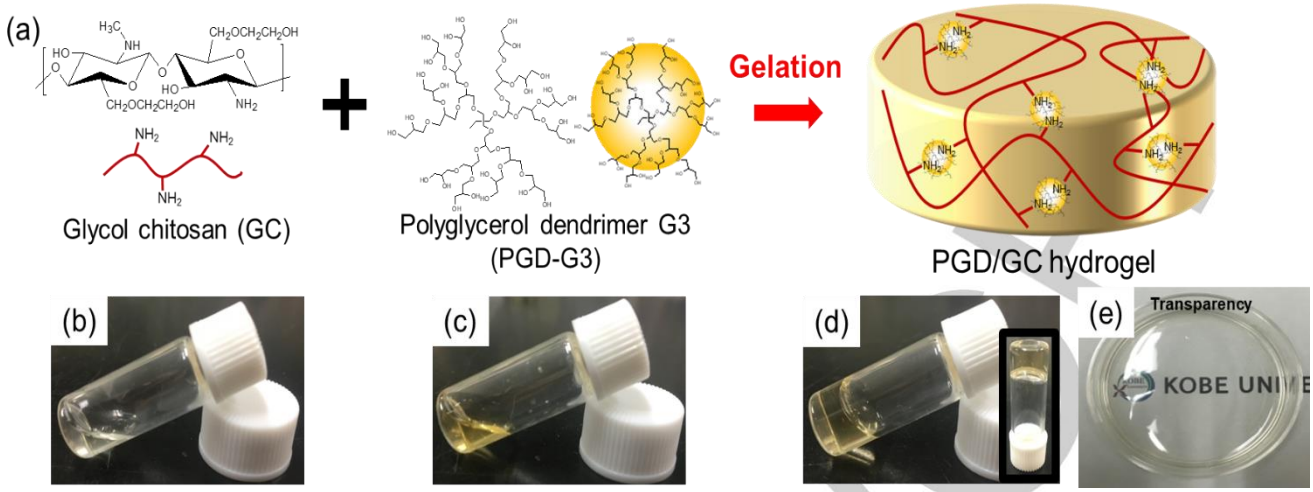

Figure 1. Gelation of the PGD-G3/GC hydrogel system. (a) Formation of the PGD/GC network hydrogel architecture induced by the self-crosslinking of a PGD host and GC guest in distilled water. Representative photos of aqueous solutions of (b) GC, (c) PGD-G3, (d) 1:1 PGD-G3 and GC mixture. (e) PGD/GC thin gel prepared on a petri dish.

Here, we report the first observation of hydrogelation via supramolecular complexation between a PGD-G3 and a natural polymer, glycol chitosan (GC) under physiological conditions. We hypothesized that a PGD-based hydrogel can be formed via host-guest crosslinking using the PGD as the host molecule and the amino group of GC (Figure 1a). When GC solutions (Figure 1b) were mixed with PGD-G3 (Figure 1c) solution at room temperature, a transparent hydrogel was observed (Figure 1d and e). The gelation procedure was simple, and no chemical additives were required. To clarify the hydrogelation mechanism, ^{1}H-NMR spectra were recorded to determine the interaction between PGD-G3 and GC. A ^{1}H-NMR spectrum of the PGD-G3/GC hydrogel (Figure S4c) shows that the peak attributed to the proton next to an amino group of GC, H_a (δ = 2.54 ppm, Figure S4b), was split and shifted to $H_{a``}$ and $H_{a`}$ (δ = 2.59 and 2.74 ppm). The observed peak splitting to $H_{a``}$ and $H_{a`}$ indicates an interaction between PGD-G3 and GC similar to that described in a previous report: PGD-G3 formed a complex with basic amino acids in aqueous media.[18c] To confirm the amino group recognition, we mixed PGD-G3 with different types of polysaccharides without amino groups (dextran (Dex), carboxymethyl cellulose (CMC), and hyaluronic acid (HA): Figure S5). After mixing PGD-G3 and each polysaccharide, PGD-G3 did not form a hydrogel (Figure 2). This indicates that PGD can selectively interact with the amino group of GC.

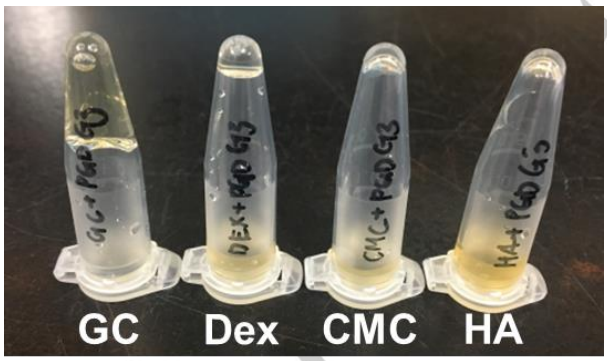

Figure 2. Photographs of the gelling behavior of PGD-G3 mixed with different polysaccharides

Although PGD-G3 recognized the amino group of GC to form a hydrogel, the generation of PGD and the degree of branching may affect the gelation behaviour. We mixed GC and PGD of generations 1 to 3 under the same conditions. Additionally, a HPG, the molecular weight of which was similar to PGD-G3, was also subjected to gelation. These dendritic polymers and GC were mixed at various feed molar ratios. Detailed information on the concentrations is given in the Supporting Information. The gelation time of this mixture was monitored by a tube inversion method at 37°C, and the results are listed in Table 1.

Table 1. Gelation times of dendritic polyglycerol hydrogels with various feed molar ratios.

Hydrogel	PGD: NH_2[a]	Gelation time[b]
PGD-G3/GC 1	0.125:1	-
PGD-G3/GC 2	0.25:1	more than 180 min
PGD-G3/GC 3	0.5:1	9.2 ± 0.1 min
PGD-G3/GC 4	1:1	2.8 ± 0.2 min
PGD-G2/GC 3	0.5:1	more than 90 min
PGD-G1/GC 3	0.5:1	-
HPG /GC 4	1:1	-

[a] Feed molar ratio of PGD to the glucosamine residue of GC. [b] The vial inversion method was used to determine the gelation time of the hydrogel.

To determine the best mixing ratio, we measured the gelation time of PGD-G3/GC hydrogels with different molar ratios of PGD-G3 to amino groups of GC. As shown in Table 1, the gelation time of the PGD-G3/GC hydrogels was dependent on the molar ratio of PGD-G3 and GC; this was more than 180 min when the ratio was 0.25:1. When the ratio was increased from 0.5:1 to 1:1, the gelation time decreased from 9.2 ± 0.1 to 2.8 ± 0.2 min, respectively. These results indicate that the equilibrium

of the interaction between PGD and NH_2 shifted toward binding. However, for the sample with a low molar ratio (0.125:1), no gelation was observed in the mixture. Thus, we determined that the best mixing ratio was 0.5–1:1. At a ratio of 0.5:1, we measured the gelation times of PGD/GC hydrogels with different generations of PGD-G*n* (*n*, generation number = 1–3). The gelation time of PGD-G*n*/GC hydrogels depended on the generation: PGD-G1 did not form hydrogel, indicating that interaction between PGD-G1 and the amino group is too weak to induce physical crosslinking. However, when *n* was increased from 2.0 to 3.0, the gelation time decreased drastically from 90 min to 9.2 ± 0.1 min, respectively. These results suggest that the ability to attract the amino group of GC into the interior of PGDs with low generations was poor, as discussed previously.[18b]

To evaluate the effect of the degree of branching (DB) on the gelation, we tested the gelation of PGD-G3 (M_n = 2,275, DB = 1) and HPG (M_n = 2,184, DB = 0.47) by mixing with GC. Unlike PGD-G3, the mixture of GC and HPG did not form a hydrogel, suggesting that an optimum DB is necessary for effective interaction; the linear sections of HPG did not participate in the interaction (Figure S6).

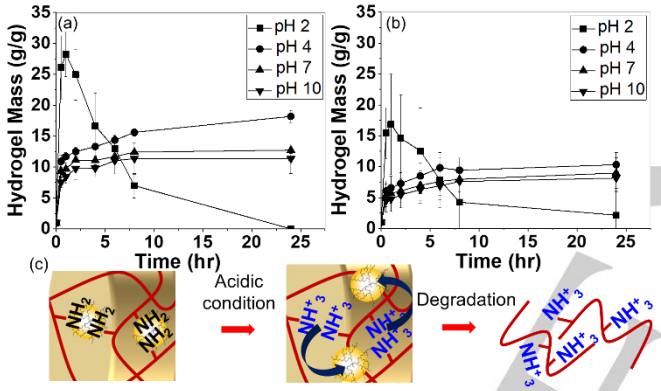

Figure 3. pH-dependent swelling behavior of PGD/GC hydrogels. (a) PGD-G2/GC 1, (b) PGD-G3/GC 1. (c) Schematic illustration of swelling and dissolution of the hydrogel under acidic conditions.

Since chitosan is a cationic polymer with pH-responsive swelling properties,[19] the obtained hydrogels may be pH-responsive. The pH-responsive properties for dried PGD-G2/GC 3 and PGD-G3/GC 3 are shown in Figure 3a and b, respectively. To determine their swelling behaviors, the dried hydrogels were immersed in solutions with different pH values (2.0, 4.0, 7.0, and 10.0). At pH 4.0, PGD-G2/GC 3 continued swelling up to 24 h (g g^{-1} ≅ 18.2). As the pH increased, the swelling ratio decreased slightly at pH 7.0 (g g^{-1} ≅ 12.7) and pH 10.0 (g g^{-1} ≅ 11.3) after 8 h. The swelling ratio of PGD-G3/GC 3 was slightly lower than that of PGD-G2/GC 3 (g g^{-1} ≅ 10.3, 8.9, 8.1 at 24 h at pH 4.0, 7.0, and 10.0, respectively). These results suggest that the crosslinking density of PGD-G3/GC was higher than that of PGD-G2/GC. Interestingly, in a much more acidic environment (pH 2.0), both PGD-G2/GC 3 and PGD-G3/GC 3 showed rapid swelling within 1 h, followed by mass loss during incubation (Figure 3a and b). The significant swelling behavior of the PGD/GC hydrogels at pH 2.0 might be due to protonation of the amino groups ($-NH_3^+$) on GC, leading to shifting of the equilibrium of the interaction between PGD and the amino groups to dissociation (Figure 3c). After 24 h, PGD-G2/GC 3 was completely dissolved and disappeared, while a little PGD-G3/GC 3 remained (g g^{-1} ≅ 2.1). These results indicate that a high generation improved the mechanical properties of the hydrogels. Thus, the PGD/GC hydrogels might exhibit pH-dependent changes in biodegradability.

Another specific property of the PGD-based hydrogels is L-arginine selectivity as PGD-G3 interacts strongly with L-arginine.[18c] The L-arginine responsive properties of the dried PGD-G3/GC 3 are shown in Figure 4a. To determine the L-arginine dependent swelling ratio, the dried PGD-G3/GC 3 was immersed in solutions with different L-arginine concentrations (10.0, 5.0, 1.0, 0 wt%) at 37°C. When the dried gels were exposed to 10 wt% L-arginine solution, the swelling ratio reached 38.2 (g g^{-1}) at 2 days, followed by a decrease over 30 days, although this degradation behavior was not observed below 5.0 wt%. These results indicate that L-arginine interacted competitively with PGD-G3 bound to the amino group of GC, resulting in dissociation (Figure 4c). Interestingly, the swelling ratio increased with the L-arginine concentration (Figure 4a: The morphological changes are shown in Figure S7). These results also supported the competitive interaction between the amino groups and PGD-G3, which led to a 'loosened' gel structure and increased water uptake. However, fully swelled PGD-G3/GC 3 showed no disintegration in 10 wt% L-arginine solution (Figure S8). This is probably because the central core, the hydrophobic part of the PGD-G3, blocks the penetration of L-arginine.

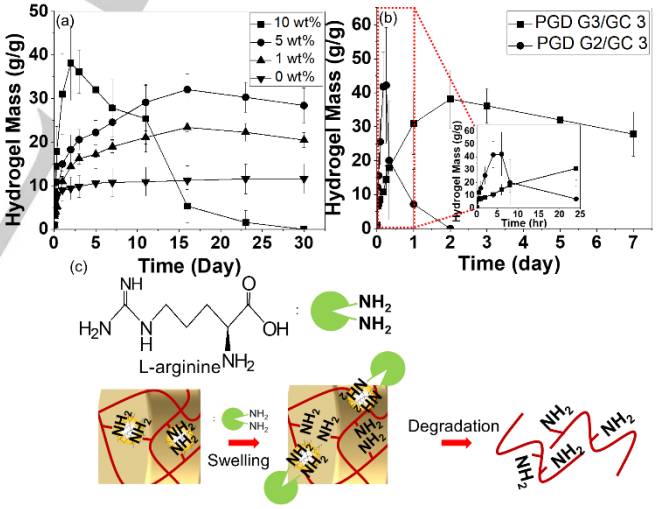

Figure 4. Swelling/degradation behaviour of (a) PGD-G3/GC 3 in aqueous L-arginine solutions with various concentrations, (b) PGD-G3/GC 3 and PGD-G2/GC 3 in 10 wt% L-arginine solution, and (c) Schematic illustration of swelling and dissolution of hydrogel in L-arginine solution.

The PGD-generation dependency of the L-arginine selectivity toward swelling/degradation properties was examined with PGD-G2 and PGD-G3/GC 3 hydrogels in 10 wt% L-arginine solution (Figure 4b). The PGD-G2/GC hydrogel showed rapid swelling behavior within 6 h, followed by a decrease in the hydrogel weight over 2 days. This is because the crosslinking density and

mechanical performance of PGD-G2/GC 3 are lower than those of PGD-G3/GC 3.

In conclusion, we achieved hydrogel formation by mixing PGD and GC, which was due to PGD-specific amino group recognition. The hydrogels exhibited both pH-responsive and L-arginine-competitive degradation properties. As far as we know, this is the first report on supramolecular hydrogels based on this formation. Since this supramolecular hydrogel formation is expected to promote differentiation of encapsulated cells,[20] the PGD/GC hydrogels are also potentially suitable for tissue engineering applications.

Acknowledgments

We gratefully acknowlege financial support from the Grant-in-Aid for JSPS Researc Fellow (JSPS KAKENHI grant number 17J09992); and a Grant-in-Aid for Scientific Research on Innovative Areas “New polymeric Materials Based on Element-Blocks (No.2401)“ (JSPS KAKENHI grant number JP15H00748)

Keywords: Gels • Dendrimers • Polyglycerol • pH responsive • Stimuli responsive

[1] (a)K. Deligkaris, T. S. Tadele, W. Olthuis, A. van den Berg, *Sens. Actuators, B* **2010**, *147*, 765-774; (b)K. Y. Lee, D. J. Mooney, *Chem. Rev.* **2001**, *101*, 1869-1880; (c)N. Peppas, P. Bures, W. Leobandung, H. Ichikawa, *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27-46; (d)N. Peppas, Y. Huang, M. Torres-Lugo, J. Ward, J. Zhang, *Annu. Rev. Biomed. Eng.* **2000**, *2*, 9-29; (e)B. V. Slaughter, S. S. Khurshid, O. Z. Fisher, A. Khademhosseini, N. A. Peppas, *Adv. Mater.* **2009**, *21*, 3307-3329.

[2] R. Dong, Y. Pang, Y. Su, X. Zhu, *Biomater. Sci.* **2015**, *3*, 937-954.

[3] T. Kureha, D. Aoki, S. Hiroshige, K. Iijima, D. Aoki, T. Takata, D. Suzuki, *Angew. Chem., Int. Ed.* **2017**, *56*, 15393-15396.

[4] (a)C. Pradal, L. Grondahl, J. J. Cooper-White, *Biomacromolecules* **2015**, *16*, 389-403; (b)A. Bin Imran, K. Esaki, H. Gotoh, T. Seki, K. Ito, Y. Sakai, Y. Takeoka, *Nat. Commun.* **2014**, *5*; (c)T. Ooya, T. Ichi, T. Furubayashi, M. Katoh, N. Yui, *React. Funct. Polym.* **2007**, *67*, 1408-1417; (d)J. Watanabe, T. Ooya, K. H. Nitta, K. D. Park, Y. H. Kim, N. Yui, *Biomaterials* **2002**, *23*, 4041-4048; (e)T. Ichi, J. Watanabe, T. Ooya, N. Yui, *Biomacromolecules* **2001**, *2*, 204-210.

[5] (a)Y. L. Cui, M. Tan, A. D. Zhu, M. Y. Guo, *RSC. Adv.* **2014**, *4*, 56791-56797; (b)L. P. Zhou, J. X. Li, Q. Luo, J. Y. Zhu, H. X. Zou, Y. Z. Gao, L. Wang, J. Y. Xu, Z. Y. Dong, J. Q. Liu, *Soft Matter* **2013**, *9*, 4635-4641.

[6] (a)L. Y. Xu, R. C. Cooper, J. Wang, W. A. Yeudall, H. Yang, *ACS Biomater. Sci. Eng.* **2017**, *3*, 1641-1653; (b)J. Wang, H. L. He, R. C. Cooper, H. Yang, *ACS Appl. Mater. Inter.* **2017**, *9*, 10494-10503.

[7] (a)M. Calderón, M. A. Quadir, S. K. Sharma, R. Haag, *Adv. Mater.* **2010**, *22*, 190-218; (b)Y. Lu, T. Nemoto, M. Tosaka, S. Yamago, *Nat. Commun.* **2017**, *8*, 1863; (c)A.-M. Caminade, S. Fruchon, C.-O. Turrin, M. Poupot, A. Ouali, A. Maraval, M. Garzoni, M. Maly, V. Furer, V. Kovalenko, *Nat. Commun.* **2015**, *6*; (d)A.-M. Caminade, D. Yan, D. K. Smith, *Chem. Soc. Rev.* **2015**, *44*, 3870-3873.

[8] (a)J. Wan, P. F. Alewood, *Angew. Chem., Int. Ed.* **2016**, *55*, 5124-5134; (b)G. Lapienis, *Prog. Polym. Sci.* **2009**, *34*, 852-892.

[9] E. Abbasi, S. F. Aval, A. Akbarzadeh, M. Milani, H. T. Nasrabadi, S. W. Joo, Y. Hanifehpour, K. Nejati-Koshki, R. Pashaei-Asl, *Nanoscale Res. Lett.* **2014**, *9*, 247.

[10] T. Ooya, J. Lee, K. Park, *J. Controlled Release* **2003**, *93*, 121-127.

[11] K. Satoh, T. Yoshimura, K. Esumi, *J. Colloid Interface Sci.* **2002**, *255*, 312-322.

[12] H. Holst, *J. Mater. Chem. B* **2013**, *1*, 6015-6019.

[13] H. Zhang, A. Patel, A. K. Gaharwar, S. M. Mihaila, G. Iviglia, S. Mukundan, H. Bae, H. Yang, A. Khademhosseini, *Biomacromolecules* **2013**, *14*, 1299-1310.

[14] U. Boas, P. M. Heegaard, *Chem. Soc. Rev.* **2004**, *33*, 43-63.

[15] (a)C. Ghobril, E. K. Rodriguez, A. Nazarian, M. W. Grinstaff, *Biomacromolecules* **2016**, *17*, 1235-1252; (b)Z. Huang, H. Lee, E. Lee, S.-K. Kang, J.-M. Nam, M. Lee, *Nat. Commun.* **2011**, *2*, 459; (c)M. Wathier, P. J. Jung, M. A. Carnahan, T. Kim, M. W. Grinstaff, *J. Am. Chem. Soc.* **2004**, *126*, 12744-12745; (d)H. Altin, I. Kosif, R. Sanyal, *Macromolecules* **2010**, *43*, 3801-3808; (e)G. R. Newkome, G. R. Baker, S. Arai, M. J. Saunders, P. S. Russo, K. J. Theriot, C. N. Moorefield, L. E. Rogers, J. E. Miller, *J. Am. Chem. Soc.* **1990**, *112*, 8458-8465; (f)A. K. Gaharwar, N. A. Peppas, A. Khademhosseini, *Biotechnol. Bioeng.* **2014**, *111*, 441-453.

[16] (a)Q. Wang, J. L. Mynar, M. Yoshida, E. Lee, M. Lee, K. Okuro, K. Kinbara, T. Aida, *Nature* **2010**, *463*, 339-343; (b)C. Wu, C. Strehmel, K. Achazi, L. Chiappisi, J. Dernedde, M. C. Lensen, M. Gradzielski, M. B. Ansorge-Schumacher, R. Haag, *Biomacromolecules* **2014**, *15*, 3881-3890; (c)M. H. Oudshoorn, R. Rissmann, J. A. Bouwstra, W. E. Hennink, *Biomaterials* **2006**, *27*, 5471-5479.

[17] (a)T. Ooya, T. Ogawa, T. Takeuchi, *J. Biomater. Sci., Polym. Ed.* **2018**, *29*, 701-705; (b)Y. Yang, A. Wang, Q. Wei, C. Schlesener, R. Haag, Q. Li, J. Li, *ChemistryOpen* **2017**, *6*, 158-164.

[18] (a)H. Lee, T. Ooya, *Chem. Commun.* **2012**, *48*, 546-548; (b)H. Lee, T. Ooya, *The J. Phys. Chem. B* **2012**, *116*, 12263-12267; (c)T. Ooya, H. Lee, *ChemNanoMat* **2015**, *1*, 264-269.

[19] L. Chen, Z. Tian, Y. Du, *Biomaterials* **2004**, *25*, 3725-3732.

[20] (a)H. Onoe, T. Okitsu, A. Itou, M. Kato-Negishi, R. Gojo, D. Kiriya, K. Sato, S. Miura, S. Iwanaga, K. Kuribayashi-Shigetomi, Y. T. Matsunaga, Y. Shimoyama, S. Takeuchi, *Nat. Mater.* **2013**, *12*, 584-590; (b)S. Zhang, M. A. Greenfield, A. Mata, L. C. Palmer, R. Bitton, J. R. Mantei, C. Aparicio, M. O. de la Cruz, S. I. Stupp, *Nat. Mater.* **2010**, *9*, 594-601.

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COMMUNICATION

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Supporting Information

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Abstract: Dendrimer-based supramolecular hydrogels have gained attention in biomedical fields. While biocompatible dendrimers were used to prepare hydrogels via physical and/or chemical crosslinking, smart functions such as pH and molecular control remain undeveloped. Here, we present polyglycerol dendrimer-based supramolecular hydrogel formation induced by a specific interaction between the polyglycerol dendrimer and an amino group of glycol chitosan. Gelation was achieved by mixing the two aqueous solutions. Hydrogel formation was controlled by varying the polyglycerol dendrimer generation. The hydrogel showed pH-dependent swelling; strongly acidic conditions induced degradation via dissociation of the specific interaction. It also showed unique L-arginine-responsive degradation capability due to competitive exchange of the amino groups of glycol chitosan and L-arginine. These polyglycerol dendrimer-based supramolecular characteristics allow multimodal application in smart biomaterials.

DOI: 10.1002/anie.2016XXXXX

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Experimental Procedures

Materials

Tetrabutylammonium bromide, allylchloride and osmoium tetroxide were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Dextran and L-arginine were purchased form Nacalai Tesque (Kyoto, Japan). Trimethylolpropane, toluene, t-butanol, glycol chitosan, carboxyl methyl cellulose, hydrochloric acid, hexane, ethylacete and methanol were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Magnesium sulfate was purchased from KANTO Chemical Industries, Ltd (Tokyo, Japan). Hyaluronic acid was purchased from Kikkoman Bio. Chem. Pha. (Tokyo, Japan).

Synthesis and characterization of polyglycerol dendrimer generation 3 (PGD-G3)

A polyglycerol dendrimer (Generation 3; PGD-G3) was synthesized according to the two-step processes based on allylation of alcohols and catalytic dihydroxylation.[1] Polyol (0.1 mol alcohol equivalents), tetrabutylammonium bromide (0.01 mol) were dissolved in 50 % NaOH solution in Teflon three-necked round-bottom flask. And then, allylchloride (0.50 mol) was added over 22 h at 45 °C under vigorous stirring. After the reaction was completed, 100mL of toluene was added to the flask. The organic phase was separated from the other phases and washed. After that reactant was dried with $MgSO_4$, filtered and concentrated under vacuum. Before the column chromatography, thin layer chromatography (TLC) was developed to ensure that the reactant was successfully synthesized. The crude was purified by a silica gel column chromatography (hexane/ethylacetate) to obtain a colorless oil. Polyallyl ether (0.1 mol equivalent), *N*-methylmorpholine-N-oxide (0.11 mol) were dissolved in acetone, distilled water and t-butanol, and the 4 wt% OsO_4 solution in water was added to the mixture and stirred for 20 h at room temperature, then concentrated under vacuum. The crude product was further purified by alumina column chromatography (methanol), then all volatile compound was removed *in vacuo*. The same procedure was repeated to obtain third generation PGD.

The PGD-G*n* (*n*=1-3) was identified by ^{1}H-NMR measurements using a JEOL ECS 400 NMR (JEOL, Japan) operating at 400 MHz.

^{1}H-NMR of PGD-G1 (400 MHz, D_2O): δ = 3.78 (t, 24H, HOC$\underline{H}_2$CHCH_2-), 3.75-3.67 (m, 12H, HOC$\underline{H}$CH_2O-), 3.66-3.59 (m, 24H, HOCHC$\underline{H}_2$O-), 3.58-3.51 (m, 9H, -OC$\underline{H}$CH_2O-), 3.51-3.18 (m, 36H, -OC$\underline{H}_2$CHC$\underline{H}_2$O-), 3.13 (t, 6H, -CC_2HOC$\underline{H}_2$CH-), 2.71 (s, 6H, -CC$\underline{H}_2$O-), 1.23 (q, 2H, -CC$\underline{H}_2$CH_3), 0.71 (t, 3H, -CCH_2C$\underline{H}_3$)

^{1}H-NMR of PGD-G2 (400 MHz, D_2O): δ = 3.75-3.67 (m, 6H, HOC$\underline{H}$CH_2O-), 3.66-3.60 (m, 3H, -OC$\underline{H}$CH_2O-), 3.60-3.48 (m, 12H, HOCHC$\underline{H}_2$O-), 3.48-3.43 (m, 12H, HOC$\underline{H}_2$CH-), 3.43-3.33 (m, 12H, -OC$\underline{H}_2$CHO-), 3.24 (q, 4H, -CC$\underline{H}_2$O-), 3.17 (s, 2H, -CC$\underline{H}_2$O-), 1.2 (q, 2H, -CC$\underline{H}_2$CH_3), 0.69 (t, 3H, -CCH_2C$\underline{H}_3$)

^{1}H-NMR of PGD-G3 (400 MHz, D_2O): δ = 3.72 (m, 3H, HOCHCH_2-), 3.51-3.46 (dd, 4H, -OC$\underline{H}_2$CH-), 3.41-3.36 (m, 6H, HOC$\underline{H}_2$CH-), 3.35-3.30 (m, 2H, -OC$\underline{H}_2$CHOH), 3.26 (q, 4H, -CC$\underline{H}_2$O-), 3.17 (s, 2H, -CC$\underline{H}_2$O-), 1.20 (q, 2H, -CC$\underline{H}_2$CH_3), 0.67 (t, 3H, -CCH_2C$\underline{H}_3$)

Gelation tests of PGD-G3 with various natural polysaccharide solution.

Aqueous glycol chitosan (GC), dextran (Dex), carboxymethyl cellulose (CMC), and hyaluronic acid (HA) solutions of 3 wt% and PGD-G3 solution of 5.65 wt% in distilled water were prepared respectively, and stored at 4 °C before use. To prepare hydrogels GC, Dex, CMC, and HA solutions were mixed with PGD-G3 solution in a ratio of 1:1, respectively. The mixtures were gently stirred for 10s with

bortex and the mixture was maintained at 37 °C for gelation. The gelation time of the hydrogels were observed by the tube inverting method.

^{1}H-NMR of L-arginine against PGD-G3

GC was dissolved in D_2O. PGD-G3 dissolved in D_2O was added to form a PGD G3/GC hydrogel. The final concentration of PDG G3 and GC was 2.83 wt% and 1.50 wt%, respectively. ^{1}H-NMR spectra of GC, PGD-G3, and PGD G3/GC hydrogel were obtained using a JEOL ECS 400 NMR (JEOL, Japan) operating at 400 MHz.

Gelation tests of GC with polyglycerol dendrimers of generations 1,2 and 3

Aqueous PGD G3 solutions of 11.3 wt%, 5.65 wt%, 2.85 wt% and 1.41 wt% in distilled water were prepare and aqueous PGD G2 and PGD G1 solutions of 1.2 wt% and 1.9 wt% were also prepared. Also, HPG solution, one of dendritic polyglycerol, was also prepare at a concentration of 11.3 wt%. The prepared dendritic polyglycerol solutions were mixed with 3 wt% GC solution in a ratio a 1:1, respectively. The mixtures were gently stirred for 10s with bortex and the mixture was maintained at 37 °C for gelation. The gelation time of the hydrogels were observed by the tube inverting method.

pH-dependent swelling properties of PGD/GC hydrogel

For swelling properties, the PGD G2/GC 1 and PGD G3/GC 1 were made as cylindrical shape (diameter of 10 mm and height of 5 mm). These hydrogels were dried using freeze-drier. To determine the pH-dependent swelling properties of dry hydrogel, pre-weighed dry samples were immersed in 5 mL solutions with pH values of 2.0, 3.0, 4.0, 7.0, 10.0. The pH values were precisely checked by a pH-meter (HORIBA, Ltd., Japan). At pre-determined time intervals, swollen hydrogels were taken out, excess water on the surface was carefully wiped off and the they were weighed on a sensitive balance (SHIMADZU Corporation, Japan). This procedure was repeated at all of the time points. The swelling ratio of the hydrogel was calculated using the following Equation.

$$Hydrogel\ mass\ (g/g) = W_w/W_d$$

Where W_w and W_d are the sample weight at time t in the wet and dry state, respectively.

L-Arginine-dependent swelling properties of PGD/GC hydrogel

For swelling properties, the PGD G2/GC 1 and PGD G3/GC 1 were made as cylindrical shape (diameter of 10 mm and height of 5 mm). These hydrogels were dried using freeze-drier. To determine the L-arginine-dependent swelling properties, pre-weighed dry samples were evaluated in 5 mL solutions with L-arginine concentrations of 0 wt%, 1 wt%, 5 wt%, 10 wt%. The swollen samples were then tested according to the above swelling test.

To compare L-arginine-dependent swelling properties of the PGD-G3/GC 3 dried-gel, the dried-gel, it was swollen in distilled water for 24 h, and then, the fully swollen hydrogel was evaluated in 5 mL solution with L-arginine concentrations of 10 wt%. The swollen hydrogel was then tested according to the above swelling test.

Supporting Figure S1-S8

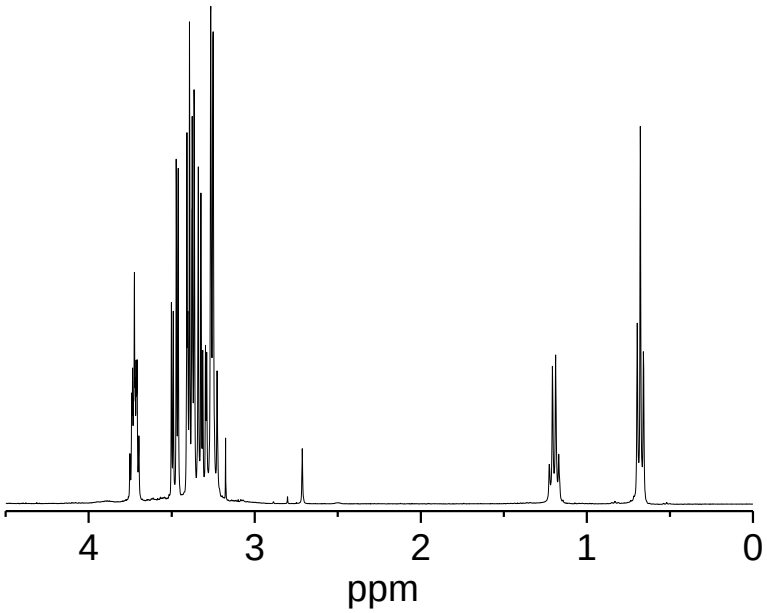

Figure S1. A ^{1}H-NMR spectrum of PGD-G1. (Immersed in D_2O, 400 MHz)

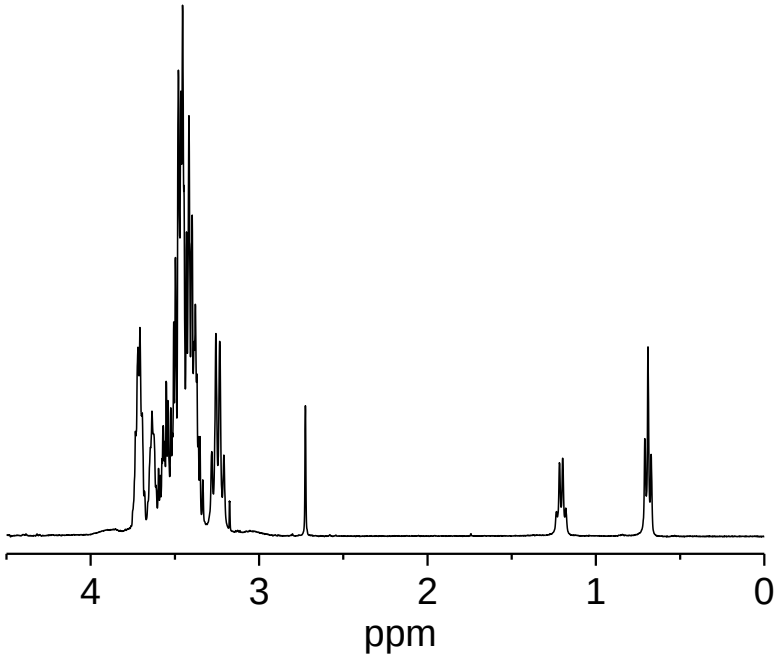

Figure S2. A ^{1}H-NMR spectrum of PGD-G2. (Immersed in D_2O, 400 MHz)

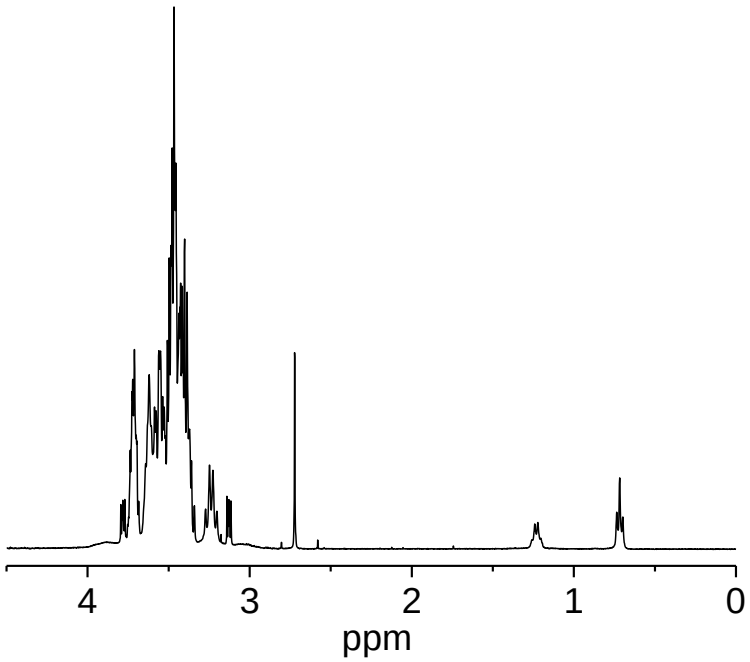

Figure S3. A ^{1}H-NMR spectrum of PGD G3. (Immersed in D_2O, 400 MHz)

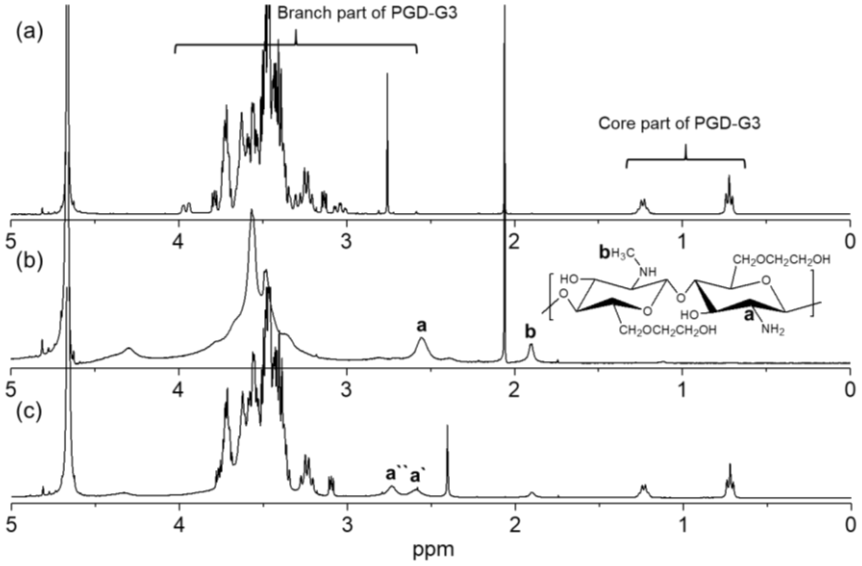

Figure S4. ^{1}H-NMR spectra of (a) PGD-G3, (b) GC, (c) PGD-G3/GC hydrogel in D_2O. The concentration of PGD-G3 and GC were 2.83 wt% and 1.5 wt%, respectively

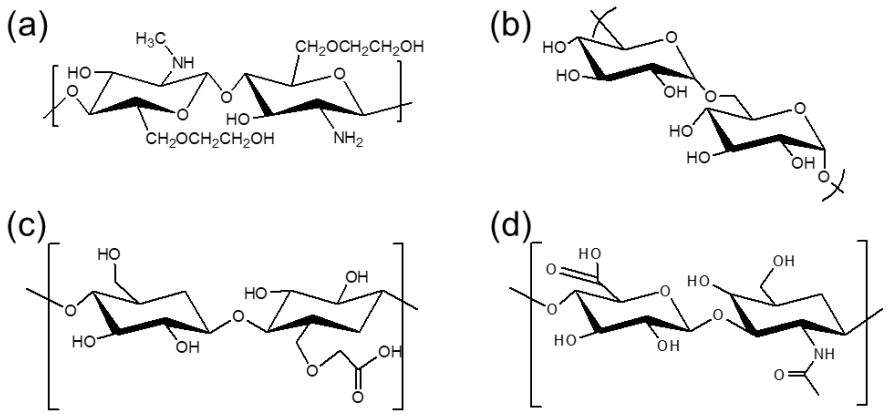

Figure S5. The chemical structures of (a) glycol chitosan (GC), (b) dextran (DEX), (c) carboxyl methyl cellulose (CMC), and (d) hyaluronic acid (HA)

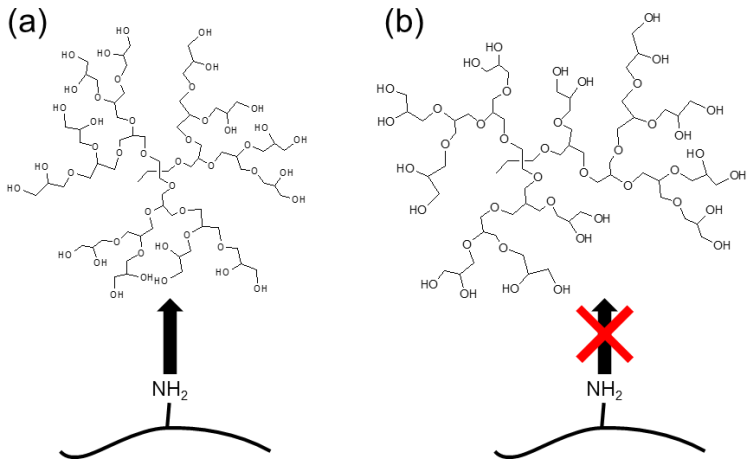

Figure S6. Interaction between GC and (a) PGD or (b) HPG

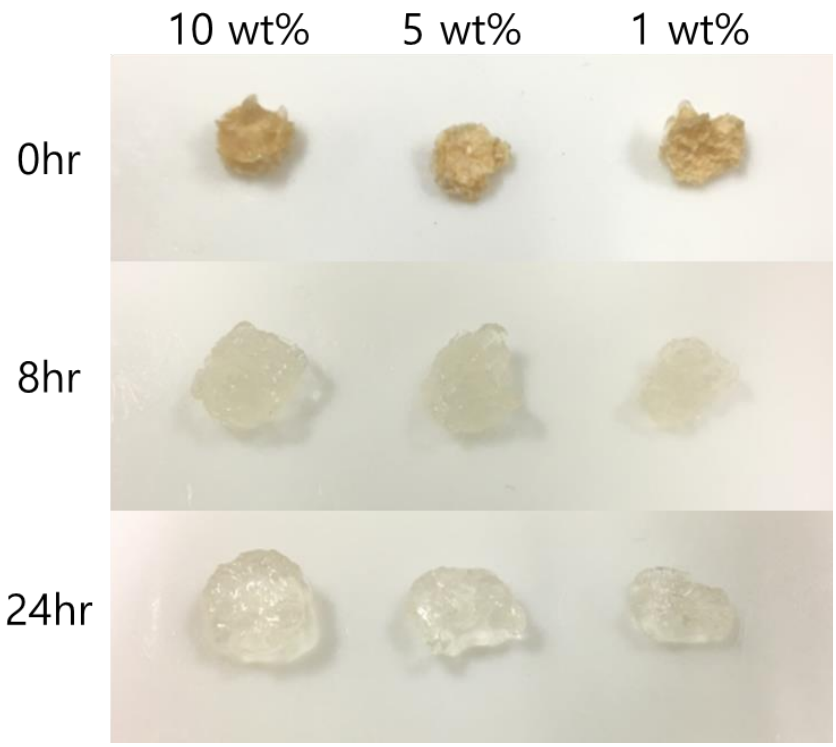

Figure S7. Digital photographs of time-dependent change of PGD-G3/GC 3 hydrogels with a different concentration of L-arginine.

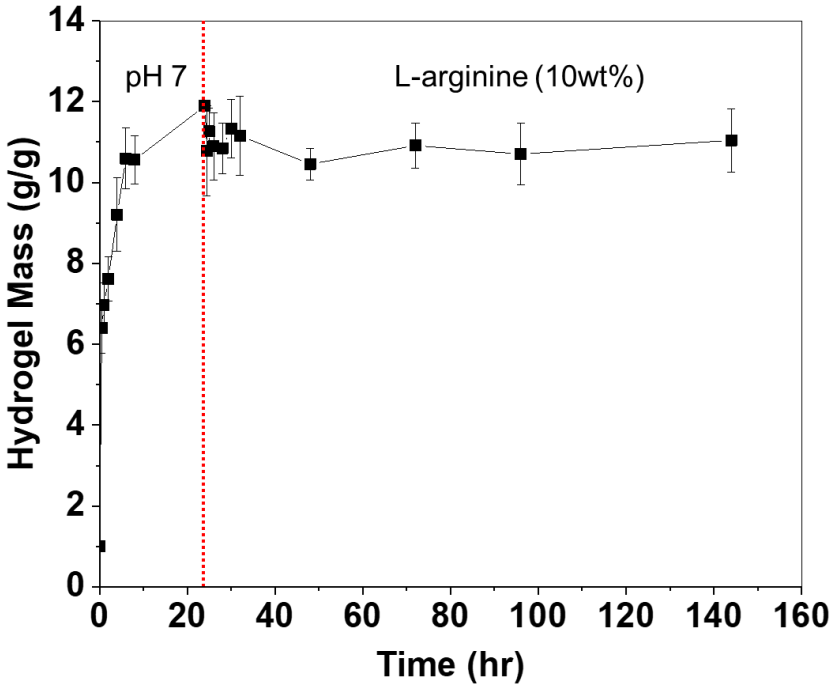

Figure S8. Swelling behaviour of PGD-G3/GC 3 at pH 7 and L-arginine (10 wt%) solution.

References

[1] R. Haag, A. Sunder, J.-F. Stumbé, *Journal of the American Chemical Society* **2000**, *122*, 2954-2955.

Author Contributions

Ik Sung Cho: type (funding acquisition, investigation, writing of original draft) the degree of contribution: 40%

Tooru Ooya: type (funding acquisition, project administration, data curation, validation, revising of the original draft) the degree of contribution: 60%