Semi-Crystalline, Three-Segmented Hybrid Gels with Multiple Shape-Memory Effect

Aslihan Argun, Umit Gulyuz, and Oguz Okay*

Natural biological systems such as intervertebral disk, tendon, and ligament consist of regions with distinctly different mechanical properties, yet these regions intermesh with each other through an extremely tough interface. Here the authors present mechanically strong, three-segmented hybrid hydrogels comprising of soft and hard components in a fused body resembling the mechanical heterogeneity of biological materials. An easy UV-initiated bulk copolymerization method of stratified monomer solutions is used to synthesize three-segmented hybrid hydrogels. In this method, stratification of the monomer solutions is created by means of the differences in their densities. Thus, the polymerization reactions in the monomer mixtures as well as in the interface regions occur simultaneously resulting in the formation of multi-segmented hybrid hydrogels consisting of segments with different chemical and physical properties. Hydrophilic and hydrophobic monomer mixtures compositions of stratified solutions selected in this study lead to the formation of supramolecular, semi-crystalline three-segmented hybrid hydrogels with adjustable thermal and mechanical properties. They also exhibit thermally induced pseudo multiple shape-memory function originating from distinctly different melting temperatures of crystalline domains belonging to the gel components of hybrids.

1. Introduction

Hydrogels are soft, intelligent materials and resemble to biomaterials in many aspects.[1–3] However, in contrast to their isotropic structures, many biological systems are anisotropic in nature and composed of hard and soft segments with an extremely strong interface between their components. One of the examples is the enthesis, which is the connective tissue between tendon or ligament and bone.[4,5] Enthesis serves as more than a simple anchor for connection of soft to hard tissue, it also minimizes the risk of damage under stress.[6] Only a few studies have been reported in the past years to acquire anisotropy in the gel architecture, Hu et al. prepared "modulated hydrogels" which was achieved by copolymerization of a gel network with another one.[7] Thus, by this technique, a harder interpenetrated network hydrogel component could be produced as merged with a softer single network hydrogel. Hybrid gels consisting of two different gel components were first reported by Raghavan et al.[8,9] Due to limited diffusion between high-viscosity monomer solutions, the components of the hybrids preserve their individual properties with a smooth and robust interface between them.[8] Another method to create hybrid gels was introduced by Leibler et al. by "gluing" separate gel samples together via nanoparticles as a binding agent.[10] This approach was extended by Yong et al. to the preparation of multilayered hydrogel sheets where the layers were stacked and effectively glued together with chemical cross-links obtained by performing atom transfer radical polymerization between the successive layers.[11,12] In order to fuse dissimilar materials in a body as macroscopic organo/hydrogel hybrids, another method was developed by Deng et al. which bases on the rapid adhesion by condensation of the aldehyde and acylhydrazine groups of poly(ethylene glycol) gelators in an organic solvent and water.[13] However, a limited number of monomers can be used in this method and the resulting hybrids suffer from low tensile strength (up to 0.1 MPa).[13] Recently, Yuk et al. reported an approach to create mechanically strong, optically transparent and electrically conductive bonding of hydrogels to nonporous solid surfaces which was achieved by covalently anchoring polymer network in the hydrogel on the silanated surfaces.[14]

Although hybrid hydrogels composed of soft and hard segments have potential application areas as biomaterials and in tissue engineering, it is crucial that they should display a high mechanical performance. Considering the mechanical properties of hybrids summarized above, they are unsuitable for stress-bearing applications. Our research group recently developed the stratification technique to produce high-strength, two-segmented hybrid hydrogels exhibiting self-healing and pseudo triple shape-memory effects.[15] By adjusting the densities of two monomer solutions, we were able to generate layers by a limited diffusion at a narrow interface. Thus, stratification of monomer solutions of different densities

A. Argun, O. Okay
Department of Chemistry
Istanbul Technical University
34469 Maslak, Istanbul, Turkey
E-mail: okayo@itu.edu.tr

U. Gulyuz
Department of Chemistry and Chemical Processing Technologies
Kirklareli University
39750 Luleburgaz, Kirklareli, Turkey

followed by polymerization produced two-segmented hydrogels. The hybrid hydrogels prepared via hydrophobic interactions have tunable mechanical and thermal properties as well as shape-memory and self-healing functions.[15–19] An important advantage of this strategy is that the method is applicable to many monomer systems.

Here we extend the stratification technique to the preparation of three-segmented hybrid hydrogels consisting of three gel components. Three-segmented hybrid hydrogels were fabricated by a simple, one-step UV-initiated bulk copolymerization of stratified solutions of hydrophilic and hydrophobic monomers followed by swelling of the resulting copolymers in water. N,N-dimethylacrylamide (DMA) and acrylic acid (AAc) were used as the hydrophilic monomers to obtain biocompatible hydrogels with associative properties.[20,21] n-Octadecyl acrylate (C18A) and stearyl methacrylate (C17.3M) were used as the hydrophobic monomers with long alkyl side chains able to form crystalline domains and hydrophobic associations in hydrogels.[17,22] By adjusting the monomer feed compositions of DMA/C18A, DMA/C17.3M, DMA/C18A/C17.3M, and AAc/C18A mixtures in the layers, we were able to create tough hybrid hydrogels that never break at the interfaces between the gel segments. Hybrids exhibit a high modulus (89–118 MPa), tensile strength (4.6–3.6 MPa), and strain (50–75%). The hybrids presented here also exhibit thermally induced pseudo multiple shape-memory behavior originating from different melting temperatures of crystalline domains belonging to their gel components.

2. Experimental Section

2.1. Materials

N,N-Dimethylacrylamide (DMA), stearyl methacrylate (C17.3M), a mixture of 65% n-octadecyl methacrylate and 35% n-hexadecyl methacrylate, n-octadecyl acrylate (C18A) and Irgacure 2959 were all purchased from Sigma–Aldrich (St. Louis, MO) and used without purification. Acrylic acid (AAc, Merck) was purified by passing through an inhibitor removal column purchased from the Aldrich Chemical Co.
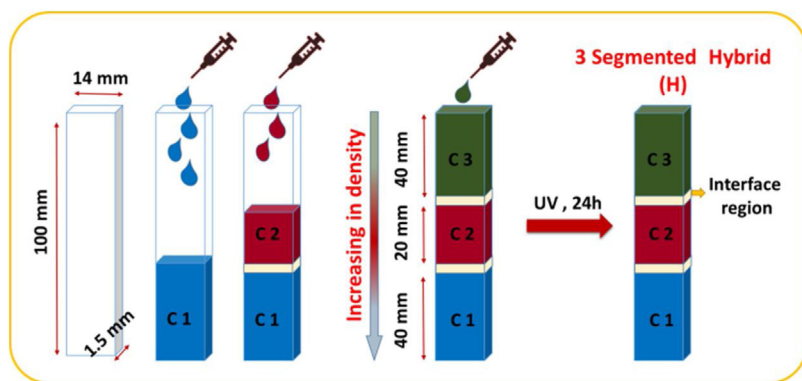
2.2. Stratification of Monomer Solutions and Preparation of Hybrid Hydrogels

The hybrid hydrogels with three distinct gel components were prepared using the density differences of the monomer mixtures, measured using a calibrated glass pycnometer at 25 °C. **Figure 1** shows schematic illustration of one-step synthesis technique of three-segmented hybrids. The solution with the highest density is first injected dropwise into the mold to form the bottom layer and subsequently, the solutions with lowering densities are successively dropped to form other layers. By this way, the monomer mixtures form layers separated by thin interface regions where they mix completely.

In this study, two kinds of three-segmented hybrid hydrogels, denoted as H1 and H2, were synthesized. Hybrid hydrogels were prepared by photo-initiated bulk copolymerization of the monomer mixtures under UV light at 360 nm in the presence of 0.1 wt% Irgacure 2959 as a photoinitiator (with respect to the monomers). The polymerization time was kept constant at 24 h.

Plastic molds of $100 \times 14 \times 1.5$ mm dimension were used to prepare flat rectangular shaped hybrids. Monomer solutions forming the segments of the hybrids were subsequently pipetted into the molds. As can be seen in Figure 1, the length of the middle segment was 20 mm which is shorter than that of other two parts. Thus, during the tensile tests of three-segmented hybrids, initial sample length between the jaws, in other words grip-to-grip separation was fixed at 60 mm providing equal lengths and hence equal attendance of the three segments of hybrid gel specimens to the tensile test. Additional 20 mm length of the end segments were used to grab the hybrid sample in jaws.

The compositions and densities (at 25 °C) of the monomer mixtures, and the properties of the resulting gel segments coded as C1, C2, and C3 are collected in **Table 1**. For comparison, the gel components of C1, C2, and C3 were also synthesized independently in the presence of 0.1 wt% Irgacure 2959, as described above.

2.3. Swelling and Gel Fraction Measurements

The resulting copolymer samples were immersed in an excess of hot water for the first day and then in water at room temperature for the following days by refreshing water every day to extract any soluble species until equilibrium swelling is reached. The water content in the gel samples was calculated as $H_2O \% = 10^2 \times (m - m_0)/m$, where m_0 and m are the initial and swollen mass of the gel samples, respectively. Then, the gel samples were freeze-dried, and the gel fraction W_g, that is, the conversion of monomers to the water-insoluble polymer, was calculated from the masses of dry polymer network and from the comonomer feed.

2.4. Differential Scanning Calorimetry (DSC) Measurements

DSC measurements were conducted on a Perkin Elmer Diamond DSC under a nitrogen

Figure 1. Illustration of one-step synthesis of three-segmented hybrids by stratification of monomer mixtures.

Table 1. Compositions and densities (at 25 °C) of the monomer mixtures, water contents, thermal and tensile mechanical properties of hybrids, and their individual gel components (standard deviations in parenthesis).

| Code | Composition (mol %) | | | | Density [g mL^{-1}] | H$_2$O [%] | T_m [°C] | T_{cry} [°C] | f_{cry} [%] | E [MPa] | ε_f [%] | $\sigma_{nom(f)}$ [MPa] |
	DMA	AAc	C18A	C17.3M								
C1	70			30	0.895	36	37	26	8	42 (2)	354 (45)	2.5 (0.2)
C2	60		40		0.888	21	51	42	19	122 (5)	8 (1)	7.1 (0.5)
C3	50			50	0.881	10	35	23	12	74 (9)	287 (35)	4.0 (0.2)
H1										118 (2)	50 (3)	4.6 (0.2)
C1	70			30	0.895	36	37	26	8	42 (2)	354 (45)	2.5 (0.2)
C2	60		15	25	0.887	18	46	36	16	92 (12)	14 (3)	8 (1)
C3		60	40		0.880	5	56	43	29	144 (14)	4 (1)	6 (1)
H2										89 (4)	75 (10)	3.6 (0.3)

atmosphere. The cooling/heating steps between 5 and 80 °C were carried out at a fixed rate of 5 °C min^{-1}. Enthalpy changes, ΔH_m, were calculated from the melting peak areas of DSC curves. The degree of crystallinity, f_{cry}, that is, the fraction of hydrophobic monomer units in crystalline domains, was estimated by $f_{cry} = x_{HM} \Delta H_m / \Delta H_m^\circ$, where x_{HM} is the mole fraction of the hydrophobic monomer in the comonomer feed and ΔH_m° is the melting enthalpy of crystalline C17.3M or C18A units. ΔH_m° was taken as 71.2 kJ mol^{-1} from previous works on the melting behavior of long n-alkyl chains forming a hexagonal crystal structure.[15,23–26] We have to note that because dyes such as crystal violet used to visualize the segments of hybrid hydrogels may affect the mechanical and thermal properties of the hydrogels, DSC and mechanical measurements were only conducted on virgin, uncolored gel specimens.

2.5. Mechanical Tests

Uniaxial elongation measurements were carried out on swollen hydrogel samples on Zwick Roell test machine with 500 N load cells. All the tests were conducted at 24 ± 1 °C. The Young's

modulus E was calculated from the slope of stress–strain curves between 1 and 3% deformations. The stress was presented by its nominal value σ_{nom}, which is the force per cross-sectional area of the undeformed specimen, while the strain is given by ε, the fractional change in the sample length. The measurements were performed on rectangular-shaped hydrogel samples at a strain rate of 3.8×10^{-2} s^{-1}. The sample length between jaws was 40 ± 3 and 60 ± 3 mm for the gel components and three-segmented hybrids, respectively.

3. Results and Discussion

As described above, we prepared two kinds of hybrid hydrogels to experimentally demonstrate the design principles of multi-segmented hybrid hydrogels. In the first three-segmented hybrid denoted as H1, DMA was used as a hydrophilic monomer whereas C17.3M and/or C18A were the hydrophobic monomers with various amounts. H1 contains two segments with C17.3M on both sides (C1 and C3 components) and one segment with C18A in the middle (C2 component). As reported before, the gels composed of DMA and C17.3M have a relatively low melting temperature T_m (35–38 °C) because of the methyl group on the backbone of C17.3M units restricting formation of ordered crystalline domains.[15,19] On the other hand, the gels composed of DMA and C18A have a higher T_m (40–51 °C) due to the longer side alkyl chain length of C18A as compared to C17.3M. The thermal properties of the gel components of H1 hybrid are shown in **Figure 2**a. T_m values for C1, C2, and C3 components of the H1 were found to be 37, 51, and 35 °C, respectively. The degree of crystallinity of the gel components calculated from the area under the melting peaks of DSC curves increases with increasing hydrophobic monomer content or by changing C17.3M with C18A (Table 1).

Previous works show that semi-crystalline physical hydrogels formed using large hydrophobes such as C17.3M or C18A exhibit self-healing and thermally induced shape-memory behavior.[15,18,19] The shape-memory effect of the hydrogels is based on the existence of
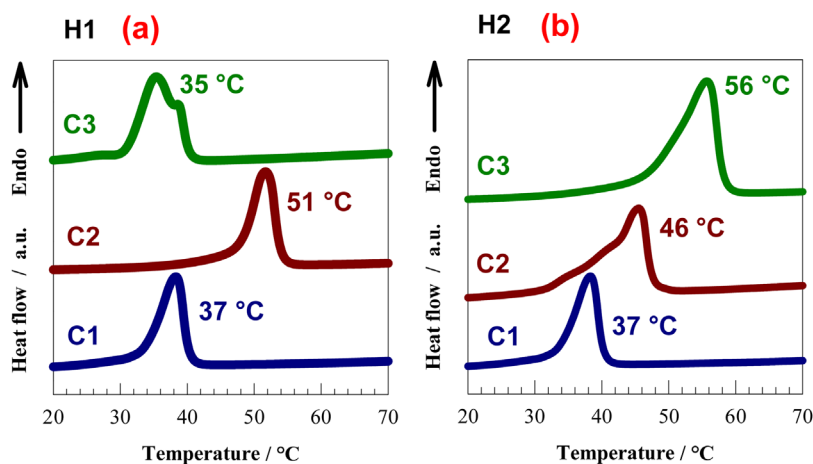
Figure 2. DSC scans conducted on the individual gel components of (a) H1 and (b) H2 hybrids. Melting temperatures are indicated.
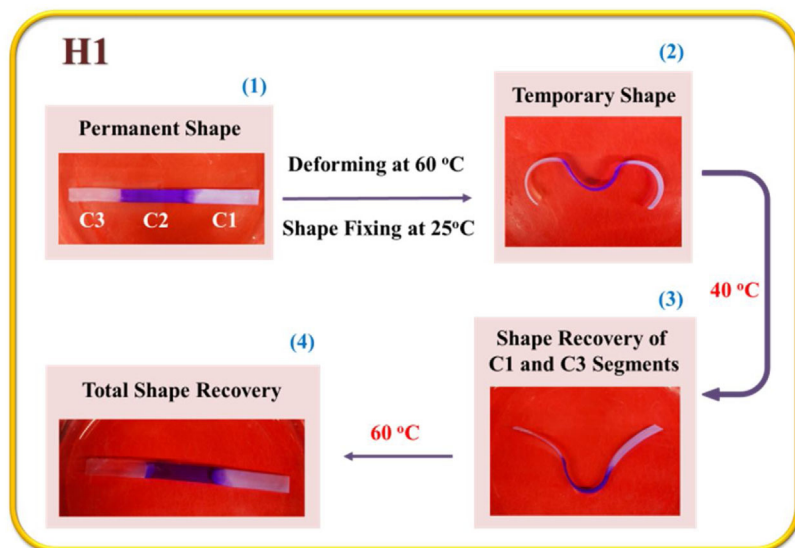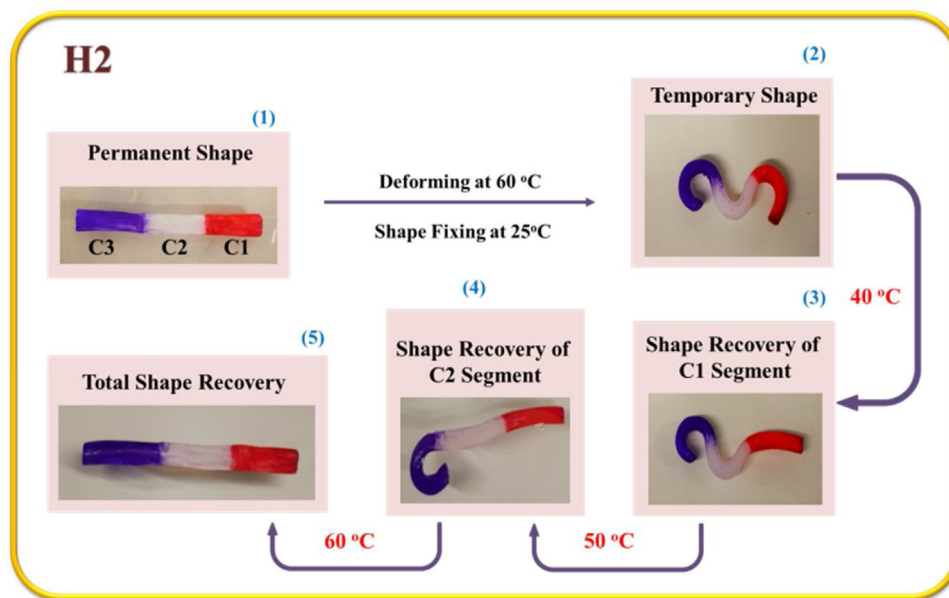
Figure 3. Images demonstrating pseudo triple-shape-memory effect of H1 hybrid hydrogel. Permanent shape (1), temporary shape (2), shape recoveries of C1 and C3 segments at 40 °C (3), and following shape recovery of C2 segment to reach total shape recovery at 60 °C (4).

violet for clarity. The rod-shaped hybrid gel specimen was first immersed in a water bath at 60 °C which is above T_m's of all segments, so that the whole specimen became soft and could be easily deformed into a temporary shape (image 2). This temporary shape was then fixed by cooling to 25 °C, which is well below the T_m's of all segments due to the formation of crystalline domains. The hybrid specimen was then heated to 40 °C to recover the permanent shapes of C1 and C3 segments whereas further heating to 60 °C recovered the total permanent shape of the hybrid (images 3 and 4). Although H1 has three segments with three different T_m's it demonstrates pseudo triple-shape-memory behavior due to the close T_m values of C1 and C3 segments (37 and 35 °C).

In the second hybrid denoted by H2, the type of the monomers and the composition of the gel components C2 and C3 were changed to create an almost 10 °C difference in the melting temperatures between the segments (Table 1). C2 component of H2 was prepared using a mixture of C17.3M and C18A hydrophobes to adjust an intermediate melting temperature among the segments. To further increase the melting temperature of C3 component in H2, AAc was selected instead of DMA because the semi-crystalline gel containing hydrophilic AAc units produced a higher degree of crystallinity as compared to DMA-containing gel (Table 1).[19] Moreover, we observed that the monomer mixture containing AAc as the hydrophilic monomer has a longer gelation time as compared to that containing DMA monomer. This different gelation times facilitated stratification of the monomer solutions and

hydrophobic associations and crystalline domains serving as netpoints and switching segments, respectively. In the present hybrid hydrogels, each segment has its own shape-memory function due to the localization of switching segments and thus, the whole hybrid exhibits triple- or multiple-shape-memory effect, which may be termed as "pseudo triple or multiple shape-memory" behavior. The images labeled with 1, 2, 3, and 4 in **Figure 3** demonstrate shape-memory capability of a H1 hybrid gel specimen. C2 segment of the hybrid was colored with crystal

Figure 4. Images demonstrating pseudo multiple-shape-memory effect of H2 hybrid hydrogel. Permanent shape (1), temporary shape (2), successive shape recoveries of C1 (3), C2 (4), and C3 (5) segments at 40, 50, and 60 °C, respectively.
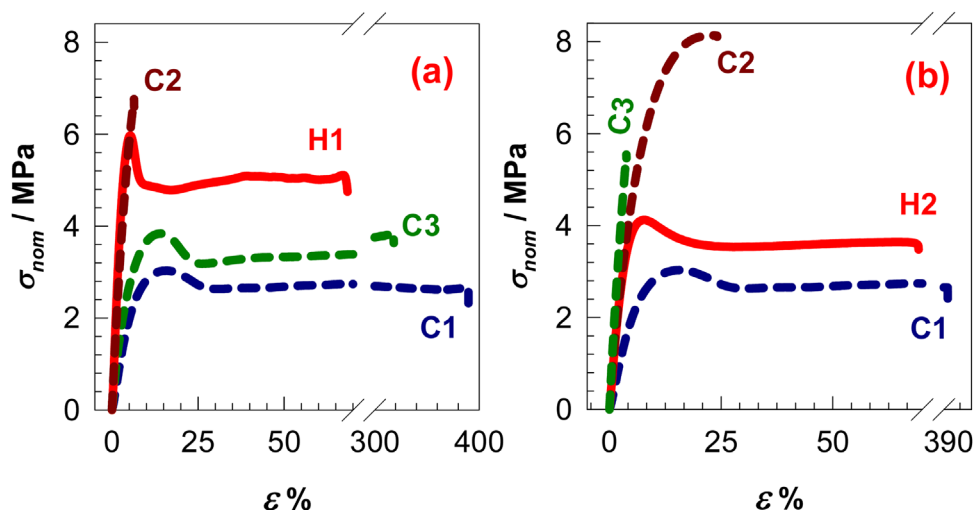
Figure 5. Tensile stress σ_{nom}–strain ε curves of three-segmented H1 (a) and H2 hybrids (b). The solid and dashed curves present the data of hybrids, and their components, respectively.

formation of a smooth interface between the stratified solutions. Thus, a new requirement for preparing hybrid hydrogels with smooth interfaces has emerged. The monomer mixture with a higher density should have a shorter gelation time than that with a lower density. The solution containing AAc should always flow on top of another solution containing DMA because otherwise the mismatch in the polymerization rates resulted in the formation of non-smooth transition in the interface region.

DSC measurements were also conducted on the individual segments of H2 hybrid gel specimens. As seen in Figure 2b, the melting peak of C1 segment appears at 37 °C whereas it increases to 46 and 56 °C for C2 and C3 segments, respectively. Thus, different from the H1 hybrid, H2 has gradually increasing three T_m values which are separated by about 10 °C from each other. We have to note that the DSC curves in Figure 2 show shoulders for C3 and C2 gel components of H1 and H2 hybrids, respectively, which is attributed to the existence of two different hydrophobic monomer units in these segments.

Shape-memory tests conducted on H2 hybrid hydrogel revealed existence of pseudo multiple shape-memory function due to the distinct differences in T_m's of its individual segments (**Figure 4**). The image labeled by (1) is the permanent shape of H2, where C1 and C2 segments were colored with red and blue, respectively, for clarity. The hybrid was first heated to 60 °C for complete melting of crystalline domains, and then the initial straight shape was deformed into a wavy shape (temporary shape), which was then fixed by cooling to 25 °C (image 2). After immersing the hybrid in a water bath at 40 °C, C1 segment of H2 returned to its initial permanent shape (image 3) whereas further successive heating to 50 and 60 °C recovered the permanent shapes of C2 and C3 segments, respectively (images 4 and 5). Thus, three-segmented H2 hybrid hydrogel exhibits quadruple shape-memory effect due to its three distinctly different melting temperatures (37, 46, and 56 °C),[27] achieved by choosing suitable comonomer pairs and compositions.

The gel fractions of hybrids and their gel components were found to be unity revealing complete conversion of the
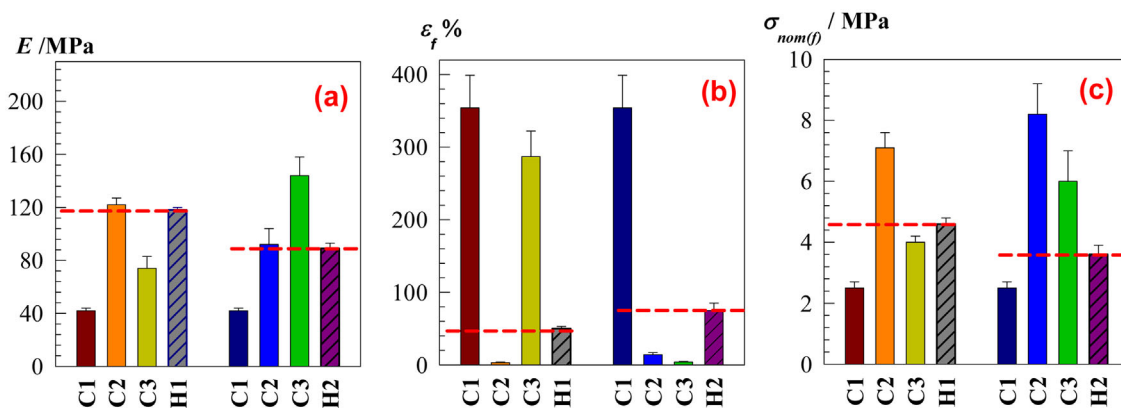
Figure 6. Comparison of elongation test results of H1 and H2 hybrids, and their individual segment. (a) Young's modulus E; (b) fracture strain ε_f; and (c) fracture stress $\sigma_{nom(f)}$. The dashed lines represent the values for the three-segmented hybrid hydrogels.

monomers into the network structure. The water content of the gel components composed of DMA and hydrophobic monomers was between 36 and 10 wt%, which decreased with increasing amount of the hydrophobic monomer (Table 1). Moreover, by replacing the hydrophilic monomer DMA with AAc at a fixed amount of C18A, it also decreased from 21 to 5 wt% due to the increasing degree of crystallinity.

Mechanical tests conducted on hybrid hydrogels revealed their extraordinary mechanical performances. The solid and dashed curves in Figures 5a, b display tensile stress–strain curves of the hybrids and their individual gel components, respectively, where the nominal stress σ_{nom} is plotted against the strain ε.

The main difference in the mechanical properties of the two hybrids is that, H1 has two stretchable and one rigid segments (**Figure 5**a) whereas H2 has two rigid and one stretchable segments (Figure 5b). As compared to the gel components, the hybrids exhibit medial mechanical properties such as stretchability, and tensile strength. H1 hybrid has a Young's modulus of 118 ± 2 MPa and tensile strength of 4.6 ± 0.2 MPa while H2 hybrid has a lower modulus and tensile strength (Table 1) but relatively higher elongation at break (50% vs. 75%). The stress–strain curves of both H1 and H2 hybrids also show a yielding behavior with a yield point at a very low elongation (about 5%). The important point is that hybrid gel specimens never break at their interfaces between the segments during the tensile tests. The interface regions remained stable under stress, and the fracture occurred in the weakest segment of hybrid hydrogels. This behavior can be explained by the formation of more ordered crystalline domains in the presence of mixed hydrophobes leading to increased strength of the interface regions.[17]

Young's modulus E, fracture strain ε_f and fracture stress $\sigma_{nom(f)}$ of three-segmented hybrids (H1 and H2) and their segments are compared in **Figure 6**. The general trend is that the hybrids represent average values of the modulus E, fracture strain ε_f and stress $\sigma_{nom(f)}$ of their gel components.

4. Conclusion

We demonstrated here that the photo-initiated polymerization of three stratified monomer solutions produces hybrid hydrogels consisting of mechanically and thermally different segments intermeshed through strong interfaces. The stratification technique presented here bases on the density differences of the monomer mixtures as well as different gelation times in stratified solutions. The hybrid hydrogels H1 and H2 formed via hydrophobic interactions exhibit tunable mechanical properties such as a high modulus (118–89 MPa) and tensile strength (4.6–3.6 MPa) at 50–75% elongations. They also exhibit thermally induced pseudo multiple shape-memory effect due to the distinctly different melting temperatures in the gel components of hybrids. An important advantage of the design strategy presented here is that it could be expanded to the numerous shape-memory systems by choosing suitable monomers.

[1] P. Calvert, *Adv. Mater.* **2009**, *21*, 743.

[2] S. Seiffert, Ed., *Supramolecular Polymer Networks and Gels; Book Series Adv. Polym. Sci.* Springer, Berlin **2015**.

[3] A. J. R. Amaral, G. Pasparakis, *Polym. Chem.* **2017**, *8*, 6464.

[4] J. Apostolakos, T. J. S. Durant, C. R. Dwyer, R. P. Russell, J. H. Weinreb, F. Alaee, K. Beitzel, M. B. McCarthy, M. P. Cote, A. D. Mazzocca, *Muscles Ligaments Tendons J.* **2014**, *4*, 333.

[5] L. Rossetti, L. A. Kuntz, E. Kunold, J. Schock, K. W. Müller, H. Grabmayr, J. Stolberg-Stolberg, F. Pfeiffer, S. A. Sieber, R. Burgkart, A. R. Bausch, *Nat. Mater.* **2017**, *16*, 664.

[6] H. M. Shaw, M. Benjamin, *Scand. J. Med. Sci. Sports* **2007**, *17*, 303.

[7] Z. Hu, X. Zhang, Y. Li, *Science* **1995**, *269*, 525.

[8] S. J. Banik, N. J. Fernandes, P. C. Thomas, S. R. Raghavan, *Macromolecules* **2012**, *45*, 5712.

[9] A. Gargava, C. Arya, S. R. Raghavan, *ACS Appl. Mater. Interfaces* **2016**, *8*, 18430.

[10] S. Rose, A. Prevoteau, P. Elzière, D. Hourdet, A. Marcellan, L. Leibler, *Nature* **2014**, *505*, 382.

[11] X. Yong, A. Simakova, S. Averick, J. Gutierrez, O. Kuksenok, A. C. Balazs, K. Matyjaszewski, *Macromolecules* **2015**, *48*, 1169.

[12] A. Beziau, R. N. L. de Menezes, S. Biswas, A. Singh, J. Cuthbert, A. C. Balazs, T. Kowalewski, K. Matyjaszewski, *Polymers* **2017**, *9*, 186.

[13] G. Deng, Q. Ma, H. Yu, Y. Zhang, Z. Yan, F. Liu, C. Liu, H. Jiang, Y. Chen, *ACS Macro Lett.* **2015**, *4*, 467.

[14] H. Yuk, T. Zhang, S. Lin, G. A. Parada, X. Zhao, *Nat. Mater.* **2016**, *15*, 190.

[15] A Argun, U. Gulyuz, O. Okay, *Macromolecules* **2018**, *51*, 2437.

[16] O. Okay, *Adv. Polym. Sci.* **2015**, *268*, 101.

[17] C. Bilici, S. Ide, O. Okay, *Macromolecules* **2017**, *50*, 3647.

[18] C. Bilici, V. Can, U. Nöchel, M. Behl, A. Lendlein, O. Okay, *Macromolecules* **2016**, *49*, 7442.

[19] B. Kurt, U. Gulyuz, D. D. Demir, O. Okay, *Eur. Polym. J.* **2016**, *81*, 12.

[20] Y. Uemura, J. McNulty, P. M. Macdonald, *Macromolecules* **1995**, *28*, 4150.

[21] P. Relogio, J. M. G. Martinho, J. P. S. Farinha, *Macromolecules* **2005**, *38*, 10799.

[22] C. Bilici, O. Okay, *Macromolecules* **2013**, *46*, 3125.

[23] Z. Mogri, D. R. Paul, *Polymer* **2001**, *42*, 2531.

[24] H. S. Bisht, P. P. Pande, A. K. Chatterjee, *Eur. Polym. J.* **2002**, *38*, 2355.

[25] M. G. Broadhurst, *J. Res. Natl. Bur. Stand., Sect. A* **1962**, *66A*, 241.

[26] E. F. Jordan, D. W. Feldeisen, A. N. Wrigley, *J. Polym. Sci. Part A-1 Polym. Chem.* **1971**, *9*, 1835.

[27] U. Nöchel, M. Behl, M. Balk, A. Lendlein, *ACS Appl. Mater. Interfaces* **2016**, *8*, 28068.