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Soft Material Chemistry

Tailoring Hydrogel Adhesion to Polydimethylsiloxane Substrates Using Polysaccharide Glue**

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With the emergence of microscale biotechnology, such as biomicroelectromechanical systems ("Bio-MEMS") and microfluidic-based microchips for sensing and diagnostics, polydimethylsiloxane (PDMS)-based elastomers have become very popular materials.[1] PDMS elastomers possess several features that are well suited for these applications: mechanical stability and elasticity, chemical inertness, optical transparency, gas permeability, ease of fabrication, and biocompatibility.[1d,2] However, the extremely hydrophobic nature of PDMS often limits its applicability (e.g. poor aqueous fluid flow and nonspecific adhesion of biomolecules).[2] Various methods have been proposed to modify the PDMS surface to impart hydrophilicity, for example, UV or plasma treatment to oxidize the surface[3] and coating the surface with hydrophilic polymers.[4] However, the treated PDMS surfaces often recover their hydrophobic traits due to the migration of unreacted PDMS oligomers to the surface and the rearrangement of PDMS polymer chains.[2b,5]

We suggest that coating PDMS with hydrophilic materials would be more effective than the molecular level modifications. Hydrogels, which are networks of cross-linked polymers taking up large amounts of water, are therefore considered promising materials. Hydrogels can also be designed to present functionalities for specific purposes, such as in vitro cell culture, cell encapsulation, and molecular capture and release.[6] Therefore, PDMS coated with hydrogels with desired properties would significantly enhance the performance of PDMS-based devices. However, it is a significant challenge to attain and sustain the adhesion between hydrogel and PDMS, due to the stark discrepancy between the bulk properties of PDMS substrates and hydrogels.

To meet this challenge, we describe a unique approach to tailor hydrogel adhesion to a PDMS substrate. Alginate, a naturally derived polysaccharide, was covalently linked to the PDMS surface. This attached alginate acted as a "glue" to allow the strong, permanent adhesion of the hydrogel onto the PDMS surface by 1) imparting hydrophilicity to improve compatibility with hydrogels, and 2) providing functional groups for the stable conjugation of hydrogels. The resulting hydrogel-coated PDMS substrate was used in the following two applications: 1) it served as an in vitro cell culture platform to study cellular behavior in response to cyclic mechanical strain, and 2) it was used in a microfluidic device with hydrogel-filled channels.

The PDMS surface was chemically grafted with alginate following a series of modification steps:[7] step 1: oxidation to present hydroxy groups (OH-PDMS, Figure 1a); step 2: silanization using 3-aminopropyltriethoxysilane to present primary amino groups (NH_2-PDMS); and step 3: conjugation of alginate by carbodiimide-mediated amide coupling between amino groups on the PDMS surface and carboxylic acid groups of alginate (alginate-PDMS). The successive modifications of PDMS were confirmed with FTIR spectroscopy (Figure S1 and Table S1 in the Supporting Information). The chemical linkage of alginate to the PDMS surface was further confirmed with fluorescently labeled alginate (Figure S2 in the Supporting Information). The decreased water contact angle of alginate-PDMS also showed that it is more hydrophilic than unmodified PDMS, OH-PDMS, and NH_2-PDMS (Figure 1b).

Next, alginate hydrogels were fabricated on the alginate-PDMS by means of activating a covalent or an ionic crosslinking reaction. We thought that the alginate glue on the PDMS surface would participate in the reaction and hold the alginate hydrogel to the surface (Figure 2). First, an aqueous mixture of alginate and adipic acid dihydrazide (AAD) was placed on the alginate-PDMS, in order to fabricate the hydrogel by carbodiimide-mediated amide coupling.[8] The resulting AAD-alginate hydrogel remained stably attached to alginate-PDMS for several months, regardless of the gel thickness, demonstrating that the alginate glue on the PDMS participated in the crosslinking reaction. No interfacial failure was observed between the bulk hydrogel and alginate-PDMS even when the construct was bent (Figure 3a).

Alginate hydrogel crosslinked with calcium ions could also be prepared on alginate-PDMS.[8] The resulting calcium-alginate hydrogel also remained stably attached to the alginate-PDMS for several months. No interfacial failure

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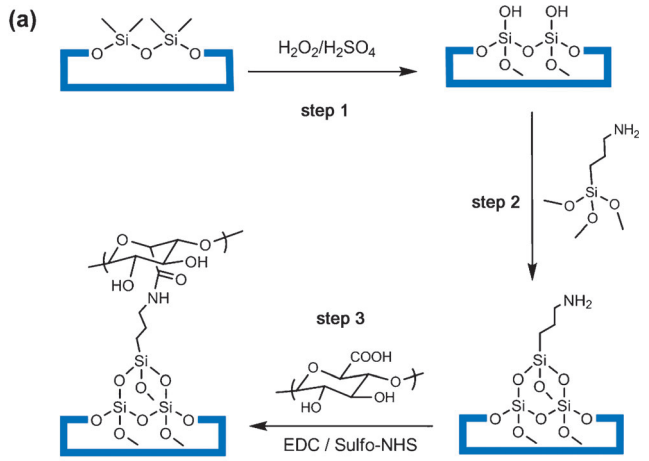

Figure 1. a) Surface modification of PDMS to attach alginate glue. b) Contact angles of water droplets on bare PDMS surfaces or those modified with hydroxy (OH) groups, amino (NH_2) groups, or alginate molecules.(*$p<0.05$). EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, Sulfo-NHS = *N*-hydroxysulfosuccinimide.

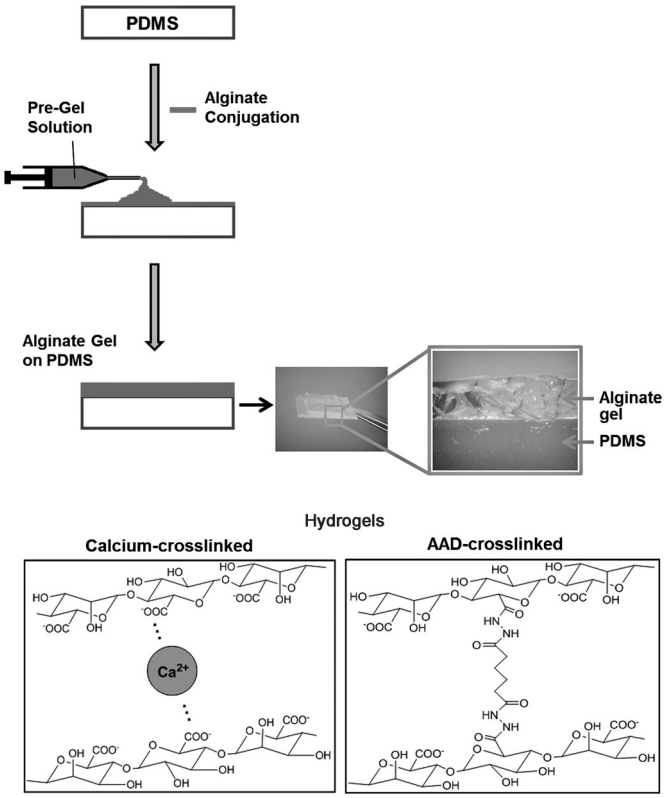

Figure 2. Hydrogels were fabricated on the alginate-PDMS surface by 1) ionic crosslinking with calcium ions or 2) covalent crosslinking with adipic acid dihydrazide (AAD).

was observed when the construct was subjected to external mechanical deformation. These results demonstrate that the alginate molecules linked to the PDMS substrate also participate in the ionic crosslinking reaction.

To further verify the role of the alginate glue in mediating hydrogel adhesion to PDMS surface, we fabricated alginate hydrogels on PDMS surfaces presenting various chemical groups, including OH-PDMS, NH_2-PDMS, and poly(ethylene glycol)-functionalized PDMS (PEG-PDMS). Neither AAD- nor calcium-crosslinked alginate hydrogels adhered to OH-PDMS and PEG-PDMS. NH_2-PDMS did allow adhesion of the AAD-crosslinked alginate hydrogel, but the hydrogel was easily detached from the PDMS surface by any mild mechanical deformation (Figure 3a) or when presented in an aqueous environment. Coupled with the fact that the contact angle of alginate-PDMS is smaller than that of NH_2-PDMS, this result implicates that the enhanced adhesion of a hydrogel to a PDMS surface results from the combined effects of increased hydrophilicity and the functional groups used for the covalent linkage of the hydrogel to the alginate glue on PDMS.

The adhesion strength of the alginate hydrogel to the modified PDMS substrate was quantitatively evaluated by measuring the ultimate strain at which interfacial failure was observed when the PDMS substrate was stretched.[9] For this study, AAD-alginate hydrogels were prepared on 1) untreated PDMS, 2) NH_2-PDMS, and 3) alginate-PDMS. Then, the PDMS substrates were stretched at a controlled rate and interfacial failure was monitored simultaneously (Figure 3b). The ultimate interfacial strain for the hydrogel assembled on the alginate-PDMS was three- and sixfold larger than that for the hydrogels prepared on the NH_2-PDMS and untreated PDMS, respectively (Figure 3b). Similar ultimate interfacial strain values were also obtained for the calcium-crosslinked alginate hydrogel (data not shown).

Additionally, the stiffness of the hydrogel prepared on the alginate-PDMS could be readily controlled by varying the molar ratio of uronic acid units in the alginate to calcium ions or AAD ($M_{UA\text{-}Ca}$, $M_{UA\text{-}AAD}$). For example, increasing $M_{UA\text{-}Ca}$ from 0.1 to 0.4 resulted in the increase of the elastic modulus from 3 to 40 kPa (Figure 3c). Similarly, increasing $M_{UA\text{-}AAD}$ from 0.01 to 0.04 led to the increase of the elastic modulus from 7 to 40 kPa (Figure 3d). Regardless of the hydrogel stiffness, all hydrogels were stably attached to the alginate-PDMS. However, these increases in elastic moduli resulted in a small decrease of the ultimate interfacial strain, which is attributed to the increase of interfacial shear stress resulting from the larger resistance of stiffer hydrogels to deformation (Figure S3 in the Supporting Information).

Such controllability of the hydrogel adhesion to PDMS substrates could not be achieved when the hydrogel was prepared by radical polymerization. It is well known that PDMS absorbs oxygen which inhibits radical polymerization at the interface, thus preventing proper formation and attachment of the hydrogel.[10] Therefore, we explored whether PDMS modified with methacrylic groups could

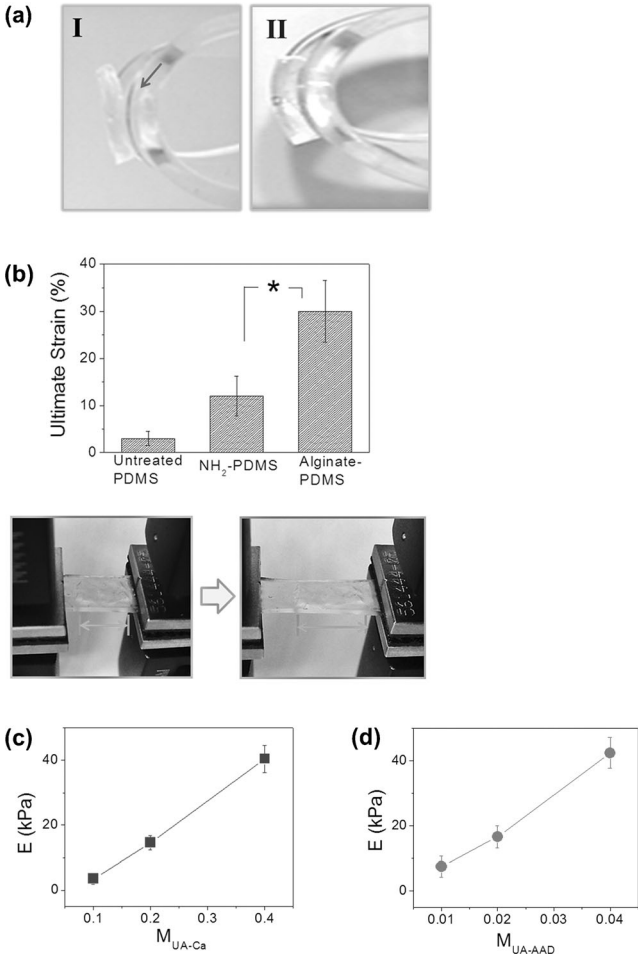

Figure 3. a) During bending, the alginate hydrogel readily detached from the NH_2-PDMS surface (I), whereas the hydrogel was stably attached on the alginate-PDMS surface (II). b) Ultimate interfacial strain of alginate hydrogel adhered to various PDMS surfaces, measured by stretching the PDMS in a uniaxial direction until the hydrogel detached from the PDMS surface (see picture); $*p < 0.05$. c,d) Elastic moduli (E) of AAD- and calcium-crosslinked alginate hydrogels controlled by the amount of crosslinker. $M_{UA\text{-}Ca}$: the molar ratio of uronic acid units of alginate to calcium ions; $M_{UA\text{-}AAD}$: the molar ratio of uronic acid units of alginate to AAD.

allow the attachment of radically polymerized hydrogels. Methacrylic groups were conjugated to the PDMS surface either by 1) treatment with 3-(trimethoxysilyl)propyl methacrylate (TMSPMA) to OH-PDMS or 2) conjugation of alginate presenting methacrylic groups, termed alginate methacrylate (Figure S4 in the Supporting Information).[11] As expected, the pre-gel solution of alginate methacrylate or poly(ethylene glycol) diacrylate (PEGDA) could not form hydrogels by the radical cross-linking reaction. In contrast, alginate methacrylate or PEGDA could be radically cross-linked to form hydrogels on glass or polystyrene substrates. Additionally, when glass was chemically modified with TMSPMA the hydrogels could permanently adhere to the glass. These results confirm the previous findings that the PDMS substrate acts as a radical scavenger and significantly limits radical-activated hydrogel formation. In addition, these results address a necessity to utilize nonradical crosslinking reaction mechanism for the hydrogel assembly on the PDMS surface.

The adhesion strength of alginate hydrogel on PDMS was further examined by applying cyclic strain to the alginate-PDMS substrate and monitoring the synchronous deformation of the attached alginate hydrogel (Figure 4a,b). The cyclic stretching device used here was originally devised to study the effects of external mechanical force on cellular organization and phenotypic activities using cells adhered to PDMS (Figure 4b).[12] The alginate-PDMS to which alginate

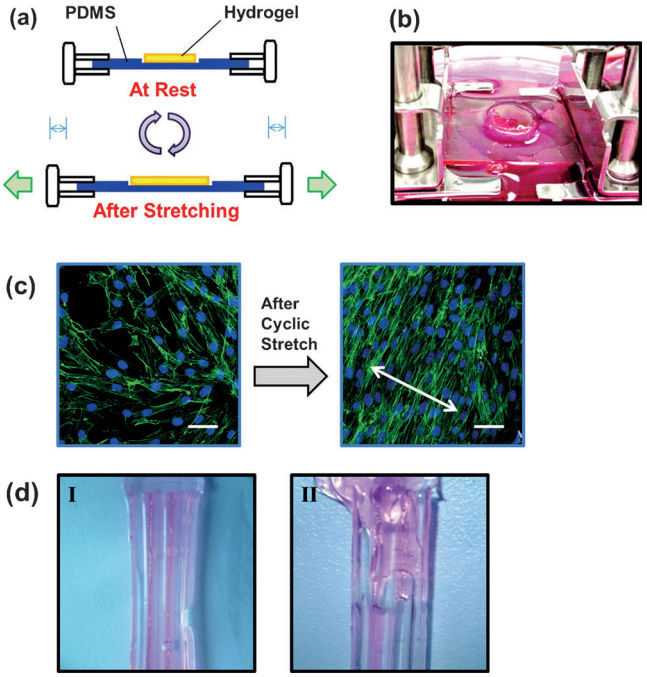

Figure 4. a) Schematic description of cyclic stretching of a hydrogel-conjugated PDMS construct. b) The experimental setup for the cyclic stretching measurements with the hydrogel crosslinked on the center of the stretchable PDMS. c) Fibroblasts cultured on an RGD-alginate hydrogel became aligned perpendicular to the direction of cyclic stretching (double-headed arrow). d) Alginate hydrogel fabricated within channels of a microfluidic device prepared with alginate-PDMS (I). The alginate hydrogel detached from the channels of NH_2-PDMS (II).

hydrogel was attached was stretched at a frequency of 1 Hz and a strain of 10% for seven days (Figure 4a, Figure S5 and Video S1 in the Supporting Information). The hydrogel stretched along with the PDMS without slippage or detachment. The limited hydrogel extension at the lower PDMS strain range (i.e. the first 5%) is likely due to interfacial resistance to deformation and the difference in stiffness between the hydrogel and PDMS substrate (Figure S5 in the Supporting Information). In contrast, the hydrogel fabricated on the NH_2-PDMS substrate was separated from the substrate within 10 min of cyclic stretching.

We examined whether the alginate hydrogel-PDMS, developed in this study, can be used to transmit cyclic mechanical strain to the cells cultured on the hydrogel surface. There have been extensive efforts to understand cellular responses to external mechanical stimuli (e.g. endo-

thelial junction remodeled by pulsatile blood flow and stem cell fates modulated by cyclic mechanical loading).[13] These studies have been conducted by applying controlled mechanical force to cells grown on silicone-based substrates coated with cell-adhesion proteins.[14] However, these substrates are much stiffer than the natural extracellular matrix and thus do not adequately reflect the in vivo mechanical environment. Therefore, it is often suggested that applying cyclic strain on cells adhered to a substrate of similar softness to the natural extracellular matrix, such as a hydrogel, would allow us to better understand and regulate the interplay of the intrinsic and extrinsic mechanical signals on cellular organization and activities. However, it is technically challenging to directly apply the mechanical force to cell-adherent hydrogels, due to their structural fragility. There were attempts to indirectly apply mechanical force by fabricating hydrogels on PDMS substrates, but poor adhesion between the hydrogel and PDMS greatly limited the cell culture period.

For this study, an alginate hydrogel presenting the integrin-binding oligopeptide Arg-Gly-Asp (RGD peptide) was fabricated on an alginate-PDMS surface.[15] The elastic modulus of the hydrogel was tuned to 15 kPa. Fibroblasts were then cultured on the hydrogel surface. Interestingly, cyclic stretching of the PDMS substrate (1 Hz, 10% strain) for seven days resulted in the cytoskeletal rearrangement of the cells, as evidenced by the alignment of the cells and the anisotropic elongation of the cell nuclei in a direction perpendicular to the axis of stretching, similar to that observed in the cells cyclically stretched on a collagen-coated PDMS substrate (Figure 4c, Figure S6 in the Supporting Information). These results demonstrate that the alginate hydrogel-PDMS construct provides a suitable platform for studying the effect of cyclic mechanical strain on cells under physiologically relevant conditions.

Our approach to enhance the adhesion of a hydrogel to PDMS surface was also useful in filling microchannels of PDMS-based microfluidic devices with hydrogels. Such microfluidic systems coupled with hydrogel are often proposed to study various biological phenomena and also to assemble biosensors or actuators.[16] However, one of the major challenges has been the interfacial bonding between the channel wall of PDMS and the hydrogel. Alginate hydrogel fabricated within alginate-PDMS microchannels remained stably attached in physiological media over seven days, without any compromise in interfacial adhesion (Figure 4d, Figure S7 in the Supporting Information). In contrast, the hydrogel fabricated within NH_2-PDMS microchannels was readily detached and squeezed out of the channels.

Altogether, this study demonstrates a simple yet innovative approach to fabricate a polymeric hydrogel adhered to PDMS-based constructs. Specifically, this study defines two important design parameters to elaborate the stable adhesion of hydrogels to PDMS: 1) surface modification of PDMS with alginate glue to provide sufficient surface hydrophilicity and reaction sites for hydrogel conjugation and 2) crosslinking reactions that do not require radical polymerization. The PDMS surface chemically modified to present alginate could permanently bond with hydrogels of controlled volume and elasticity. The ionic or covalent crosslinking mechanism could circumvent the radical scavenging activity of PDMS. The adhesion strength of the hydrogel to the PDMS was strong enough to withstand significant mechanical deformation. This hydrogel-coated PDMS was successfully used as an in vitro platform to study the effect of external mechanical force on cells adhered to the hydrogel, and also in the fabrication of microfluidic devices filled with hydrogel having controlled properties. This new approach will be highly useful to engineering the surface of a wide array of PDMS-based constructs, and ultimately improving the performance of various soft-material-based devices.

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