Published in final edited form as:

Angew Chem Int Ed Engl. 2017 April 03; 56(15): 4224–4228. doi:10.1002/anie.201700628.

Moldable nanocomposite hydrogel as a fit-to-shape tissue sealant based on mussel-inspired chemistry

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Abstract

Engineering bioadhesives to bind and conform to the complex contour of tissue surfaces remains a challenge. We developed a novel moldable nanocomposite hydrogel by combining dopamine-modified poly(ethylene glycol) and nano-silicate, Laponite, without using cytotoxic oxidants. The hydrogel transitioned from a reversibly crosslinked network formed from dopamine-Laponite interfacial interactions to a covalently crosslinked network through the slow autoxidation and crosslinking of catechol moieties. Initially, the hydrogel can be remolded to different shapes, can recover from large strain deformation, and can be injected through a syringe to adhere to the convex contour of a tissue surface. With time, the hydrogel solidified to adopt the new shape and

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Supporting information <http://onlinelibrary.wiley.com/doi/10.1002/anie.201700628/abstract>

Conflict of interest

The authors declare no conflict of interest.

sealed defects on the tissue. This fit-to-shape sealant has potential in sealing tissues with non-flat geometries, such as a sutured anastomosis.

Graphical Abstract

A novel moldable nanocomposite hydrogel was developed by combining a mussel-inspired polymer and nano-silicate. The hydrogel transitioned from a predominantly physically crosslinked network to a covalently crosslinked one. During this dynamic process, the hydrogel was initially remoldable and injectable, and can adhere to tissue with a convex contour. With time, the hydrogel was fixed in its new shape and functioned as a fit-to-shape sealant.

Keywords

gels; biomimetic synthesis; polymer-nanoparticle bonds; moldability; tissue sealant

Marine mussels secrete adhesive proteins that enable these organisms to attach to various surfaces (rocks, boats, etc.) in a wet, saline environment.^[1] The catecholic moiety found in these proteins plays an important role in interfacial binding and intermolecular crosslinking.^[2] Catechol-modified synthetic bioadhesives have demonstrated potential in suture-less wound closure,^[3] fetal membranes sealing,^[4] Achilles tendon repair,^[5] and immobilization of therapeutic drugs^[6] or cells.^[7] However, existing mussel-inspired adhesives are not ideal for sealing tissue surface with a complex and non-flat geometry (i.e., sealing the convex contour of a sutured anastomosis) due to the slow curing rate (~30 s) of the applied liquid precursor solution.^[7a] A moldable hydrogel that can be processed in the gel form and remolded to fit a desired geometry can potentially address this issue. Although various moldable hydrogels have been reported, these materials are activated by triggers that are potentially unsuitable for biomedical applications (i.e., high pressure,^[8] high temperature,^[9] large change in pH,^[10] and redox reagent^[11]). Most importantly, none of these moldable hydrogels has demonstrated adhesive property. Designing a hydrogel which can be remolded under mild physiological conditions and applied as a bioadhesive remains a challenge.

Herein, we report a simple approach to construct a moldable nanocomposite hydrogel that can function as a fit-to-shape tissue sealant. The hydrogel combines a mussel-inspired polymer, dopamine-modified multi-armed poly(ethylene glycol) (PEG-D) and a synthetic inorganic nano-silicate, Laponite ($\text{Na}^{0.7+}(\text{Mg}_{5.5}\text{Li}_{0.3}\text{Si}_8\text{O}_{20}(\text{OH})_4)^{0.7-}$) (Scheme 1A). The key to this strategy is the dynamic crosslinking process of PEG-D (Scheme 1B) involving the interfacial interactions between polymer-bound dopamine and Laponite (Scheme 1C).^[12] A light brown hydrogel was formed from mixing solutions of PEG-D and Laponite (Figure S1). This color change is attributed to the basic nature of Laponite solution^[13] leading to the autoxidation of dopamine and the subsequent covalent crosslinking between catechol

residues (Scheme 1D).^[14] UV-Vis spectra (Figure S2) revealed the steady increase of oxidation intermediates of dopamine (i.e., dopamine quinone) and dimerized dopamine when incubated with Laponite over time. Using 4-methylcatechol as the model compound, both nuclear magnetic resonance (Figure S3) and mass spectrometry (Figure S4) studies confirmed the formation of dimerized catechol after incubation with Laponite. Fourier transform infrared spectroscopy spectra confirmed the presence of Laponite in the solidified hydrogel (Figure S5).

During the early stage of curing (i.e., incubation time < 24 h), the hydrogel was stretchable and adhesive (Scheme 1E), able to be remolded to different shapes (Scheme 1F and Figure S6), and can be applied around the contour of a tissue surface (Scheme 1G and H). When the hydrogel was incubated for prolonged periods of time (i.e., > 2 d), it progressively lose its ability to be reshaped (Figures S4G and H). During which time, the hydrogel transitioned from a light brown color to a dark brown color, resulting from progressive intermolecular crosslinking of the catechol group.^[14] As the crosslinking density increased, the hydrogel became shape-fixed and functioned as a fit-to-shape sealant.

To investigate the factors that affect the curing process, PEG architecture (4-, 6-, and 8-armed PEG-D (PEG-D4, 6, and 8, respectively; Scheme S1)) and the concentrations of PEG-D and Laponite (10–15 wt% and 2.5–7.5 wt%, respectively) were varied. Nanocomposite hydrogel was denoted as Dx-a/Lapo-b, where x, a, and b denoted the arm number of PEG, weight percent of PEG-D and Laponite in the hydrogel, respectively. At all the concentrations tested, PEG-D4 did not cure even after 48 h of incubation (Table S1). For PEG-D6 and PEG-D8, the concentration of Laponite needed to be 5 wt% or higher to achieve gel formation. For PEG with a reduced number of arms (i.e., 4-armed) or a solution with lower Laponite contents, the polymer chains were unable to connect neighboring Laponite particles to form a network.^[15] Both a higher degree of branching and an elevated content of Laponite provided more physical crosslinking points.

Oscillatory rheology was performed to investigate the hydrogel mechanics during the crosslinking process. For D8-15/Lapo-5, the crossover between the storage and loss moduli (G' and G'' , respectively) values (i.e., gel point) occurred after 5 h of incubation (arrow in Figure S7). G' values continued to increase over 4 d, indicating an increased stiffness and crosslinking density over this time period (Figure 1A). G' values also became less frequency dependent with time, indicating a more elastic behavior. Similarly, the damping ratio (G''/G') also decreased with incubation time (Figure 1B), as the hydrogel transitioned from a predominantly physically crosslinked network to a more chemically crosslinked network. The damping ratio of the fully crosslinked D8-15/Lapo-5 (~ 0.1 at $t = 4$ d) is much greater than a purely covalent crosslinked PEG-D8 hydrogel that do not contain nanoparticles (~ 0.02),^[16] and the elevated damping ratio can be attributed to the presence of reversible dopamine-Laponite interactions in the hydrogel. Catechol likely formed strong interfacial bonds with silica oxide found on the surface of the nano-silicate through the combination of hydrogen bonding and the dispersion interactions.^[17] From the stress relaxation experiment, hydrogel incubated for 1 d exhibited rapid stress relaxation (Figure 1C), indicating that the hydrogel dissipated the strain energy via the disassociation of dopamine-Laponite reversible interactions.^[15] Relaxation time increased with incubation time (Figure 1D), resulting from

the formation of permanent chemical crosslinks with the continuous oxidation of dopamine. Both scanning electron microscopy images (Figure S8) and equilibrium water content values (Figure S9) confirmed that the crosslinking density of hydrogel increased with incubation time.

The time dependent recovery properties of the hydrogel were assessed after applying a large oscillatory shear strain. Hydrogel incubated for 1 d repeatedly recovered its initial G' value immediately after failure caused by 1000% shear strain (Figure 1E and F). This mechanical reversibility suggested that the hydrogel is loosely chemically crosslinked and able to re-establish its network via strong dopamine-Laponite interactions. In contrast, the hydrogel incubated for 4 d did not fully recover its initial G' value (Figure 1F), potentially due to irreversible breakage of covalent bonds. The rheological experiments collectively demonstrated that the hydrogel exhibited reduced ability to rearrange its structure with the formation of covalent crosslinks over time.

The adhesive property of the nanocomposite hydrogel was evaluated using burst adhesion testing (Figure 2A). The pericardium tissue with a central concentric defect was placed over a pipette tip to simulate the application of the hydrogel on a convex surface (Figures 2B and C). D8-15/Lapo-7.5 exhibited the highest burst pressure (320 ± 22 mmHg) among all the formulations tested, which was superior than the reported values for sealing renal vein^[18] and intestinal anastomosis.^[19] The hydrogel formulated with a reduced concentration of Laponite (D8-15/Lapo-5) or PEG with reduced branching (D6-15/Lapo-5) demonstrated a significantly reduced burst pressure (Figure 2D). The effect of hydrogel formulation on burst pressure coincided with the corresponding hydrogel bulk property in rheological testing (Figure S10), which confirmed that an increased bulk materials property contributed to improved adhesive performance.^[20] For all the formulations tested, the hydrogels failed cohesively (i.e., at the bulk of the hydrogel), indicating strong interfacial binding between the hydrogel and the pericardium tissue. The adhesive is tightly bound to the pericardium tissue and resisted the externally applied tension and torsion forces (Figure S11). However, when the hydrogel was incubated for a longer period of time (30 h) prior to application, it demonstrated a remarkable decrease in the measured burst pressure and the adhesive failed at the interface (Figures 2E). Tissue adhesion of catechol-containing adhesives involves the formation of interfacial bonds between the oxidized quinone with the reactive functional groups present on the tissue surface (i.e., $-\text{NH}_2$ and $-\text{SH}$ of lysine and cysteine, respectively; Scheme 1I).^[2] As the incubation time increased, more dopamine-dopamine covalent bonds were formed while reducing the number of available quinone for tissue adhesion, leading to the adhesive failure at the hydrogel-tissue interface.

We further examined the feasibility of sealing the sutured anastomosis performed on a collagen tubing using the nanocomposite hydrogel (Scheme 1H). D8-15/Lapo-5 incubated for 20 h was applied via a syringe around the contour of the tubing over the suture line and demonstrated no leakage when phosphate buffered saline (PBS) was pumped through the tubing (Movie S1). For comparison, PEG-D8 was applied via a double-barreled syringe using sodium periodate (NaIO_4) to initiate the curing (Movie S2). The liquid adhesive noticeably dripped below the tubing when applied and cannot seal the anastomosis during

the pumping of PBS, which confirmed the disadvantage of using a slow curing liquid sealant on a non-flat surface.

Inducing the oxidation of the catechol adhesive moiety is the critical first step for initiating the curing and interfacial crosslinking for catechol-containing bioadhesives.^[21] However, the application of cytotoxic chemical oxidant such as the oft-used NaIO₄ may not be desirable for many biomedical applications.^[22] Additionally, *in situ* activation using an oxidizing reagent requires mixing of two precursor solutions during application. Ion-induced slow, oxidative crosslinking of catechol have been recently reported to form hydrogels that transitioned from a physically crosslinked network into a predominantly covalently crosslinked network.^[14, 23] Our nanocomposite hydrogel exhibited similar transition without the addition of oxidizing reagents. This unique dynamic crosslinking process enabled the stretching, remolding, and adhesion of the hydrogel onto a tissue surface with convex contour. The hydrogel then adapted to a new shape with the formation of intermolecular covalent crosslinks. Additionally, incorporation of Laponite into the highly biocompatible yet bioinert PEG-based material supported human dermal fibroblast attachment (Figure S12), which is needed for rapid tissue repair and regeneration.^[16, 24]

In summary, a nanocomposite hydrogel was constructed by a simple combination of PEG-D and Laponite. The hydrogel transitioned from a reversibly crosslinked network formed from dopamine-Laponite interfacial interaction to a more covalently and densely crosslinked network through the gradual autooxidation and crosslinking of catechol moieties. The hydrogel initially exhibited the ability to be remolded and adhered to the convex contour of a tissue surface. With time, the hydrogel is fixed in its new shape and functioned as a fit-to-shape sealant. This novel strategy overcomes existing challenges associated with *in situ* curable sealants that are unable to seal tissue with a non-flat geometry, the need for cytotoxic oxidizing reagent, and the need for a mixing tip to for mixing precursor solutions. The incubation time prior to application of the nanocomposite hydrogel described here is crucial to obtaining the optimized adhesive property. To further advance the hydrogel for clinical applications, there is a need to better control the crosslinking process (i.e., temperature^[25] or oxygen contents^[26] during incubation) for adapting the hydrogel to different clinical situations.

Acknowledgments

This project was supported by the National Institutes of Health under the award numbers R15GM104846 (awarded to BPL) and R15CA202656 (awarded to FZ). The authors thank Yuting Li, Rattapol Pinnaratip, and Shari Konst for the synthesis of PEG-D.

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Figure 1.

Rheological studies of D8-15/Lapo-5 during the crosslinking process of the hydrogel. Frequency dependence of the storage (G' , close symbols) and loss (G'' , open symbols) moduli (A) and damping ratio (G''/G' ; B) performed at a strain of 1%. Stress relaxation experiment with fitted relaxation curves (solid lines; C) and the calculated relaxation time (D) for an applied step strain of 10 %. Data were presented as mean $\pm$ SD ($n = 3$). ^a $p < 0.05$ when compared to day 1. ^b $p < 0.05$ when compared to day 2. Normalized G' values of D8-15/Lapo-5 (incubated for 1 d) subjected to repeated cycles of 1% and 1000% shear strain with 10 s resting time in between (E). Normalized G' values of D8-15/Lapo-5 (incubated for 1 and 4 d) subjected to increasing applied strain (0.1 – 1000% at 1 Hz) followed by a reduced applied strain of 1% at 1 Hz (F).

Figure 2.

Adhesive property of nanocomposite hydrogels. **(A)** Schematic illustration of the burst adhesion testing set up. PBS was pumped against the hydrogel until failure. **(B)** Side and **(C)** top view of hydrogel sealing a convex pericardium with a central concentric defect. Scale bar: 10 mm. **(D)** The effect of hydrogel formulation and **(E)** incubation time prior to application on the max burst pressure. Data were presented as mean $\pm$ SD ($n = 4$). ^a $p < 0.05$ when compared to D6-15/Lapo-5. ^b $p < 0.05$ when compared to D8-15/Lapo-5. ^c $p < 0.05$ when compared to 30 h incubation time. # Cohesive failure at max burst pressure. * Adhesive failure at max burst pressure.

Scheme 1.

Schematic illustration of the preparation of moldable nanocomposite hydrogel. **(A)** Laponite was combined with PEG-D to prepare the adhesive hydrogel. **(B)** During the early stage of curing, the hydrogel consists of reversible interaction formed between dopamine and Laponite **(C)** and is loosely covalent crosslinked **(D)**. Hydrogel was stretchable and adhesive **(E)**, can be remolded to different shapes **(F)**, and can applied around a convex pericardium **(G)** and collagen tubing **(H)** via a syringe. As the covalent crosslinking density increased, the hydrogel was shape-fixed and bound to tissue via interfacial covalent bonds **(I)**. Scale bar: 10 mm