

Bonding of Macromolecular Hydrogels Using Perturbants

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This Communication describes the use of low molecular weight solutes to bond patterned macromolecular hydrogels and provides evidence that bonding occurs via reversible perturbation of the gels. The ability to form biologically relevant gels with complex microscale architectures may provide useful scaffolds for studies in cell biology and physiology and for microfluidic devices.¹ One promising route to these structures is layer-by-layer stacking, a widely used technique in the microfabrication of polydimethylsiloxane (PDMS).² Passive adhesion between hydrogels, however, is much weaker than that between PDMS structures (most likely due to an intervening thin layer of water). Stacked gels are thus unlikely to resist stresses imparted by mechanical pumping or by cells. Recent work by Stroock and co-workers has shown that alginate gels can be irreversibly bonded by removal and reintroduction of free calcium ion.³ Here, we describe a general strategy for bonding extracellular matrix gels (such as collagens and fibrin) into mechanically robust structures with a resolution of $\sim 20 \mu\text{m}$.

Figure 1 describes the bonding procedure, which started with patterned⁴ and flat gels that passively adhered to form a microfluidic network. This adhesion was sufficient to confine a suspension of microspheres to perfused channels. Mechanical stress, however, readily fractured networks along their adhesion planes and allowed microspheres to pass between the gels (Figure 1B).

We reasoned that solutes that can antagonize gelation would serve as effective bonding agents for gels. Given that many macromolecular gels form by self-assembly in water, we tested solutes known to affect hydrogen bonding in water (bond-weakening chaotropes and bond-forming kosmotropes⁵) for their ability to bond gels. Delivery of a candidate solute proceeded by convection through the microfluidic network and subsequent outward diffusion into the gel; after 1 h, a similar procedure with phosphate-buffered saline (PBS) flushed out the solute. Only perfusion and removal of certain solutes resulted in gels that withstood mechanical agitation (Table 1 and Figure 1B) and bursting pressures $>80 \text{ cm H}_2\text{O}$. For these solutes, bonding occurred up to a maximum concentration, beyond which deformation or disintegration of the gel took place. At the doses indicated in Table 1, these solutes preserved the geometry of microfluidic networks. Cross-sections of treated structures indicated that the sharp features normally obtained by micromolding remained after exposure to solute (Figure 1B, inset).

Our results imply a bonding mechanism that relies on reversible perturbation (de- and repolymerization) of the gel, for several reasons: First, bonding at the indicated solute concentrations invariably led to a loss of opacity in type I collagen and fibrin gels as the solute was introduced, and a return of opacity as the solute was flushed out (Table 1). During exposure of collagen gels to guanidine hydrochloride (GnHCl), second harmonic generation (SHG) output by surface fibrils decreased over time in the forward and backward directions (Movies S1 and S2), with a decrease in the forward/backward signal ratio. The decrease in opacity and SHG output and ratio suggest that bonding solutes decrease the number

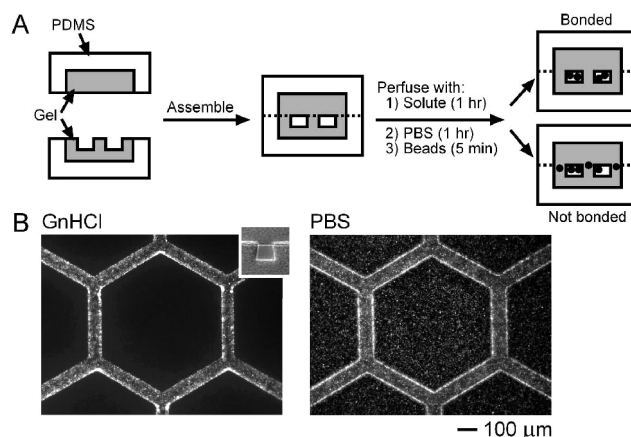


Figure 1. (A) Schematic diagram of perturbant-mediated bonding. (B) Fluorescence images of $80 \mu\text{m}$ wide hexagonal networks in collagen gels that were treated with 0.42 M GnHCl or PBS, perfused with a suspension of $1 \mu\text{m}$ diameter fluorescent microspheres and stressed. Inset: phase-contrast image of a cross-section of a GnHCl-bonded gel (same scale).

Table 1. Bonding of Gels by Solutes

| solute | bonding concn (M) ^a | decrease in absorbance (%) ^b |
|--|--------------------------------|---|
| Type I collagen (rat) | | |
| GnHCl | 0.42 | 93 ± 2 |
| glycerol | 2.1–2.2 | 93 ± 3 |
| NaSCN | 0.82 | 95 ± 9 |
| (+ 0.2 M NaCl) | 0.90 | 89 ± 5 |
| (+ 0.2 M Na ₂ SO ₄) | 1.10–1.20 | 42 ± 4 |
| NaI | 0.82–0.90 | 98 ± 1 |
| NaBr | 2.6–3.0 | 45 ± 1 |
| NaCl | did not bond | 27 ± 2^c |
| Na ₂ SO ₄ | did not bond | 17 ± 2^d |
| none | did not bond | 0 ± 1 |
| Fibrin (human) | | |
| GnHCl | 0.40–0.42 | 88 ± 4 |
| NaCl | did not bond | 18 ± 2^e |
| none | did not bond | -2 ± 6 |

^a Values are the maximum concentrations in PBS before deformation of gels in three independent experiments. ^b Values are means \pm SD ($n = 3$). ^c Gels were treated with 4.5 M NaCl . ^d Gels were treated with $1.5 \text{ M Na}_2\text{SO}_4$. ^e Gels were treated with 0.42 M NaCl .

and thickness of fibrils;⁶ the reverse effects occurred during removal of solute. Second, both perfusion and removal of solute were essential to form gels that confined $1 \mu\text{m}$ diameter microspheres under mechanical stress (Figure 1). Collagen gels that were perfused with GnHCl but not subsequently flushed with PBS led to partial bonding. Third, treatment with bonding solutes led to partial depolymerization of gels. For collagen gels treated with GnHCl, at least 10% of the total protein was solubilized; this effect was kinetically limited, and using a longer treatment with solute ($\sim 3 \text{ h}$) decreased the bonding concentration required for bonding by $\sim 10\%$. Fourth, gels fixed with 1% paraformaldehyde did not bond.

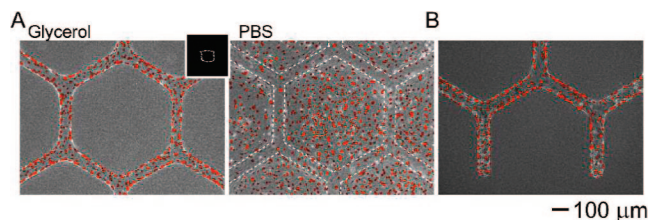


Figure 2. Compatibility of perturbant-treated gels with EC cell culture. (A) Phase-contrast and fluorescence images of a 80 μm wide hexagonal network in collagen gel that was treated with 2.2 M glycerol or PBS (dotted lines indicate channels), seeded with cells, and stained with Hoechst 33342 to visualize nuclei (red). Inset: fluorescence image of a cross-section of a GmHCl-bonded and seeded gel stained with Hoechst 33342 (same scale). (B) Analogous images of a semiopen seeded network.

Nearly all bonding solutes were chaotropes (GmHCl, NaSCN, NaI, NaBr). Addition of the kosmotrope Na_2SO_4 antagonized the bonding effect of chaotropes, while the neutral salt NaCl had no effect (Table 1). The ranking of bonding “strength” for anions followed the Hofmeister series, which correlates with their ability to disrupt macromolecular complexes and fibrils.⁷ Although the kosmotrope glycerol did bond collagen gels, this solute acts as a collagen-specific perturbant, by competing for hydrogen bonds between self-assembled helices.⁸

High concentrations of NaCl did not bond gels, ruling out a nonspecific osmotic effect. Treatment of the gels with 0.05% Triton X-100 did not interfere with bonding, implying that bonding is not due to interfacial hydrophobic interactions.

Taken together, our data are consistent with the following bonding mechanism: Solute is rapidly transported throughout the gel (<5 min, by numerical modeling). Depolymerization then slowly occurs to release oligomers, which can slowly diffuse between gels and/or out of the gels. We expect that, as with any linear self-assembling network, a variety of oligomers are released during this phase.⁹ Removal of solute allows repolymerization to take place, which interlocks the gels across the interface.

To determine whether bonded gels could serve as effective scaffolds for cell culture, and as an additional test of bonding strength, we cultured cells on the surface of the channels and within the bulk of the gel. We used primary human endothelial cells (ECs) as a model of monolayer forming cells and human fibroblasts as a model of mesenchymal cells. When perfused as a suspension through the microfluidic network in a bonded gel, ECs readily attached and grew to confluence over the span of a few days. These cells remained confined to the surface of the channels, as shown by a nuclear stain (Figure 2A, left and inset). In contrast, cells did not remain confined in untreated gels (Figure 2A, right). When human fibroblasts were embedded within collagen gels that were subsequently bonded with glycerol, the levels of cell viability did not differ significantly from those of cells that were not exposed to the solute: For both treated and untreated gels, >90% of embedded fibroblasts remained viable after 2 days. We limited exposure to hyperosmotic stress introduced by the bonding solute to 20–30 min, which may explain why the embedded cells remained largely viable after bonding.¹⁰ Our data suggest that the bonding procedure preserved enough native structure in the gel to support cell adhesion and proliferation, although we cannot rule out the possibility that some epitopes were irreversibly denatured.

The bonding procedure can be extended to semiopen networks, in which the channels do not extend across the full extent of the gel. Here, transport of the solute took place both through the

channels and in the bulk of the gel. Because these structures had a higher resistance to water flow than fully open networks did, we perfused them with solute for a longer time (2 h perfusion and 2 h flushing) and at a larger pressure drop. As with the open hexagonal networks, ECs formed a confluent layer in the channels, resulting in semiopen networks in which the cells were confined to channels (Figure 2B).

Our results demonstrate that certain small solutes (chaotropes and other perturbants) can be used to bond macromolecular hydrogels into monolithic structures that preserve the initial geometries of the gels. This technique can be used to form both open and semiopen networks in biologically relevant gels, and the resulting materials support cell culture. Our data are consistent with a bonding mechanism of reversible perturbation, which is similar to the fusion of alginate gels.³ In theory, other self-assembling macromolecular gels such as Matrigel and agarose may be bonded with similar solutes. Compared with recently described subtractive methods for making microfluidic gels,¹¹ the additive method described in this work has the potential to form three-dimensional (3D) networks by stacking and bonding layers. The development of methods to register layers in 3D (similar to what has been described for PDMS²) will be an important step toward this goal. We believe that the structures described here will provide interesting opportunities to generate spatially complex biological tissues for the study of physiological flows or multicellular biological processes.

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Supporting Information Available: Experimental details and time-lapse movies of SHG in treated gels. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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