Supplementary material

Methods

The adhesive secretion from the dorsal surface of *A. subfuscus* was collected as described previously (3). Each sample was collected from a single slug. Samples were collected rapidly, with the goal of collecting a large (>5 mm diameter) and relatively uniform mass before it set fully. Based on visual observations, setting of the glue takes place within several seconds. Each sample was then placed in a microcentrifuge tube on ice. The samples were stored at -80 C until use.

For tensile testing, frozen samples were partially thawed so that they were firm, but could be cut with minimal effort. They were cut into strips with paired razor blades taped together 1.5 mm apart (1.6 in some cases) [3]. The first cut of the ball-shaped sample created a 1.5 mm thick disc; cutting this disc orthogonally to the first cut created rectangular strips. Dry ice was used to keep the sample partially frozen so that it could be cut easily without deformation. This is important because the glue’s adhesiveness would normally cause it to adhere to the razor blades such that it could not be removed without extensive deformation. These strips were slightly wider than those used by Wilks et al. [3] to reduce the possible effect of evaporation on the samples. Note that no evidence was seen of deterioration of the samples in these tests, which typically took place in less than five minutes. Also, previous work showed that freezing did not measurably affect the peak stress, yield strain, failure strain or toughness [3]. Strips were typically at least 5 mm long. Two to four strips of glue could be cut from each sample. Only one strip was used for most tests, but in some, a separate strip was used as a paired control. All samples were fully thawed before they were used in any experiments, and they were tested at room temperature.

*Stress relaxation and tensile testing*

Stress relaxation tests were performed using a 100 Series Electromechanical Universal Test Machine with a 5.6 lbf load cell. Samples were clamped in flat metal grips. Although this deformed the sample substantially, that was unavoidable for these gels. Samples were stretched to a strain of 2 (ie. three times the original length), at a strain rate of 3 mm sec-1. Samples had an original length of 3-5 mm between the clamps, so they reached a strain of 2 in two seconds. The strain was then held constant and the stress was monitored for five minutes. Three trials were performed with different samples.

In preliminary tests, stress relaxation tests were performed at strains of 1, 2, 3, 4 and 5. Three samples were tested at each strain. These measurements were made with the custom-built tensometer described by Wilks et al. (3). The advantage of the custom device is that its relative simplicity made it easier to work with small, deformable samples, but it was less precise due to the use of a less sensitive force transducer and less precise measurement of strain. Nevertheless, the results gained from the custom apparatus closely matched those from the other device.

Samples were also tested to failure at varying strain rates. The 100 Series Electromechanical Universal Test Machine was used for these tensile tests, with strain rates between 0.01 sec-1 and 1 sec-1. The elastic modulus was calculated based on the slope of the stress-strain curve during elongation, before the sample yielded by necking.

In order to analyze the strain rate dependence further, several samples were also tested using a dynamic rheometer. Because the glue sets within seconds into a firm ball when collected, it is difficult to get in a uniform sheet between rheometer plates without substantial deformation, especially given the amount of glue collected from one slug (usually 30-60 mg). To get sufficient material between the plates, the samples were soaked for three hours, allowing them to swell substantially. Additional samples soaked overnight or for two days gave similar results. The samples were tested on an AR-550 rheometer (TA Instruments) at 1% strain using a frequency sweep from 0.01 to 50 radians per second. Swollen samples were still firm and held their shape, thus pressing them between rheometer plates caused substantial deformation and often fracture due to inhomogeneity within the sample. Nevertheless, this provided an independent test of the strain rate dependence of the glue.

*Cyclic stress-strain tests*

In order to determine if sacrificial bonds failed as the glue was strained, cyclic stress-strain tests were performed and the sample hysteresis was measured. For these trials, an AR-G2 Magnetic Bearing Rheometer (TA Instruments) was used. This had custom clamps to allow tensile testing. Samples with an original length of roughly 4 mm were taken through a series of stress-strain cycles, extending 4 mm, then reversing to the starting position, then repeating this process to 8 mm, then to 12 mm. This corresponded roughly to strains of 1, 2 and 3. For comparison, a separate strip of glue was cut from each of the samples and taken through a single stress-strain cycle extending directly to a strain of three and back without the intermediate steps. In one additional experiment, strains of 1, 2, 3 and 4 were tested. For all trials, the extension rate was 0.25 mm sec-1, thus a strain rate of roughly 0.06 sec-1. In preliminary trials, cyclic stress-strain tests were performed as described using the custom tensometer at a strain rate of 0.5 to 1 sec-1.

The elastic moduli of the samples was measured by fitting a linear regression to the data points during the extension phase. For the repeated trials, the modulus was estimated by fitting a regression to a line created by the linear parts of the three extension curves. Specifically, the regression was based on the points representing the first recording for any given strain value during extension, thus excluding regions of subsequent trials that repeated the previous, shorter extensions. The energy dissipated in each hysteresis loop was measured by measuring the area enclosed within the loop using a spreadsheet to calculate Riemann sums.

To test whether failure of sacrificial bonds was reversible, after each sample was stretched through three cycles, it was allowed to rest at its initial length for five to ten minutes then re-tested. Longer recovery periods were not tested due to the difficulty in ensuring that the sample hydration did not change.

*The effect of pH on glue stiffness and ion content*

To test the impact of pH on stiffness, test strips prepared as described above were soaked in 50 mM acetate buffers with pH values ranging from 2.9 to 7.4. The samples were soaked for five minutes to allow equilibration to the pH while minimizing the swelling that typically occurs when samples are soaked for longer periods. After soaking, stress-strain tests were performed using the custom tensometer described by Wilks et al. (3). This has clamps that are suited for the slightly swollen samples that result from soaking. The initial sample length between clamps was measured with calipers. Samples were stretched to a strain of three at a rate of 1.1 mm sec-1, which corresponded roughly to a strain rate of 0.3 sec-1. The elastic modulus of the samples was calculated using linear regression. In some trials the glue strip had an anomalous stress strain curve, often due to irregularities in the sample, or errors induced during clamping. To deal with outliers, eight trials at each pH were performed and the highest and lowest modulus values for each pH were discarded. To ensure that the data from this tensometer matched the data from the other devices, a set of eight samples were tested without soaking. To provide context for these experiments, the pH of three samples of the native glue was measured by homogenizing the glue in 25 volumes of distilled water using a rotor-stator homogenizer, then measuring the pH of the solution with litmus paper, and assuming that the buffering capacity of the polymers would prevent the pH from changing much with dilution.

The role of calcium and magnesium as potential cross-linkers was tested by determining how tightly they were bound to the polymers in the gel at different pH values. This showed whether there was a correlation between the binding of these metal ions and the stiffness of the gel. If the gel lost stiffness at the pH at which the ions dissociated from the gel, this would indirectly implicate those metal ions in cross-linking. To test this, glue samples of roughly 2 x 2 x 2 mm were cut and the mass of the cube was recorded (typically 5-15 mg). Each sample was then soaked for one hour in one of the six different acetate buffers used in the tensile tests (pH 2.9 to 7.4). This range was chosen because metal ions typically dissociate from their ligands at acid pH. At the end of the hour, the solid glue was separated from the bathing solution. The bathing solution containing any metal ions that dissociated from the gel was mixed with 5.25 volumes of 0.7% nitric acid, and the calcium or magnesium content was measured in an atomic absorption spectrometer (Shimadzu AA-6300). For comparison, the total metal concentration of samples was measured by soaking five samples for an hour in 0.7% nitric acid to remove all metals, then measuring the metal content as described previously. The spectrometer was calibrated for the range of concentrations analyzed using commercial standards in dilute nitric acid. Blanks containing only the buffers diluted in 0.7% nitric acid were measured to confirm that the buffers did not contribute a measurable amount of ions. Finally, a time series of these experiments collecting samples at intervals from 5 minutes to 3 hours was performed using the pH 2.9 and 7.4 buffers for both calcium and magnesium.

Results

Supplemental Figure 1

**Supplemental Fig. 1**. The effect of strain rate on two swollen glue samples in a dynamic rheometer. Samples had been soaked for three hours to swell them sufficiently for use in the rheometer. Samples were pressed between plates and tested at a strain of 1% across a range of strain rates. Storage modulus (G’) is indicated by black circles and loss modulus (G”) is indicated by gray circles.

Supplemental Figure 2

**Supplemental Fig.2**. Time course of calcium diffusion from the glue. Ion content values are given as percentage of the total, which was measured after one hour incubation in 0.7% nitric acid.

Comparison of modulus and yield strain data to results from Wilks et al. 2015

The measured mechanical characteristics reported in this paper are similar to those of Wilks et al. (3), but there were some differences. Wilks et al. (3) measured an elastic modulus of 30 kPa, as opposed to 10-20 kPa in these experiments. Wilks et al. used a strain rate closer to 1/sec. The modulus predicted for that strain rate in the experiments described here was just over 20 kPa. The yield strain found by WIlks et al. (3) was also slightly lower (3.8 vs. 5.0). A slight underestimate of strain could lead to an overestimate of stiffness, and the data from Wilks et al. were based on a custom tensometer that did not have precise control over strain. Nevertheless, the same device was also used in this study, and gave modulus values that were consistent with those measured by the commercial machines. Thus, it is possible that there is year to year variation in glue samples, or some as yet unknown aspect of sample preparation was changed and impacted the results. More interestingly, the post-yield plateau measured in this study was not as extensive as that described by Wilks et al. The material extended past the yield point of 5 to a strain of 6.6. In contrast, Wilks et al. found that the glue continued to extend to a strain of 10 after yielding, holding at a constant stress. These differences are most likely due to the nature of the clamps used. Because the material is a deformable gel, it cannot be clamped without substantial distortion. There is a trade-off between gripping it securely by squeezing it between two solid clamps as with the commercial instruments, and gripping it with the toothed alligator-style clamp of the custom device. Although neither caused any obvious, repeatable weakening of the glue, an impact on the measured mechanics was likely.