Supporting Information

*Electro-responsive aqueous silk protein as “smart” mechanical damping fluid*

Rod R. Jose, Roberto Elia, Lee W. Tien, David L. Kaplan*

Department of Biomedical Engineering
Tufts University
Science and Technology Center
4 Colby Street
Medford, MA 02155, US

KEYWORDS

damper; viscous damping; dashpot; friction; electrogelation; electrorheology; silk
Supplemental Experimental Details

Materials
Cocoons of the silkworm *Bombyx mori* were supplied by Tajima Shoji Co. (Yokohama, Japan). Sodium carbonate, lithium bromide, were purchased from Pierce Sigma-Aldrich (St. Louis, MO, USA). Slide-a-Lyzer dialysis cassettes were purchased from Pierce, Inc. (Rockford, IL, USA).

Fabrication of Device Components
The simplest design of our device is similar to a standard dashpot damper, but it can alternatively be mounted with spring assemblies similar to a strut. There is no limitation to the size of the components. The device geometry can be sized appropriately according to the application. The main housing acts as the working cylinder which contains the components and fluid. The working cylinder was fabricated by boring out the internal chamber from a solid piece of aluminum stock. A window was cut through the wall of the working cylinder to provide visibility of the fluid gelling process. The cylinder was protected and insulated by a clear polypropylene tube. The piston rod was fabricated from a stainless shaft. The rod seals were standard urethane. The piston rod enters the assembly through a guide seal. The piston rod guide was Teflon. The rod terminated with three 1 mm thick Teflon orifice plates which formed the damper piston. The orifice plates contained four large orifices and a 1.5 mm clearance from the cylinder wall which left 45% of the cross-sectional area free for fluid travel. The valve piston assembly can be modified by adjusting the number, spacing, conductivity and size of the piston valve rings, which will affect the interaction with the electrogelating fluid thereby altering the damping effect. The piston valve rings can be conductive or non-conductive.
Preparation of Aqueous Silk Polymer Fluid

Silk fibroin solutions were prepared following published procedures. In brief, B. mori silk cocoons were boiled in 0.02M aqueous Na$_2$CO$_3$ for 10 minutes to extract the sericin component and isolate the silk fibroin protein. Isolated silk fibroin was then washed three times for 20 minutes in deionized water and allowed to dry for 48 hours at room temperature. Dried silk was dissolved in 9.3M LiBr at 60°C for 4 h, and the resulting 20% (w/v) solution was dialyzed against water using a Slide-a-Lyzer dialysis cassette (molecular weight cutoff 3500) for two days to remove salts. The ensuing concentration of aqueous silk fibroin ranged from 5-7% (w/v), which was calculated by weighing the remaining solid after drying. All silk fibroin solutions were stored at 4°C until use.

Measurement of Viscosity

Solution viscosities (n=3) were measured using a cone & plate viscometer (model RVDV-II+P CP, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) following standard procedures. Plastic viscosities were reported as the slope of the shear stress/shear rate line above the corresponding yield point. Variability is reported, with the mean, as standard deviation.

Mechanical Testing of Dashpot Damper with Electrogelation

Silk solution viscosity was reversibly altered using an electric field following published procedures. The working cylinder and piston rod were conductive. Electrode leads were located at the mounting locations. The piston orifice plates were fabricated from Teflon. A positive or negative charge was applied at the piston rod mounting, while the opposite charge was applied at the working cylinder. The prototype device was configured to emit the gel-
initiating electric field from the inner wall of the working cylinder or the piston rod. Application of the electric field initiates gelling of the fluid at the positively charge component. Reversal of the charge reversed the gelation of the fluid. Gelation was induced by supplying a charge of 5 V to 15 V at current of 0.01 A continuously or discreetly for 30, 60, 90, or 120 seconds. For discrete gelation, the fluid was subjected to electrogelation for each time period, and then the electrical field was disabled thereby halting the gelation process before mechanical testing. After 120 seconds of electrogelation, the field was reversed for 30 seconds. Mechanical testing was conducted throughout ten stroke cycles without applied voltage. **Dunnett's multiple comparison post-test was used to compare the damping of each stroke (n=40) using stroke 2 as the control in order to account for the initial sloughing of gel.** Variability in damping per each stroke is reported as standard deviation (n=40). Average damping of the ten strokes was reported with standard deviation. Alternatively, the electrogelation charge time was applied continuously during 450 actively reciprocating stroke cycles during which the piston rod traveled 10 mm at a velocity of 1 mm per second per cycle. Solution was brought to the damping plateau then charge was reversed and damping was reduced to the minimal capability after this preconditioning step. Damping coefficient was calculated as the quotient of the force in Newtons caused by viscous drag divided by the velocity of the piston in meters per second. The damping coefficient was given in units of newton-seconds per meter. Fold damping enhancement was normalized to the minimal damping capability. The change in mean damping coefficients between strokes (n=5) during gelation was reported. Discrete and continuous tests were compared using standard unpaired t-tests. Associated error bars depict standard deviation. Asterisks centered over the error bar indicate the relative level of the p-value (*p< 0.05, **p< 0.01, ***p< 0.001).
Quantification of Friction

Pull-out tests were performed using a uniaxial mechanical tester (model 3366, Instron Inc., Norwood, MA, USA). Stainless steel plates were used for pull-out tests. Three plates were left uncoated. Three additional plates per time period were coated in silk fluid and then electrogelated for 0, 15, or 60 seconds using a current of 0.01 A at 15 V. Each plate was then mounted between clamps using a large normal force of 1.5 kN. Clamp surfaces featured a standard diamond-pattern knurling with a spiral angle of 30°, a pitch of 1 mm, and a 90°profile angle. Clamping area was 15 x 25 mm. Friction force (n=3) was reported as one-half of the peak force required to initiate linear motion, to account for the two surface interfaces. Variability is reported, with the mean, as standard deviation.

Prevention of Surface Marring by Fluid Coating

Stainless steel plates (n=3) were subjected to surface marring via brass bristles held at an interfacial pressure of 175 kPa. Surface marring proceeded for 350 rotational cycles. Sample surfaces were either uncoated or coated with silk fluid which was electrogelated for 0, 15, or 60 seconds using a current of 0.01 A at 15 V prior to marring. Variability is reported, with the mean, as standard deviation.
Supplemental Figure 1. Effect of electrogelation time and reversal of charge on damping when gelation propagates from the piston rod. Damping effect is continuously enhanced several fold with continuous electrogelation of the fluid as shown here. The fluid was continuously subjected to electrogelation using a weak electric field throughout mechanical testing stroke cycles. After 160 cycles, the 15 V electrical field was reversed thereby reversing the gelation process and reducing the damping enhancement during continuous mechanical testing.
Supplemental Figure 2. Effect of electrogelation charge time on damping during active stroke cycles when gelation propagates from the working cylinder. Gel initiating charge is emitted from the inner wall of the working cylinder. Damping effect is continuously enhanced several fold with continuous electrogelation of the fluid as shown here. The fluid was continuously subjected to electrogelation using a weak 15 V electric field throughout mechanical testing stroke cycles. After 190 cycles, the 15 V electrical field was reversed thereby reversing the gelation process and reducing the damping enhancement during continuous mechanical testing. The damping transition from gelation to reverse gelation is smoother when charge is emitted from the wall compared to the rod.
Supplemental Figure 3. Effect of electrogelation charge time on damping during active stroke cycles when gelation propagates from the working cylinder. Gel initiating charge is emitted from the inner wall of the working cylinder. Damping effect is continuously enhanced several fold with continuous electrogelation of the fluid as shown here. The fluid was continuously subjected to electrogelation using a weak 5 V electric field throughout mechanical testing stroke cycles. After 360 cycles, the 5 V electrical field was reversed thereby reversing the gelation process and reducing the damping enhancement during continuous mechanical testing. The damping transition from gelation to reverse gelation is smoother when charge is emitted from the wall compared to the rod.
**Supplemental Figure 4.** Comparison of stainless steel plate surfaces after subjection to surface marring. Sample surfaces were either non-protected or protected by a coating of pre-gelated silk fluid, or fluid which was electrogelated for 60 seconds prior to marring.
REFERENCES

(1) Lovett, M. L.; Cannizzaro, C.; Daheron, L.; Messmer, B. Silk Fibroin Microtubes for Blood Vessel Engineering. *Biomaterials* **2007**, *28*, 5271–5279.

(2) Yucel, T.; Kojic, N.; Leisk, G. G.; Lo, T. J.; Kaplan, D. L. Non-Equilibrium Silk Fibroin Adhesives. *J. Struct. Biol.* **2010**, *170*, 406–412.

(3) Lu, Q.; Huang, Y.; Li, M.; Zuo, B.; Lu, S.; Wang, J.; Zhu, H.; Kaplan, D. L. Silk Fibroin Electrogelation Mechanisms. *Acta Biomater.* **2011**, *7*, 2394–2400.