A BIO-INSPIRED, CHEMO-RESPONSIVE POLYMER NANOCOMPOSITE FOR MECHANICALLY DYNAMIC MICROSYSTEMS

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ABSTRACT

This paper reports the development of a biologically-inspired, variable-modulus nanocomposite material for mechanically dynamic biomedical microsystems. This nanocomposite is comprised of a poly(vinyl acetate) matrix polymer that is reinforced with rigid cellulose nanofibers, and becomes very flexible when exposed to water. A direct-write CO_2 laser was used to pattern structures in this chemical- and temperature-sensitive material. Tensile testing of laser-cut, micron-scale nanocomposite beams was performed using a custom-built tensile tester. These samples displayed a significant reduction in Young's modulus from 4.1 GPa to 6.1 MPa when the nanocomposite was exposed to phosphate buffered saline. Additionally, the modulus change was observed to be reversible upon drying of soaked tensile samples. As a well-suited application of this nanocomposite, cortical probes utilizing this material as a substrate were fabricated. Gold-coated, dual-shank cortical probes utilizing this nanocomposite as a substrate were shown to record action potentials from a single neuron in a cockroach brain.

KEYWORDS

mechanically dynamic, nanocomposite, cortical probe, neural interfacing

INTRODUCTION

The advancement of micromachining techniques for polymers like polyimide, PDMS, and parylene has enabled the development of MEMS structures from mechanically flexible materials. The mechanical properties of these polymers can be "tuned" during synthesis, but cannot be reversibly altered once the material is formed. A novel polymer nanocomposite, exhibiting reversible chemo-responsive mechanical behavior, has recently been developed by members of our team [1]. A particular version of this nanocomposite consists of a low modulus polymer (PVAc) infused by a network of stiff cellulose fibrils. The stiffness of the nanocomposite is dependent on the hydrogen bond interactions between the fibers, which are regulated by the presence or absence of water (Fig. 1). This material was inspired by the sea cucumber, which can modify the stiffness of its dermis by chemical regulation of collagen fibrils. Bulk specimens of the bio-inspired material have exhibited a reduction in tensile modulus from 5.4 GPa to 12 MPa upon exposure to water at 37°C.

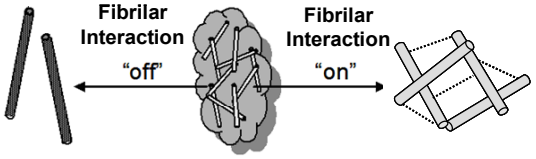


Figure 1: Schematic representation of the mechanism responsible for the stimuli-responsive behavior of the PVAc nanocomposite.

The purpose of this work was to develop the requisite micromachining techniques to produce multilayered MEMS structures from this material and to determine how well the chemo-responsive behavior translates to microfabricated structures. Additionally, this paper discusses the utilization of this material as the substrate material for an intracortical probe for neural recording.

NANOCOMPOSITE PATTERNING

The nanocomposite consisted of cellulose nanofibers (~12% v/v) extracted from sea tunicates and synthesized using a nanofiber template and a sol-gel process. Briefly, the cellulose fibers are first isolated from sea tunicates by acid hydrolysis in sulfuric acid. After purification and redispersion in dimethyl formamide, the fibers are mixed with a solution of the polymer in the same solvent. The mixture is then cast into a Teflon dish and dried to remove the solvent from the resulting nanocomposites. Finally, the nanocomposite is compressed to form planar specimens 50 to 150 μm in thickness.

The development of micromachining techniques for devices that utilize this material is very challenging because the PVAc matrix polymer is highly sensitive to both chemicals and temperature. PVAc is incompatible with many chemicals that are commonly used in photolithographic processing. It also exhibits a glass transition temperature (~35°C) lower than temperatures typically used for a photoresist soft bake in photolithography, which results in distortion of the patterned features. As a result, laser micromachining with a direct-write 50 W CO_2 laser was used to pattern the PVAc nanocomposite. The operation parameters for the

laser were customized for this material with a laser power of 2.5 W, a cutting speed of 560 mm/s, and a resolution of 600 laser pulses per inch. The number of laser passes required to completely cut through the sheet was dependent upon the thickness of the sheet, with approximately one pass required for every 10 μm in thickness. The use of the laser for patterning did not result in any charring of this material.

MECHANICAL EVALUATION OF NANOCOMPOSITE STRUCTURES

Standard tensile testing was performed to assess if, and how well, laser-patterned micron-scale nanocomposite structures reflect the strength and stiffness of the bulk material, as well as how well these structures respond to the stimulus. The testing was performed using a custom built tensile tester designed specifically for the widely varying stiffness of the nanocomposite. The test structures (Fig. 2) consisted of beams 150 μm in thickness, 150 μm in width, and 2000 μm in length, anchored on both ends by pads 3000 μm x 2000 μm.

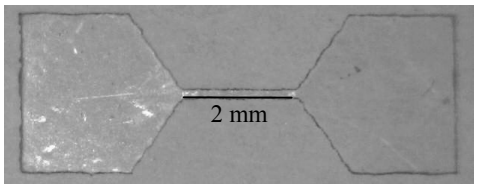


Figure 2: PVAc nanocomposite tensile test specimen.

The tensile tester (Fig. 3) utilizes a MicroMo linear piezomotor capable of displacement in 20 nm increments. Displacement of the drive rod of the piezomotor is measured using an indicator (Mitutoyo 543-562A) with 0.5 μm resolution. Force is measured using one of two load cells, MDB-25 with a resolution of 56 mN and the GSO-10 with a resolution of 49 μN (Transducer Techniques). Typically, the MDB-25 is used for the stiff dry samples and the GSO-10 is used for the flexible wet samples. The sample beams are secured on one end by a mobile grip, and on the other end by a fixed grip. The grips consist of acrylic blocks held tightly together by three small screws. The mobile clamp is attached to the drive rod of the linear motor, which is set on a wheeled platform to minimize friction between the platform and the bottom surface of the test setup. A screw connects this platform and the load cell. As the linear motor pulls the sample, the opposing force pushes the wheeled platform toward the load cell, which is fixed in position. The displacement of the motor is measured by the indicator. The indicator and the load cell are interfaced with a computer using DAQFactory (Azeotech) to allow for automated recording of the force and displacement data. The force is then converted to stress by dividing by the cross sectional area of the beam, and the displacement is converted into strain by dividing by the length of the beam. The initial slope of the stress versus strain curve in the elastic region is the Young's modulus of the sample.

The test procedure involved first clamping the dogbone-shaped test structures at both ends. The Young's modulus of dry (as-fabricated), wet, and dried samples was measured using this setup. The wet samples were soaked in saline for 18 hours, then kept moist during testing by adding drops of saline to the beam with an eyedropper. The dried samples were soaked in saline for 18 hours, then air-dried at room temperature for 6 hours before being tested.

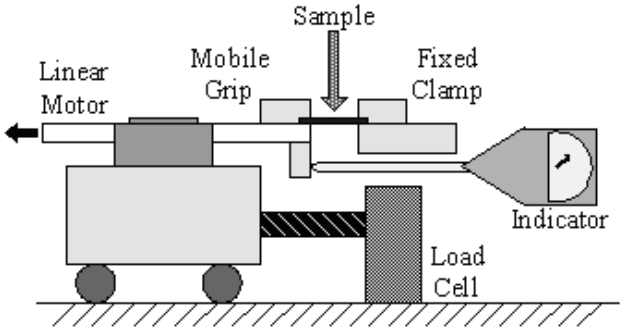


Figure 3: Tensile test setup for measuring the Young's modulus of widely varying stiffness of micron-scale beams.

Stress-strain curves from a typical dry sample and a typical wet sample are shown in Fig. 4, and measurement results for all three types of samples are summarized in Table 1. The Young's modulus of the dry sample was measured to be 4.1 GPa, which is consistent with the dynamic mechanical analysis (DMA) measurement of the bulk dry sample, indicating that the stiffness of the cellulose-fiber-reinforced PVAc nanocomposite is not impaired by laser-patterning or scaling down to 150-μm features.

Table 1: Tensile test measurements on an as-fabricated sample, a sample soaked in saline, and a soaked sample that was allowed to dry.

Parameter	*Dry Sample*	*Wet Sample*	*Dried Sample*
Young's Modulus [MPa]	4120	6.1	2200
Tensile strength [MPa]	82	5.0	9.4
% Strain at Break	3.5	560	5.0

The Young's modulus of the wet sample was measured to be 6.1 MPa, which is slightly lower than the reported modulus of bulk samples of 12 MPa, yet clearly displays a large change in stiffness. This difference may be attributed to the difference in measurement techniques and experimental error.

The Young's modulus of the dried samples was found to be 2.2 GPa, indicating that the change in Young's modulus is reversible. Additional investigation is underway to determine if the sample will regain more of the stiffness if the sample is allowed to dry further. These

mechanical data show that the nanocomposite retains much of its dynamic behavior when rendered into MEMS-scale structures by laser micromachining in spite of the potentially damaging aspects of the technique.

In addition to a greatly reduced Young's modulus, the PVAc nanocomposite is able to accommodate a very large amount of strain when exposed to water. This behavior is not displayed in either the dry or the dried sample. Furthermore, the dried samples exhibited a much lower tensile strength compared to the dry sample that had not been exposed to water. Investigation is ongoing to determine if this strength improves when more water is removed from the nanocomposite.

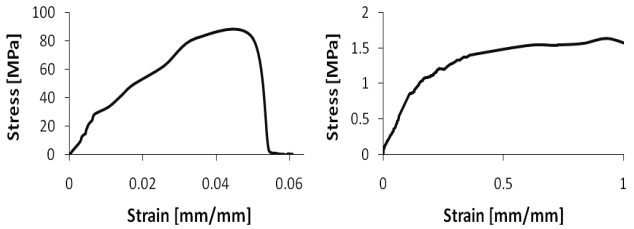


Figure 4: Stress-strain curves for (left) dry sample and (right) sample soaked in saline.

CORTICAL PROBE

As a technology demonstrator, we have targeted micromachined cortical probes, such as those shown in the schematic diagrams in Fig. 5, as the first application of this material. These cortical probes are designed to interface neurons in the cerebral cortex with external electronics for neural recording. Conventional micromachined cortical probes utilize a thin silicon substrate. These Si-based probes have the advantages of compatibility with standard integrated circuit fabrication techniques and allowing for the incorporation of on-chip active electronics for signal processing [2]. However, a large mechanical mismatch between the stiffness of the silicon neural probe and the neural tissue in which it is embedded results in relative micromotion between the probe and the brain, changing the location of the probe with respect to the neurons from which it is recording, and possibly damaging the surrounding brain tissue. Additionally, a build-up of a glial sheath around chronically implanted silicon-based probes has been consistently observed. This growth of scar tissue around the probe increases the impedance between recording electrodes and neurons, leading to a reduction in the signal-to-noise ratio, until the probe becomes inoperable [3].

It has been suggested that this sheath may not form, or may be reduced in thickness, if the mechanics of the probe closely match the mechanics of the surrounding brain tissue. This would require a probe substrate material with a Young's modulus on the order of 1 kPa. Probes with flexible polymer substrates such as polyimide [4] and parylene [5] have been previously fabricated. However, these devices still exhibit a considerable mechanical mismatch between the probe and the brain tissue, as these materials typically have Young's moduli in the range of 1-4 GPa. The dynamic nanocomposite is particularly well-suited for this application as it allows for the probe to be rigid upon insertion, yet highly flexible after deployment.

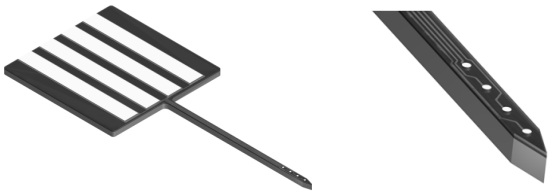
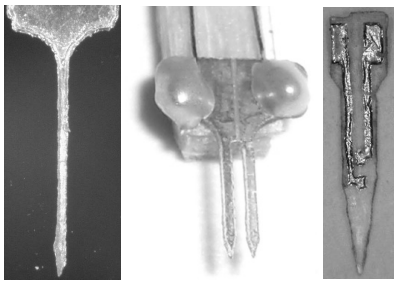


Figure 5: Schematic of a planar microprobe with a close-up of the probe shank.

Fabrication

Several versions of the cortical probe have been fabricated using the nanocomposite as a substrate, as shown in Figure 6(a)-(c). The single-shank probe shown in Fig. 6(a) has a 50-µm x 50-µm thick x 2000-µm long shaft, and was patterned using laser micromachining.



(a) (b) (c)

Figure 6: Microfabricated neural probe based on a PVAc nanocomposite: (a) plan-view micrograph of a laser micromachined probe;(b) photograph of Au-coated, dual-shank probe for in vivo *testing; (c) micrograph of a neural probe with PVAc substrate, parylene barrier layer, and Ti/Pt electrodes.*

The dual-shank probe shown in Fig. 6(b) has two electrically isolated probes spaced 500 µm apart. This probe was fabricated by first sputter-depositing a 300-nm thick Au film onto the surface of one side of a PVAc nanocomposite sheet. The outer shape of the probes was defined using the CO_2 laser with the parameters described above. Next, a single laser pass at a power of 2.25 W and a speed of 490 mm/s cut through the thin-film Au layer down the center of the structure. This power was sufficient to ablate the metal down the centerline of the structure without inducing appreciable damage to the underlying PVAc nanocomposite. Tungsten wires were attached to the probe using conductive epoxy (Chemtronics) for connection to external electronics.

The probes were designed to support electrodes, but as the varying modulus is dependent on the absorption of water, the electrodes need to be isolated from the nanocomposite with a moisture barrier. Parylene C was selected to be an electrically insulating, moisture-barrier

coating. The probe shown in Fig. 6(c) was fabricated by first vapor-depositing a 1-μm thick layer of Parylene C on one side of a nanocomposite sheet. The probe shape was laser-cut from this sheet and a micromachined stencil mask was then used to selectively pattern a sputter deposited Ti/Pt film on the parylene layer.

Coating compatibility

Adhesion of a thin-film Au coating to the PVAc nanocomposite was assessed by immersing a 300 nm Au-coated PVAc nanocomposite in room temperature saline for nine days. There was no evidence of the Au coating delaminating from the nanocomposite substrate during or after this period of time. Additionally, dynamic mechanical analysis of thin bulk films with the Au coating showed a dry modulus of 4 GPa, and a wet modulus of 8 MPa, indicating that the thin metal film had very little influence on the mechanical properties of the material.

A sheet of the PVAc nanocomposite was also coated on one side with a 1-μm thick parylene film. Rectangular samples were then laser-cut from the sheet and subjected to a saline soak test at 37°C for 19 days to simulate biological conditions. No delamination of the parylene films was observed at any point during the test. Furthermore, these samples clearly displayed the expected dynamic behavior when placed in saline.

In vivo testing

Gold-coated dual-shank probes like the one shown in Fig. 6(b) were tested *in-vivo* for electrical functionality in a cockroach brain to assess their ability to record single unit action potentials. The dual-shank allowed for two isolated electrodes, providing for a single reference electrode and a recording electrode. Measurements were made using a TDT RX-5 neural recording system and sampled at approximately 24 kHz. The data were passband filtered from 300 Hz to 3000 Hz.

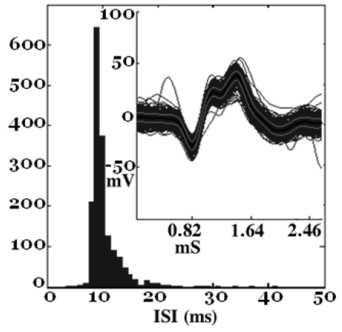


Figure 7: Interspike interval histogram and (inset) sorted spikes both indicate that single-unit action potentials were recorded from the Au-coated dual-shank electrodes.

Spike detection and sorting was performed using an algorithm in which a spike was identified as being greater in amplitude than five times the standard deviation of noise. The standard deviation of noise for a six minute recording with the gold-coated dynamic probe was estimated to be 3.64 μV, yielding a spike threshold set to 18.20 μV. The algorithm produced the interspike interval (ISI) histogram and the sorted waveforms shown in Fig. 7. The probe recording shows clear unit activity, and the ISI data indicate that this was a well-isolated unit as all intervals between spikes were greater than the minimum time for action potential duration and refractory period.

CONCLUSION

A mechanically dynamic, chemo-responsive polymer nanocomposite with a poly(vinyl acetate) matrix has been utilized as a material in the fabrication of variable-modulus neural probes. Tensile testing on microscale test beams has shown that laser-micromachining this nanocomposite and scaling down to the microscale does not interfere with the ability of the nanocomposite to respond to the presence of a chemical stimulus. Additionally, the modulus change was shown to be reversible by removing the stimulus and allowing the samples to dry. This nanocomposite was demonstrated in its use with cortical probes for neural recording. These probes were shown to be successful in recording neural action potentials from a single neuron in a cockroach brain.

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