Conductive, Mechanically Robust, and Biocompatible Hydrogels for Neural Probe Coatings

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Currently, surgeons face challenges when implanting microelectrodes into neural tissue. The challenges arise mainly because there is a significant mismatch in the modulus of elasticity of conventional metallic or microfabricated electrodes (∼10 GPa) and that of the soft brain tissue (∼10 kPa). The modulus mismatch leads to local scarring at the neural interface. Here we present a strategy to design elastic, electrically conductive, biocompatible, and tough hydrogel systems that create a seamless interface between metal electrodes and biological tissues by better matching the mechanical and physiological properties of neural cells and tissues in the central nervous system. The design strategy is to infuse conductive polyaniline (PAni) polymer chains into an intrinsically biocompatible polyacrylamide-chitosan (PAAm-Chitosan) hydrogel matrix, coat the hydrogels with collagen, and then to covalently anchor the long chain polyacrylamide polymer networks to inert metal substrates via silanation of the surfaces. The hydrogels demonstrated potential as neural probe coatings with a high conductivity greater than or equal to 0.1 S/cm, modulus of elasticity of 50 kPA, interfacial toughness of approximately 285 J/m^2, and positive cell viability test results. This possible neural probe coating technology could greatly advance the neural science and therapy fields.

Conductive hydrogel | mechanical properties | neural probe coating

Recently, many large-scale initiatives have been aimed at revolutionizing our understanding of the brain. An ongoing goal is to develop neural interface systems that can reveal the interactions of individual cells and entire neural circuits. More specifically many are interested in developing methods to conduct electrical measurements and stimulations of the brain. Conducting electrial measurements and stimulations of the brain will enable clinicians to acquire more information about and also possibly develop treatment procedures for various brain diseases such as dementia, epilepsy, Alzheimers disease, Parkinsons disease, and depression.[1]

The approach to developing neural interface systems has consisted of implanting inert microelectrodes into neural tissue. The main issue with the current approach is that there is a significant mismatch in the modulus of elasticity of conventional metallic or microfabricated electrodes (∼10 GPa) and that of the soft brain tissue (∼100 kPa). The modulus mismatch leads to local scarring (gliosis) at the neural interface. This scarring ultimately creates a nonconductive sheath around the electrode, preventing further electrical communication between the electrode and neurons.[1,2]

To address this problem, scientists have recently been using hydrogels to create a seamless interface between electronics and biological tissues for the purposes of measurement and/or stimulation. This field is promising because hydrogels have a major advantage over existing neural probes; hydrogels can be engineered to be conductive,[3] tough,[4] more elastic,[5] and biocompatible.[6] In short, hydrogels could better match the mechanical and physiological properties of the central nervous system.

As suggested by the works above, recent advances in the production of soft materials including hydrogels to optimize various properties such as conductivity, toughness, elasticity, etc., have been made, but little work exists on the combination and optimization of these processes for the application of long term implanted neural probes. This project aims to engineer hydrogels to be conductive, mechanically robust, and biocompatible so that they can be used for neural

probe coating applications (Figure 1).

Here we present a highly electrically conductive, mechanically robust, and biocompatible tough hydrogel system. This system was integrated with a metallic electrode via a robust interface. To achieve that we infused conductive polyaniline polymer chains into an intrinsically biocompatible polyacrylamide-chitosan hydrogel matrix, coated the gels with collagen, and then covalently anchored the long chain polyacrylamide polymer networks to inert metal substrates via silanation of the surfaces (Figure 2). The Polyaniline Acrylamide-Chitosan (PAni-PAAm-Chitosan) tough hydrogel demonstrated potential as neural probe coatings with a high conductivity of 0.1 S/cm to 0.24 S/cm between 1 kHz to 100 kHz, modulus of elasticity of 50 kPa, interfacial toughness of approximately 285 J/m^2, and positive cell viability test results. This is the first described conducting polymer hydrogel that has exhibited high electrical capabilities in addition to mechanical robustness and biocompatibility.

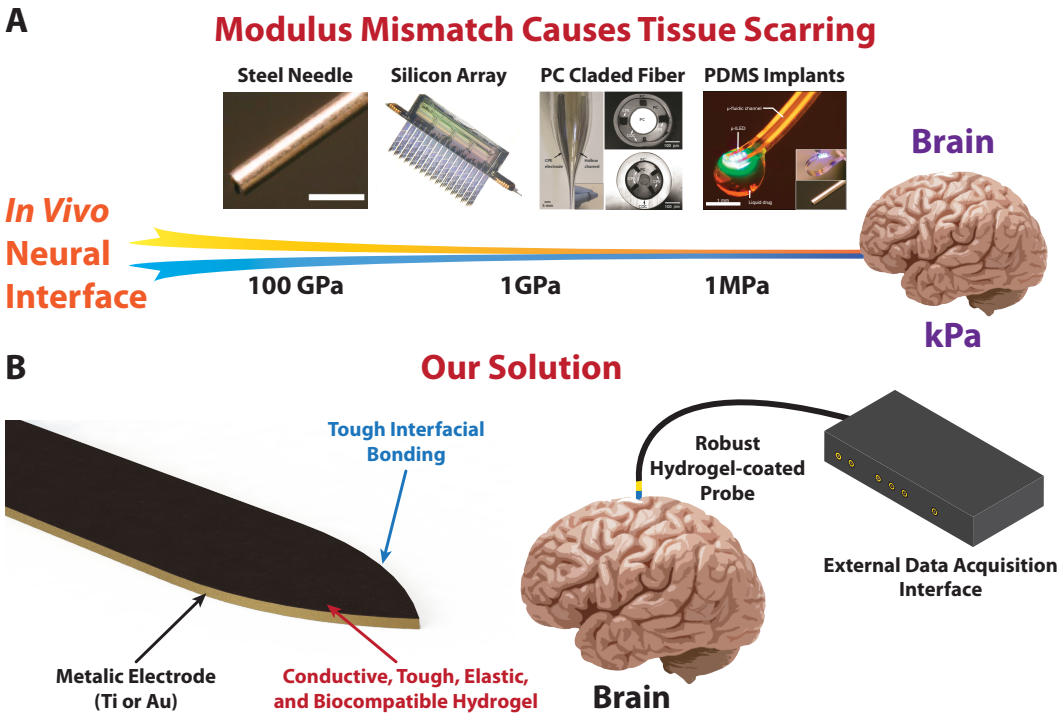

Figure 1: Overview of solution to the modulus mismatch-tissue scarring issue. We developed a highly electrically conductive, mechanically robust, and biocompatible tough hydrogel coating for neural probes. The hydrogel coated neural probes better match the mechanical and physiological properties of the central nervous system and can be used to acquire data from the brain. This will enable clinicians to acquire more information about and also develop treatment procedures for various brain diseases such as dementia, epilepsy, Alzheimers disease, Parkinsons disease, and depression.

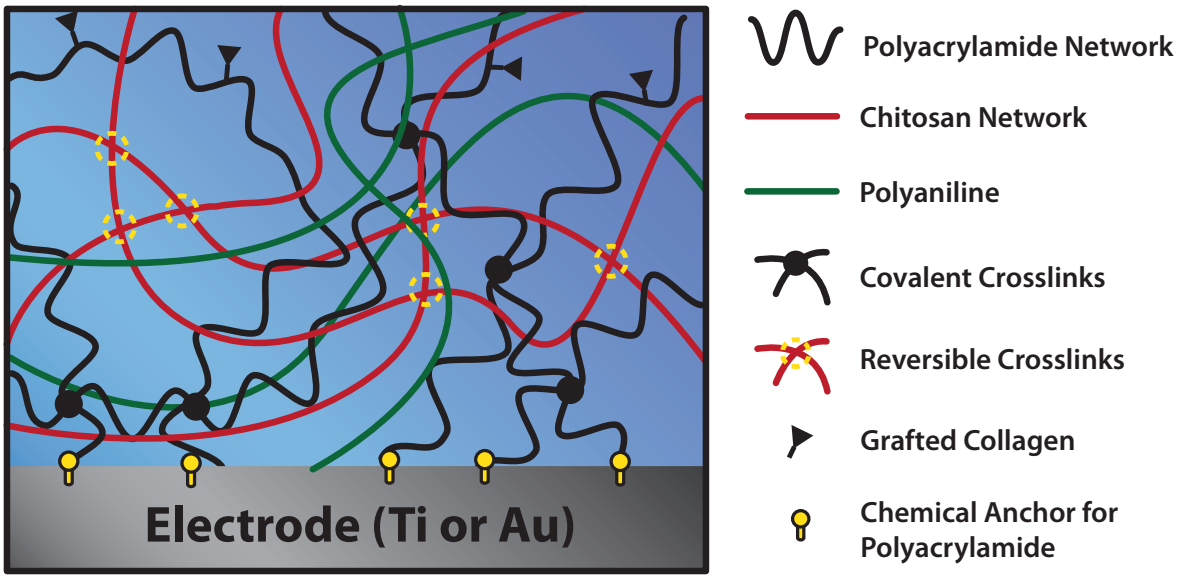

Figure 2: A design strategy for conductive, mechanically robust, and biocompatible hydrogel systems for neural probe coatings. The system consists of conductive polyaniline polymer chains infused into an intrinsically biocompatible polyacrylamide-chitosan hydrogel matrix and coated with collagen. The long chain polyacrylamide polymer networks are covalently anchored to inert metal electrodes via silanation of the surfaces.

Results and Discussion

Electrical Conductivity

To evaluate the electrical conductivity of the PAni-PAAm-Chitosan hydrogels, we performed electrochemical impedance spectroscopy (EIS) (Figure 3). Electrical impedance analysis was used since the impedance of the hydrogels vary with frequency. The PAni-PAAm-Chitosan hydrogels yielded an electrical conductivity of approximately 0.1 S/cm at 1 kHz and 0.24 S/cm at 100 kHz. These conductivity values are equivalent to or greater than the highest values reported previously for a PAAni hydrogel.[3]

Mechanical Properties

To evaluate the mechanical robustness of the PAni-PAAm-Chitosan hydrogels, we performed a series of mechanical tests including tension tests (elasticity) and 90 degree peeling tests (interfacial toughness).

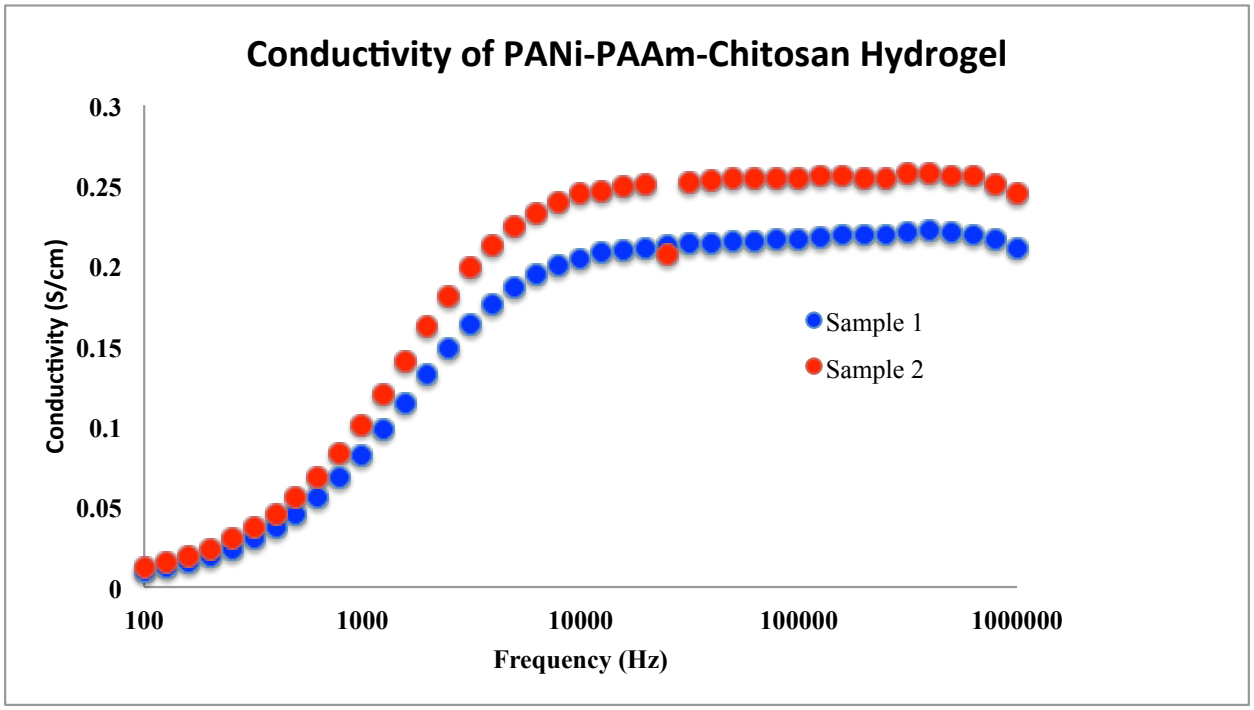

Figure 3: Electrical Conductivity Results. The PAni-PAAm-Chitosan hydrogels yielded an electrical conductivity of approximately 0.1 S/cm at 1 kHz and 0.24 S/cm at 100 kHz. These conductivity values are equivalent to or greater than the highest values reported previously for a PAAni hydrogel.[3]

Elasticity of the PAni-PAAm-Chitosan hydrogels was evaluated using Tension Tests. The PAni-PAAm-Chitosan hydrogels yielded young′s modulus of elasticity of approximately 50 kPa (Figure 4). This modulus of elasticity is orders of magnitude lower than that of current neural probes. This is significant because the rigidity mismatch between glial tissue (∼100 kPa) and that of current metallic neural probes (∼10 GPa) causes significant local glial scarring. The elasticity of the PAni-PAAm-Chitosan hydrogels is significantly closer to that of glial tissue. Hence, by coating the PAAm-Chitosan hydrogels onto metallic neural probes, we have potentially developed a technique to attenuate the occurrence of local scarring at the interface of the neural probe and glial tissue. Locally, cells will no longer detect the rigid nature of the metallic neural probe, but instead will detect the elastic nature of the hydrogel neural coating. Thus the cells will be more likely to proliferate.[2]

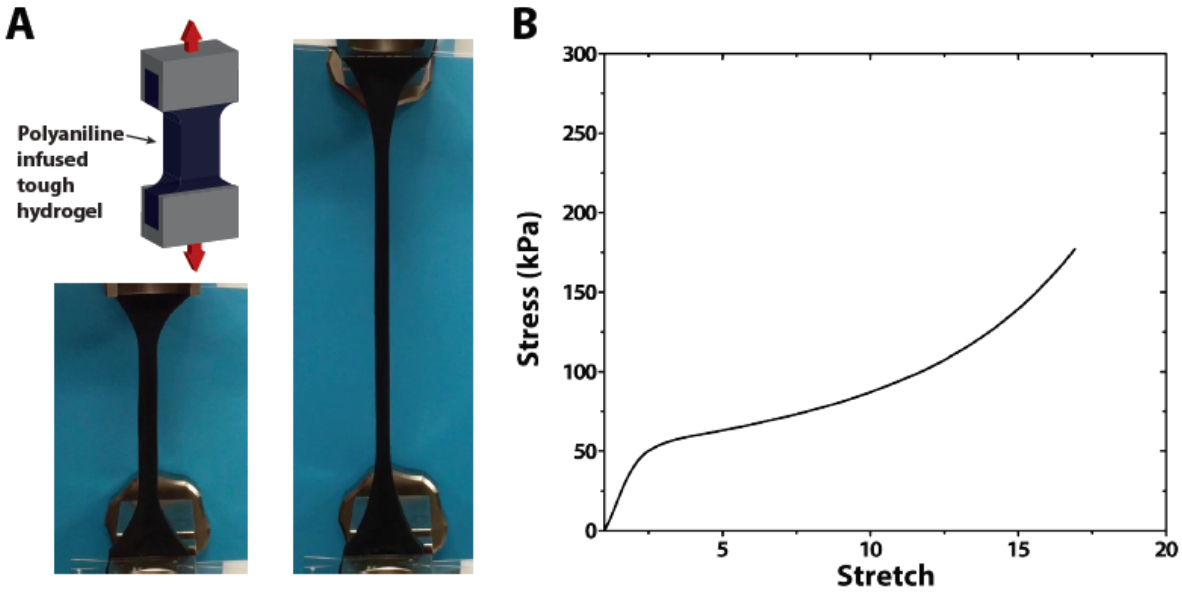

Figure 4: Elasticity Results. Elasticity of the PAni-PAAm-Chitosan hydrogels was evaluated using Tension Tests (A). The PAni-PAAm-Chitosan hydrogels yielded young′s modulus of elasticity of approximately 50 kPa (B).This modulus of elasticity is orders of magnitude lower than that of current neural probes and is significantly closer to that of glial tissue. By coating the PAAm-Chitosan hydrogels onto metallic neural probes, we have developed a technique to attenuate the occurrence of local scarring at the interface of the neural probe and glial tissue.

Interfacial toughness of the PAni-PAAm-Chitosan hydrogels was evaluated using 90 degree peeling tests. The PAni-PAAm-Chitosan hydrogels yielded an interfacial toughness of 285 J/m^2. This interfacial toughness is significantly higher than that obtained by physically anchoring the PAAm chains to the inert metal substrates and is comparable to that obtained by chemically anchoring nonconductive PAAm-Chitosan (without PAAni) tough hydrogels to inert metal substrates[7] (Figure 5). This relatively high interfacial toughness was achieved by chemically anchoring the long polymer PAAm chains of the PAAni-PAAm-Chitosan hydrogels to inert metal substrates (gold or titanium) via silanation of the surfaces. The high interfacial toughness of the PAni-PAAm-Chitosan further supports its proposed performance as an excellent neural probe coating by reducing the chance of coating failure by delamination from the metal neural probe electrode. This design criterion is of great importance for neural probe coating fabrication.

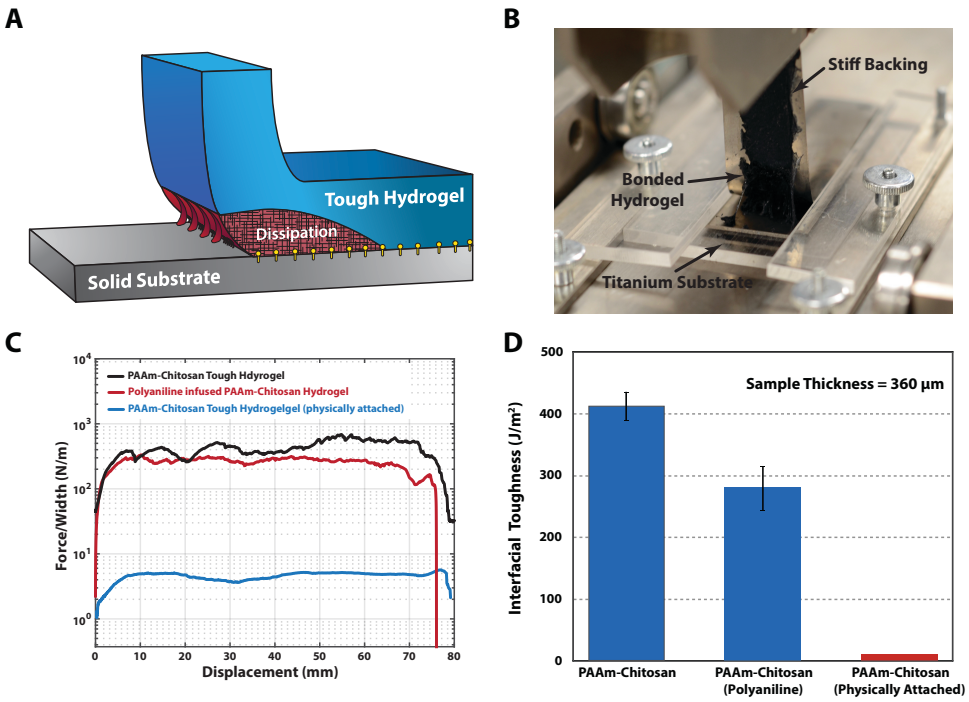

Figure 5: Interfacial toughness Results. Interfacial toughness of the PAni-PAAm-Chitosan hydrogels was evaluated using 90 degree peeling tests (B). The PAni-PAAm-Chitosan hydrogels yielded an interfacial toughness of 285 J/m^2 (D). This interfacial toughness obtained by chemically anchoring the PAAm chains to an inert metal substrate (A) is significantly higher than that obtained by physically anchoring the PAAm chains to the inert metal substrates and is comparable to that obtained by chemically anchoring nonconductive PAAm-Chitosan (without PAAni) tough hydrogels to inert metal substrates (D).[7]

Biocompatibility

Biocompatibility of the PAni-PAAm-Chitosan hydrogels was evaluated using cell viability tests (Figure 6). [Note: *These results are presented for a slightly different hydrogel system conducted earlier in the semester. The results for the PAni-PAAm-Chitosan hydrogels have not been finalized yet.*] Mesenchymal stem cells (MSCs) proliferate and spread well on PAni-PAAM-Chitosan coated gold electrodes. This is likely due to both intrinsically biocompatible nature of the PAAm-Chitosan hydrogel network and the collagen coating on the hydrogel, which has been shown to allow cell attachment and further enhance cell proliferation.[2]

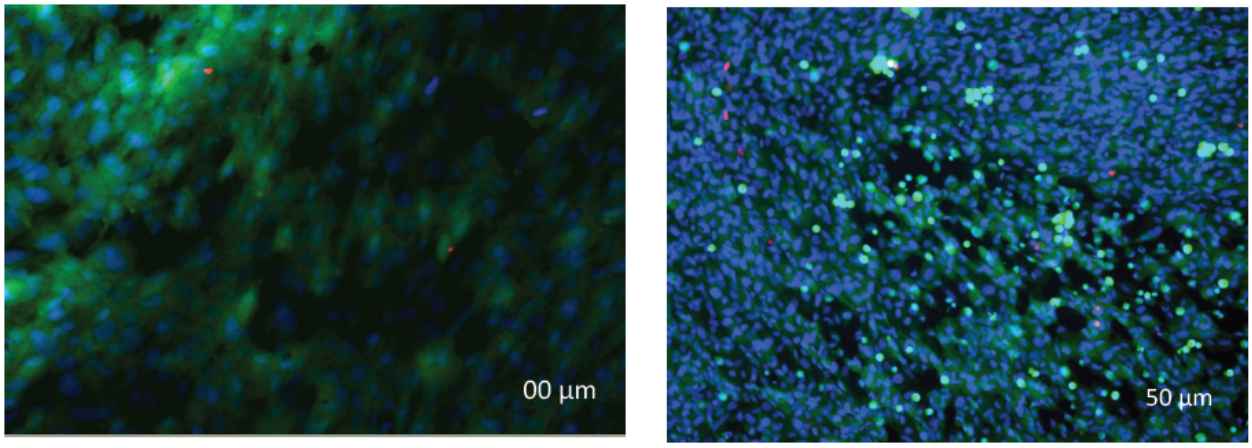

Figure 6: Cell Viability Results. The first image displays the normal growth of Mesenchymal stem cells (MSCs). The second image depicts the live-dead assay of MSCs on PAni-PAAM-Chitosan coated gold electrodes. Blue represents the nuclei of live cells. Green represents the cytoplasm of live cells, and red represents dead cells. MSCs proliferate and spread well on PAni-PAAM-Chitosan coated gold electrodes.

Conclusion

In summary, we demonstrate that the infusion of the conductive polymer PAAni into PAAm-Chitosan tough hydrogels followed by the coating of collagen on the hydrogels and the chemical anchorage of the long chain PAAm polymer networks onto titanium or gold surfaces is a promising strategy to design conductive, elastic, tough, and biocompatible hydrogels for neural probe coating applications. Following the design strategy, we use simple methods to fabricate conductive, elastic, tough, and biocompatible hydrogels with conductivity greater than or equal to 0.1 S/cm, elasticity of approximately 50 kPa, and interfacial toughness of 285 J/m^2. [Note: *Results for fracture toughness have not been finalized yet, but we expect them to be relatively high since the technique for developing tough hydrogels has been effective as reported in previous works by this research group.*] These hydrogels are promising because when coated on electrodes, they can improve the biocompatibility of rigid metallic neural electrodes, while retaining their functionality. Essentially, these hydrogels could create a seamless interface between electronics and biological tissues by better matching the mechanical and physiological properties of neural cells and tissues in the brain and spinal cord. This novel technology could completely transform the way medical implantation problems are solved and greatly advance the neural science and therapy field. We expect that these results will lead to exciting research into the next generation of conductive hydrogel neural probe coatings.

Materials and Methods

All chemical materials were purchased from Sigma Aldrich.

Synthesis of PAni-PAAm Chitosan tough hydrogels

We synthesized the PAAm-chitosan tough hydrogel following by mixing 10 ml of degassed precursor solution (24 wt % AAm, 2 wt% chitosan, 1 wt% acetic acid, 0.034 wt% MBAA and 0.084 wt% APS) with 60 µl of TPP solution (0.05 M) and TEMED (0.0025 times the weight of AAm). The PAAm-chitosan tough hydrogel was synthesized by first soaking the gel in 10 mL of DI water, 0.2 wt% HCl and 7 wt% aniline for 30 minutes. We then made the hydrogels conductive by infusing the hydrogels in conductive aniline. The gel was soaked in 10 mL of DI water, 0.2 wt% HCl, 8 wt% APS for 30 minutes. The two-step soaking procedure detailed above was repeated so that the gel was soaked in both solutions twice (double infusion). Then the conductive PAni-PAAm gel was extracted and soaked in DI water to remove any unreacted chemicals.

Chemically Anchoring PAAm on Ti and Au Substrates

We introduced a tough interface between PAAm and Ti or Au following previously reported methods.[7]

Electrical Impedance Spectroscopy Measurements
Electrical Impedance of the PAni-PAAm-Chitosan tough hydrogels was measured using an electrical impedance analyzer (Solartron 1287A) following standard procedures.[8] Electrical impedance measurements were taken over the range 1 kHz to 100 kHz because this is the typical range used to report conductivity of coatings for neural probe applications.

Interfacial Toughness Measurements
We measured the interfacial toughness of the PAni hydrogel on titanium substrates using 90 degree peeling tests following previously reported methods.[7]

Biocompatibility Assessments
We investigated the biocompatibility of the hydrogels following previously reported procedures.[7]

Acknowledgments

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