Durability of photografted zwitterionic hydrogel coatings for reduction of the foreign body response to cochlear implants

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Abstract

Objective: Durability of photografted zwitterionic hydrogel coatings on cochlear implants was examined to determine viability of these anti-fouling surfaces during insertion and long-term implant usage. Approach: Tribometry was used to determine the effect of zwitterionic coatings on lubricity of surfaces, varying hydration level, applied normal force, and timeframe. Additionally, flexural resistance was investigated using mandrel bending. *Ex vivo* durability was assessed by determination of friction between tissues and treated surfaces, as well as cochlear implantation force measurements using cadaveric human cochleae. Main results: Hydrated zwitterionic hydrogel coatings maintained decreased friction for 20 hours under normal force. For loosely crosslinked systems, films remained stable and retained lubricity after complete drying. All films were able to remain hydrated and functional for at least 30 minutes under normal force, with lower crosslink densities showing the greatest longevity. Under flexural force, zwitterionic films underwent dehydration for up to 60 minutes before failure. Furthermore, photografted zwitterionic hydrogel samples showed nearly identical lubricity before and after implantation. Importantly, zwitterion-coated cochlear implants experienced a significantly lower mean force during insertion than uncoated implants. Significance: This work demonstrates that photografted zwitterionic hydrogel coatings are sufficiently durable to maintain viability before, during, and after implantation. Mechanical properties, including greatly increased lubricity, are preserved after complete drying and rehydration for various applied forces. Additionally, the results show retention of up to 90% increase in lubricity relative to uncoated samples which translates to decreased force and overall less trauma during insertion of implants.

Keywords: photografted coating, zwitterion, antifouling, lubricity, sensorineural hearing loss

1. Introduction

Medical implants have advanced significantly in recent years, expanding capability and function.[1] In particular, cochlear implants (CIs) have increasingly been used in restoring hearing to many who suffer from different degrees of sensorineural hearing loss. For example, recent advancements in hybrid CIs have allowed significant restoration of higher frequency hearing while preserving residual lower frequency hearing.[2, 3] While CIs and other neural implants provide beneficial and restorative functions, the response of the body over time may impede function and induce deleterious effects. When a CI is inserted, non-specific proteins adsorb to the surface which then recruit other proteins and cells, such as macrophages.[4] Recent work showed that over 95% of explanted cochlea from CI patients contained significant quantities of foreign body giant cells.[5] In some cases, cellular digestion of the foreign object has been demonstrated by phagocytosed bits of titanium and plastic throughout the body.[6] However, if the cells cannot digest the foreign object, a fibrous capsule is formed around the implant. This dense tissue development around a CI can significantly impede the electrical signal from reaching the target neural cells, which can dramatically reduce the hearing quality.[7-9] Additionally, the tissue buildup and resultant scarring may even cause damage that leads to partial or complete loss of residual low-frequency hearing for those with hybrid implants.

Various solutions have been proposed to negate the foreign body response, thus enhancing the lifetime and efficacy of numerous medical implants. Most commonly, some modification is made to the surface to make the implant more similar to native tissue, leading to a reduction in the fibrotic response. For example, polyethylene glycol (PEG) is often used as a coating for biomaterials. The PEG molecules readily interact with water and render the surface more inert by recruitment of water by limiting binding sites for biomolecules .[10] While PEG-derivatives are

antifouling to an extent, these coatings often do not sufficiently inhibit fouling to prevent deleterious long term fibrosis effects.[11, 12] Zwitterions have more recently been explored as a means to negate the foreign body response.[13, 14] The Jiang group has shown the high propensity of zwitterionic polymers to swell and strongly bind surface water, leading to a large decrease in protein and cellular response.[15]

Due to excellent water binding resulting in minimal biofouling, zwitterionic systems have gained significant interest for antifouling applications. The positive and negative charges of the zwitterions strongly bind water, which readily repels proteins and cells from attaching to the material surface. Though all use of zwitterions for antifouling purposes relies on the same repelling mechanism, methods of use have been widely varied in how zwitterionic monomers are combined and attached to the material surface. Most typically, zwitterions are applied to a material surface as a brush polymer which provides for easy attachment. However, the brush polymers lack durability to remain as a viable coating for high-abrasion implant situations such as CIs. Crosslinker can be added to zwitterionic systems to form a network which swells and becomes a more stable hydrogel.[16-18] Hydrogels are relatively weak though and may not be sufficiently durable to be used as a coating for implants made out of much more durable materials, such as metals and stiffer polymers. [19, 20] In addition to requiring stability of coating itself, the attachment must be sufficient to withstand the insertion process and remain for the lifetime of the implant.[21] Some zwitterionic coatings are hydrogels formed using the graft-to method: creating a hydrogel and then subsequently attaching to the material surface. While the use of a hydrogel instead of a brush polymer increases stability and leads to greater antifouling, the process is less efficient and also may compromise either the coating or the attachment due to the two-step process. To use a zwitterionic hydrogel coating for antifouling of implants such as

CIs, stability must be sufficient to withstand mechanical deformation or detachment during the coating process, while stored in physiologic solutions, throughout the insertion, or long-term within the body.

Previous work demonstrated that zwitterionic thin films could successfully coated to biomaterials by simultaneous photografting and bulk photopolymerization, using Type II (benzophenone) and Type I (Irgacure) photoinitiators, respectively.[22] Grafting results in stronger adhesion generally,[23] and photografting allows for rapid and spatially-controlable covalent attachment to the sample surface.[24] By including a crosslinker in the system, a hydrogel is formed, along with photografting to the biomaterial surface, which significantly decreases biofouling.[22] However, a trade off in mechanical integrity with antifouling capability as a function of crosslink density was observed for the photografted zwitterionic hydrogel thin film system.[16] Based upon previous findings, these experiments focus on the application of zwitterionic thin films to CIs for antifouling and lubricity. CIs are a unique geometry and undergo bending during implantation,[25] requiring that coatings be robust and well attached. Because the portion of the cochlear implant which is inserted into the cochlea is largely housed in PDMS, experiments will be largely be performed on PDMS samples. This work demonstrates the durability of photografted zwitterionic hydrogel films as they are exposed to different extreme conditions including dessication in ambient conditions, increasing normal forces, or bending around smaller mandrels. *In vivo* testing shows the translatability to the application for CIs implanted into the body. By understanding the limits of the zwitterionic thin films coatings, implants can successfully be coated and kept in conditions such that the durability will remain sufficient during both implantation and the functional lifetime of the implant.

2. Materials & Methods

2.1 Monomer Solutions

Irgacure 2959 (2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone, Sigma Aldrich) was dissolved into deionized water to obtain 0.0769 wt% solution. The two zwitterionic monomers examined (structures shown in Figure 1) were sulfobetaine methacrylate (SBMA, 2-Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide, Sigma Aldrich) and carboxybetaine methacrylate (CBMA, 3-[[2-(methacryloyloxy)ethyl]-dimethylammonio]propionate, TCI Chemicals). Poly(ethylene glycol) methacrylate (PEGMA, MW 400, Polysciences) and 2-hydroxyethyl methacrylate (HEMA, Sigma Aldrich) were used as non-zwitterionic monomer controls. Poly(ethylene glycol) dimethacrylate (PEGDMA, MW 400, Polysciences) was used as the crosslinker (structure shown in Figure 1C) for all monomer systems. Monomer and crosslinker were added at various ratios as 35% of the total prepolymer solution, with the remaining 65% composed of the water/Irgacure mixture. Monomer solutions and resultant hydrogel films are identified what percent of the total monomer is difunctional (crosslinker).

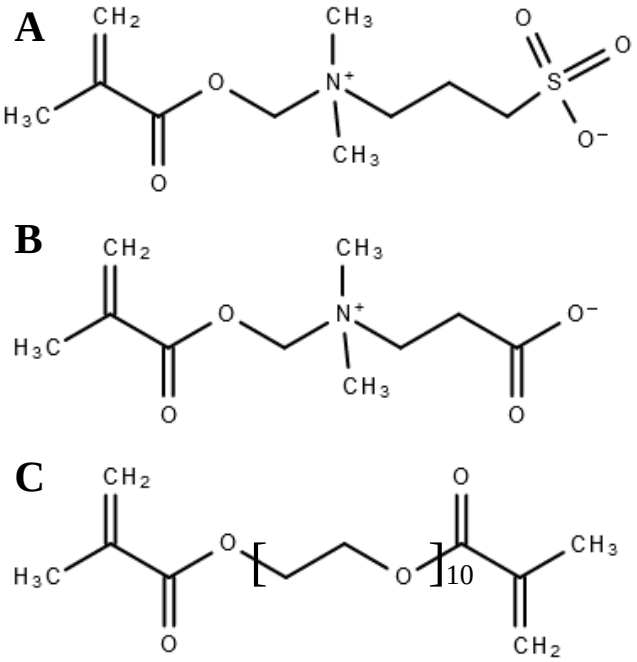

Figure 1. Chemical structures of A) sulfobetaine methacrylate (SBMA) B) carboxybetaine methacrylate (CMBA) and C) poly(ethylene glycol) dimethacrylate (PEGDMA).

2.2 Coated Samples

Reinforced medical grade PDMS (Bentec Medical) was cut into disks (2.54 mm thick with 25

mm diameter or 0.016 mm thick with12 mm diameter) or rectangles (35x75x1.016 mm). PDMS samples were soaked in a 50 g benzophenone (Sigma Aldrich)/L acetone solution for one hour. The PDMS samples were removed from the solution and vacuum dried for at least 20 minutes to evaporate any residual acetone. Prepolymer solution (20 µL for 25mm diameter disks, 5 µL for 12mm diameter disks, 200 µL for rectangles) was pipetted onto the benzophenone-treated PDMS and dispersed with glass coverslips (Fisher Scientific). The solution was then polymerized using an Omnicure S1500 lamp at 30 mW/cm^2 for 10 minutes using the full spectrum of UV light (300-520 nm) to simultaneously photograft and form the hydrogel coating. Coated PDMS samples were placed in Dulbecco's phosphate buffered saline solution (PBS, gibco) for at least 24 hours to allow hydrogel coatings to swell to equilibrium.

2.3 Tribometry

Disk samples were evaluated for lubricity using a pin-on-disk tribometer (TRB^3, Anton Paar) as previously described.[26] Typically, each sample was examined for 20 cycles (~8 minutes) at a rotational speed of 1.3 mm/s while using the tribometer liquid setting with PBS as the immersive solution, 1 Newton normal force, and a sapphire probe (representative curve in Supplemental Figure 1A). To determine longevity of samples, tribometer data were collected over 2500 cycles (~1000 minutes) determining the coefficient of friction for coated and uncoated samples, then normalizing coated samples to the average uncoated value. To examine varying force, the normal force applied for each run was varied between 1, 3, 5, 10, and 15 Newtons. Certain samples were initially swollen to equilibrium followed by drying in ambient conditions for 24 hours and 10 minutes under vacuum to complete desiccation. The samples were then rehydrated in PBS over 24 hours prior to testing. Samples were tested with the tribometer and compared to control samples which were only initially swollen (no drying allowed). Further, to ascertain the

effects during drying on the coefficient of friction, coated PDMS samples were tested without additional PBS added to the tribometer sample holder—only the PBS initially in the coating from equilibrium swelling was present for testing. The tribometer exerted normal force and collected coefficient of friction measurements for 90 minutes or until the coefficient of friction reached an approximate asymptotic maximum value (representative curve shown in Supplemental Figure 1B). Data were analyzed by determining the range from beginning coefficient of friction to maximum, then calculating T10 and T90 values where T10 is the time when the sample increases to 10% of the range for the sample and T90 the time to reach 90%.

2.3.1 Tribometry ex vivo *and* in vivo

Tissues were harvested from guinea pigs following approved methods by the University of Iowa Institute of Animal Care and Use Committee (IACUC #9092245). Selected tissues were cut into 1x1 cm pieces. These samples were then used to cover the steel probe head prior to tribometry as illustrated previously.[26] Coefficient of friction was measured between the tissues and coated- or uncoated-PDMS surfaces. For an additional control, the coefficient of friction for dermis on dermis was measured after fixing dermis tissue to PDMS using tissue glue. Coefficient of friction for uncoated PDMS with each tissue was also measured. Smaller diameter (12 mm) disks were implanted subcutaneously in 10-week old CBA/J mice for three weeks, following protocols approved by the University of Iowa Institutional Animal Care and Use Committee. After removal from the mice, lubricity of the disks was determined. For comparison, pristine samples identical to those implanted but simply stored in PBS for the duration of implantation were also examined.

2.4 Mandrel bend

Flexural failure of coated rectangular samples was examined using a mandrel bending

apparatus.[27, 28] Samples containing monofunctional monomer (not 100% crosslinker) withstood all bending diameters (2-32 mm) when fully hydrated. To ascertain the effect of dehydration, samples were subjected to ambient conditions and tested on the 5mm-diameter mandrel every five minutes. The time to failure was noted when cracking was observed.

2.5 Viability for in vivo *implantation*

To examine *in vivo* durability, coated and uncoated PDMS samples were implanted subcutaneously in mice for six months or 16 days and then explanted. For six-month implants, coatings were imaged using scanning electron microscopy. For 16-day samples, representative images were collected using confocal microscope. Uncoated PDMS, both implanted and non-implanted, was included as a negative control.

2.6 Insertion Force of Cochlear Implants

SBMA thin films were coated on cochlear implants using a previously published method.[26] In brief, cochlear implants were pretreated for one hour in an acetone solution with 50 g/L benzophenone. After removal from the solvent and drying under vacuum, the implants were inserted into rigid cylindrical sleeves of transparent PDMS (inner diameter 0.76 mm) filled with 10% crosslinker CBMA monomer solution. This system was exposed to UV light at 30 mW/cm^2 for five minutes in an oxygen-free environment to form photografted SBMA coatings. The sleeves were removed without disrupting the nascent hydrogel, followed by a 10 minute UV post cure to ensure complete polymerization. To enable the best dispersion of the hydrophilic monomer solution over the hydrophobic PDMS surface, 0.8wt% surfactant (dimethylsiloxane-acetoxy terminated ethylene oxide block copolymer, 75% nonsiloxane, Gelest, Inc.) was added to the monomer solution. Coated and uncoated cochlear implants were inserted into human

cadaveric cochleae as previously described.[26] The cochleae were mounted on a force transducer to assess the increase in force associated with an insertion run. The raw output was reported over time for each implant.

3. Results

3.1 Coefficient of friction as a function of crosslink density and runtime

In addition to considering the durability required for hydrogel coatings to remain viable before and during implantation, the durability once the cochlear implant is placed must be considered. One consideration is the length of time that the implant will remain in the body. This time can vary widely depending on the intended use and person to person. To determine the effects over time on zwitterionic hydrogel coatings, the coefficient of friction was measured for SBMA coated PDMS using tribometry over 2500 cycles (equating to nearly 17 hours of time under force) as a function of crosslink density. The average coefficient of friction for the first and last hundred cycles can be compared to show what difference the time makes, seen in Figure 2. For all samples with any amount of zwitterion, the relative coefficient of friction was not statistically significantly different between the first and last 100 cycles. For low crosslink density samples, the two values were almost identical. At higher crosslink densities, the coefficient of friction

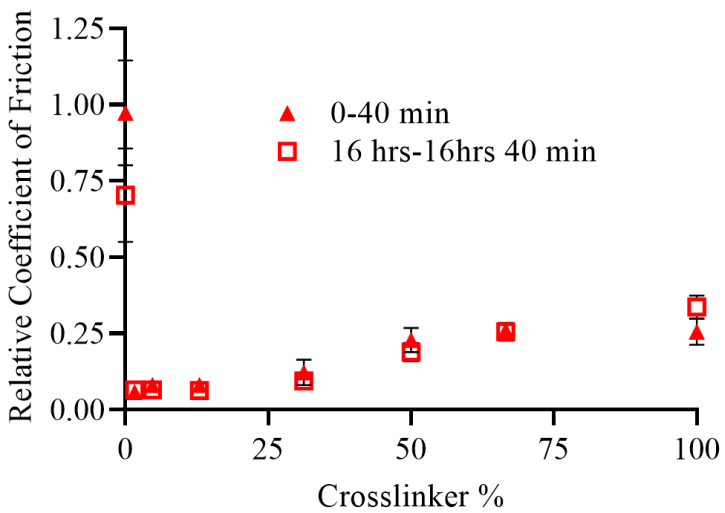

Figure 2. The average coefficient of friction (relative to uncoated PDMS) for the first and last 100 cycles of a 2500 cycles test of SBMA coated PDMS as a function of crosslinker percent. The cycle time equates to a total testing period of 1000 minutes, with sampling occurring over the first and last 40 minutes.

appears to decrease over time, which is likely resulting from the tendency of the tribometer probe to become coated in the hydrogel during testing. On the other hand, 100% PEGDMA samples did see a significant increase in the coefficient of friction over the time tested. Additionally, SBMA samples were tested at increased speed from the standard 1 mm/s to 6 mm/s, which is faster than maximum observed cochlear implantation rate.[29] For all crosslink densities, no significant difference was noted in coefficient of friction values between the two speeds (Supplemental Figure 2).

3.2 Coefficient of friction given different applied normal forces

An additional concern that could impact durability of coatings, both during insertion and while in the body, is varied normal forces to which the implant may be subjected. To understand the effect on the hydrogel coatings when an increased normal force is applied, further tribometry measurements were gathered using increasingly greater weights in the setup. SBMA hydrogels with 5% crosslinker were chosen as the representative hydrogel coating because previous work showed this to be a crosslink density with good compromise between biological efficacy and mechanical properties.[16] The data, displayed in Figure 3, demonstrated that there was nearly a linear increase in the observed coefficient of friction until the 10-Newton weight was reached. However, the 5% coatings still maintained a coefficient of friction much lower than uncoated PDMS (0.08<<0.19). Qualitatively, the hydrogels were observed following

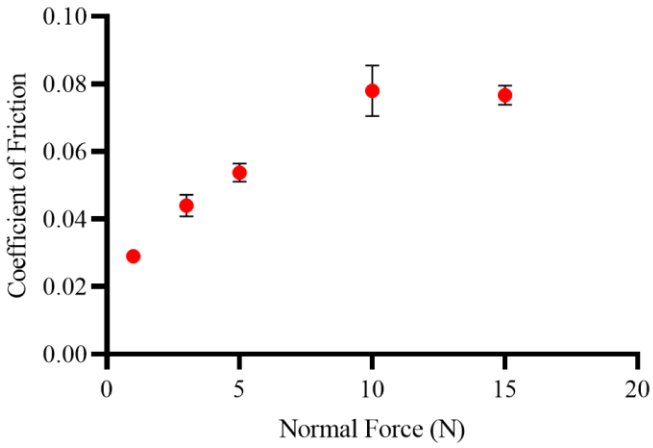

Figure 3. Coefficient of friction for SBMA hydrogels (5% crosslinker) with increasing normal force applied. Uncoated PDMS value for normal force 1N = 0.19.

measurement for any noticeable defects. At low normal force, no difference was noted. But for the higher forces, the wear track became visible after testing.

3.3 Comparison of lubricity before and after complete drying

Because coatings could become dry in the process of implantation, packaging, or at other times prior to use, the changes due to drying were investigated. The coefficient of friction was compared for SBMA hydrogel coatings before and after complete drying occurred (with all samples tested swollen to equilibrium just prior to testing) as shown in Figure 4. At low crosslink densities, the coefficient of friction measured was nearly identical whether the coating was allowed to dry or not. However, as the crosslinker percent increases, the disparity between the rehydrated sample and the sample only swollen once begins to increase. At 50% crosslinker (and similarly for 67%), the rehydrated sample exhibits a coefficient of friction value similar to that of uncoated PDMS. Qualitatively, the coating was visibly flaky when dried and largely came off when the sample was rehydrated in PBS, suggesting that the rehydrated sample had actually lost the coating and was simply then uncoated PDMS. While the 100% crosslinker sample did exhibit a slight increase in friction following drying, the change is not as large. This discrepancy to the pattern likely can be attributed to lower initial swelling due to the absence of the zwitterionic moiety, so the complete drying does not lead to as great of a breakdown in the coating induce as great of an

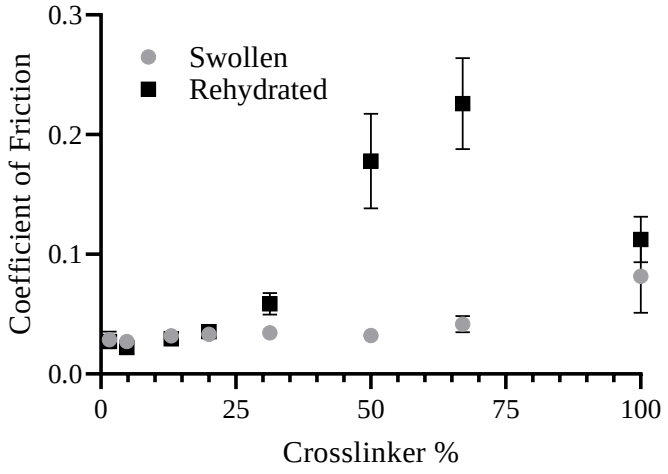

Figure 4. Coefficient of friction for SBMA hydrogels as function of crosslinker percent when swollen immediately after polymerization and following complete drying.

overall change. Samples with no crosslinker were also tested; however, these samples displayed a high coefficient of friction after initial swelling and no significant change was noted after drying.

3.4 Stability of coatings with applied normal force as drying occurs

For hydrogel coatings to be successfully used on biological implants, they need to be sufficiently durable to retain desired properties and remain intact for insertion and implant lifetime. Because the properties of hydrogels are largely determined by water content, loss of water will lead to significant changes in the hydrogels, which could interfere with effective implantation and function. As such, the lubricity of zwitterionic hydrogel coatings was investigated as a function of drying time and crosslinker concentration. Specifically, the coefficient of friction was measured for hydrogel-coated PDMS samples using tribometry. The hydrogels were swollen to equilibrium in PBS prior to testing and then exposed to air, allowing the system to lose water, while measuring friction. To quantitatively compare when hydrogel coatings lose lubricity and fail, the T10 and T90 (times at which the coefficient of friction has increased 10% and 90%, respectively, from the minimum of the observed range) were determined. T10 value is indicative of when the coating first begins to dry/fail, whereas T90 demonstrates failure has occurred. Both values indicate the stability of the film and give markers of how long a coating can remain non-submersed and remain viable, thus higher values are desirable. T10 provides an idea of how long a film can be exposed and maintain lubricity, for example how long a coated CI can be removed from solution prior to implantation and still provide the lubricious benefits. T90, on the other hand, indicates when the coating itself becomes changed and represents the maximum time a coating should be exposed to air to maintain the coating integrity.

Because CBMA and SBMA bind water differently, both zwitterionic monomers were tested to determine differences in the behavior of hydrogels as deswelling occurs. CBMA coatings at the lowest crosslink densities (Figure 5A) retained some lubricity up to 50 minutes, shown by T90 times, whereas the higher crosslinked films lost lubricity and failed around 30 minutes. The T10 times, indicating when lubricity just begins decreasing and failure is occurring, followed the same trend of decreasing time with greater crosslink density: the least crosslinked films only began to fail around 40 minutes and more crosslinked films began failing as early as 20 minutes. On the other hand, SBMA coatings (Figure 5B) required up to 60 minutes to reach failure at the low crosslink densities, with the similar trend of decreasing retention time of lubricity as

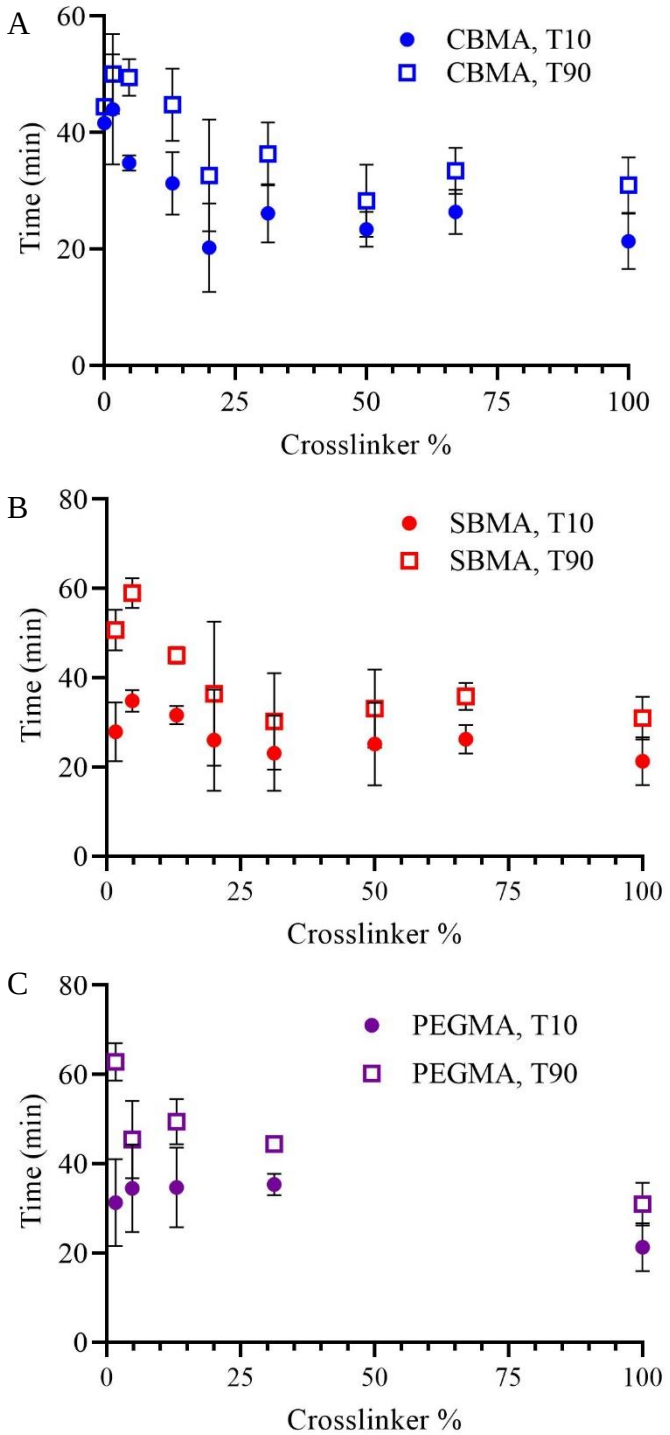

Figure 5. The time for A) CBMA, B) SBMA, and C) PEGMA coatings to reach 10% (T10) and 90% (T90) of maximum coefficient of friction value over a range of crosslink densities. Hydrogels were swollen to equilibrium in PBS prior to testing but coefficient of friction was measured without additional PBS.

crosslinker percent increases. While SBMA films resisted total failure (T90 times) for longer than CBMA, T10 times were comparable between the two zwitterionic thin films with CBMA slightly lasting longer at low crosslink densities. Overall, both zwitterions demonstrate the capability to retain hydration for significant amounts of time. PEGMA, a non-zwitterionic hydrogel, was also dried during tribometry for comparison. PEGMA (Figure 5C) exhibited durability up to approximately 60 minutes at the lowest crosslink densities; PEGMA did not exhibit a clear trend relative to crosslink density. While the times for PEGMA are higher as T10 and T90 times, the actual coefficient of friction on PEGMA-coated samples also starts higher (for example, the initial coefficient of friction for PEGMA was 0.058 compared to 0.041 and 0.047 for CBMA and SBMA, respectively).

3.5 Resistance to failure when exposed to drying and flexural bending

In addition to the measurement of frictional force, the hydrogels were examined for robustness when subjected to bending forces. Certain implants, such as cochlear implants, do not have a direct insertion path and will undergo bending during the process. Insertion will, however, be made smoother by the increased lubricity of zwitterionic hydrogels. The coatings must remain attached and withstand the forces (bending and normal) during insertion to maintain lubricity and the antifouling effects desired once the implant is in the body. To assess the ability of hydrogel coatings to remain viable after bending, a modified mandrel bend test was performed. For all hydrogel coatings except 100% crosslinker (PEGDMA), the hydrated coatings remained unaffected initially when bent around cylinders varying in diameter from 2 to 32 mm. Coatings were left exposed to ambient air to dry and bent around the 5-mm cylinder every five minutes to determine time until failure as the coatings progressed from swollen to dry. Both CBMA and SBMA again displayed high levels of water retention, suggesting robust hydrogel films,

especially at lower crosslink densities (Figure 6). At five percent crosslinker, CBMA and SBMA did not exhibit signs of failure until 75 and 50 minutes, respectively. CBMA follows a nearly linear trend relative to crosslinker percent for time to failure with films remaining viable for up to half an hour even with over 60% crosslinker. These results are quite promising to show the durability of the zwitterionic hydrogel coatings, even when allowed to both dry and experience bending forces. Whereas when only subjected to the light normal force (Figure 5) CBMA, SBMA, and PEGMA did not appear statistically different, CBMA and SBMA displayed much greater durability compared to PEGMA for bending force. The PEGMA samples failed at much earlier times when subjected to bending (Figure 6). The brittleness of the PEGMA hydrogel coatings was noted while performing the mandrel bend protocol much sooner than for either SBMA or CBMA. The crosslinker (PEGDMA) as a hydrogel alone was so brittle it failed upon initial bending.

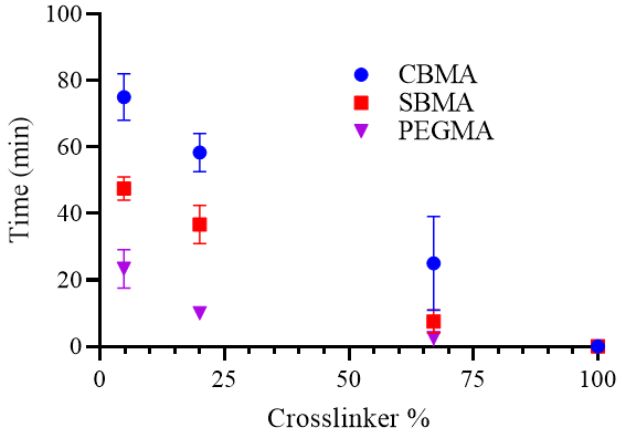

Figure 6. Time until failure was first noted for hydrogel coatings when subjected to 5mm diameter mandrel bend test for a range of crosslink densities. Hydrogel coatings were swollen to equilibrium and then exposed to air (allowed to dry) starting at time 0.

3.6 Lubricity of coatings relative to guinea pig tissue

The previous coefficient of friction measurements all occurred between the sapphire probe and the hydrogel coatings, purely characterizing the lubricity of the hydrogel surfaces. In order to provide a better understanding of how increased lubricity translates to biological settings, tribometry was performed where guinea pig tissue was used to cover the probe prior to testing. This setup provides a more directly translatable perspective on how hydrogel coatings improve

the lubricity between implants and surrounding tissue, especially for implantation. A variety of guinea pig tissues were tested against a 5% crosslinker SBMA coating, all showing at least a 90% reduction in friction relative to uncoated PDMS (Figure 7A). Dermis and trachea tissues exhibited the highest coefficient of friction values, which were similar to the value for steel (with no tissue covering) against the zwitterionic hydrogel coating. Different coatings were then further explored using dermis as the representative tissue, both for ease of use and because it showed the highest coefficient of friction of the tissues tested, suggesting that the other tissues will experience similar reduction in friction. SBMA coatings with a variety of crosslink densities were tested with the dermis tissue (Figure 7B). A slight increase in coefficient of friction was noted as the crosslink percent increased, similar to the trend that can be observed in Figures 1 and 3, as well as previous work with the zwitterionic hydrogel system.[16] At 5% crosslinker, CBMA and PEGMA coatings also showed a similar reduction in friction (~95%) to SBMA relative to dermis tissue.

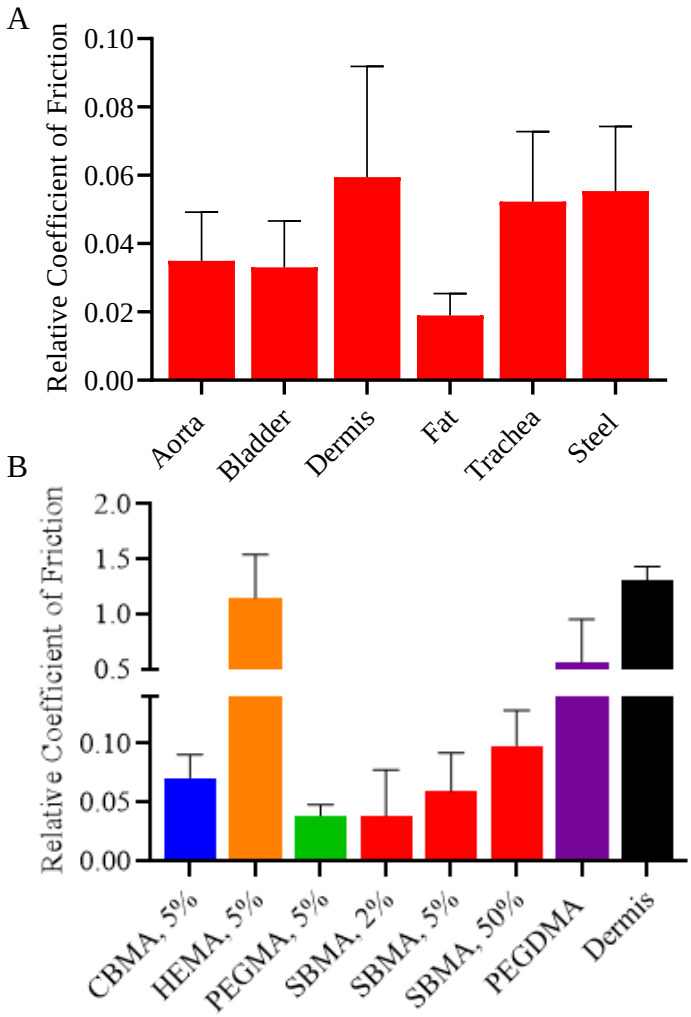

Figure 7. Coefficient of friction relative to uncoated PDMS for A) 5% SBMA hydrogel coated PDMS against various guinea pig tissues or non-tissue covered steel probe and B) different hydrogel coatings (percents are crosslinker %) or dermis itself against the dermis covered probe. Uncoated PDMS value for dermis-covered probe 0.301.

Conversely, HEMA and PEGDMA (pure crosslinker for polymerizable units) showed much higher friction, with PEGDMA reducing friction only about 50% and HEMA demonstrating similar friction value to uncoated PDMS. An additional interesting point is that when dermis was tested against itself, the coefficient of friction was close to that of dermis with uncoated PDMS showing that the zwitterionic hydrogels increase the lubricity beyond what might be naturally occurring in the body.

3.7 Maintenance of lubricity following implantation in mice

Coated and non-coated PDMS samples were implanted subcutaneously into mice to verify that the lubricity imparted by zwitterionic hydrogel coatings would be maintained after some time in the *in vivo* environment. Following removal of samples from the mice, coefficient of friction was determined for both samples stored in PBS (pristine) and those placed in mice (explanted), all samples having been generated on the same day and subsequently tested on the same later date. A non-significant increase in friction was observed from the pristine to explanted samples for uncoated and SBMA samples (Figure 8). However, the reduction in friction for either CBMA- or SBMA-coated samples, whether pristine or explanted, relative to uncoated PDMS remains the same (~90%) showing that the lubricity imparted by zwitterionic hydrogel coatings remains effective following implantation and time in the biological environment.

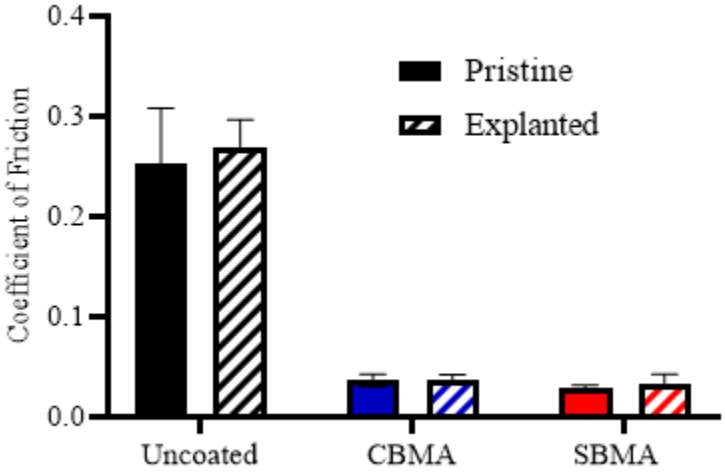

Figure 8. Coefficient of friction of uncoated, CBMA-coated, and SBMA-coated PDMS f or pristine samples and samples explanted from mice.

3.8 Durability of thin film over in vivo *incubation*

For a hydrogel coating to remain robust and effective *in vivo*, acute shearing during insertion or gradual degradation need to be minimized. To determine the durability of the photografted thin film hydrogels, various crosslink density zwitterionic hydrogel coated, as well as uncoated PDMS sample were subcutaneously implanted, incubated, removed, and imaged. As shown in Figure 9, long-term incubation of six months was performed as a function of crosslink density. Each film remained intact and is clearly visible on scanning electron microscopy (Figure 9B-E). The film of lowest crosslinker, 1.6%, appears to have an irregular surface. However, the films of 5-31% crosslinker clearly display delineated films of sharp geometry. Using another method of imaging for a short implantation (Figure 9F-G), thin films hydrogels of 10% crosslinker were found to retain their mechanical integrity and hydrogel character.

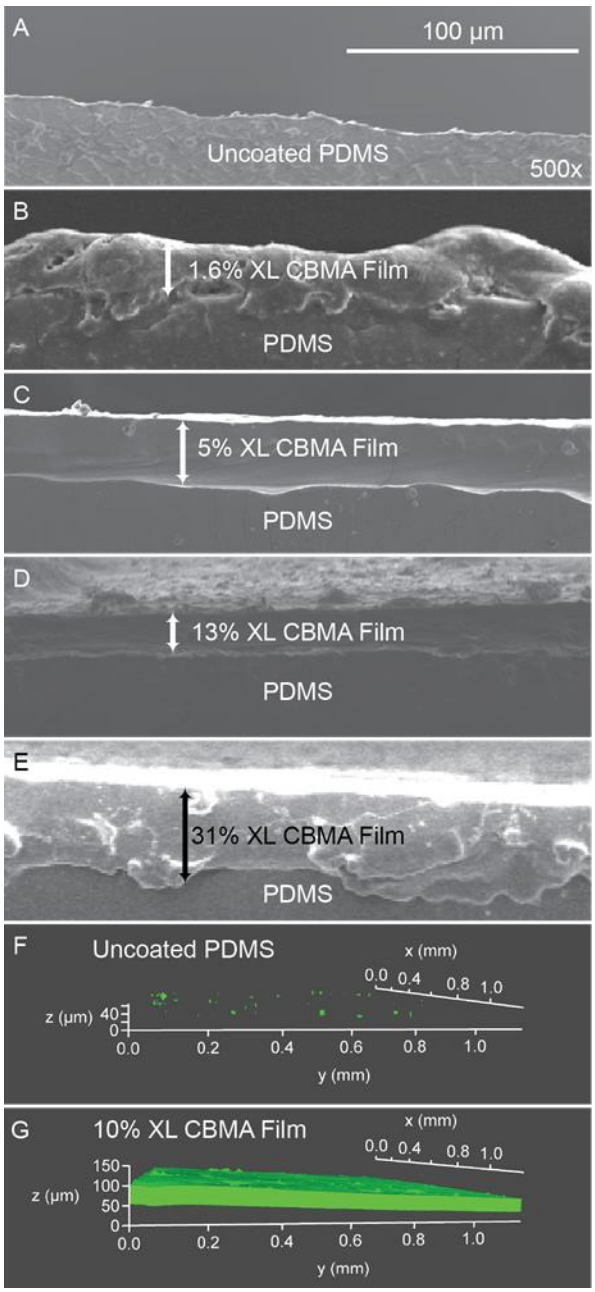

Figure 9. Scanning electron microscope cross-section images of six-month implants of A) uncoated PDMS and B-E) CBMA coated PDMS with indicated crosslinker (XL) percentages. Scale bar and magnification for A-E. Confocal microscopy images of 16-day implants are also shown of F) uncoated and G) 10% XL CBMA coated PDMS.

3.9 Reduction of realized insertion force with zwitterionic coating present

As cochlear implants are placed in the body, force is experienced by the surrounding tissue.[25, 30] The force and friction experienced by the surrounding tissue leads to trauma and scarring. To determine how the decrease in friction measured by tribometry correlates to actual insertion forces, force measurements were determined using a force transducer as cochlear implants (SBMA-coated and uncoated) were inserted into cadaveric human cochleae. Two differing array types were used for comparison, and the force was determined over time during the implantation (Figure 10, representative insertion force profiles). For array type I, halfway through the

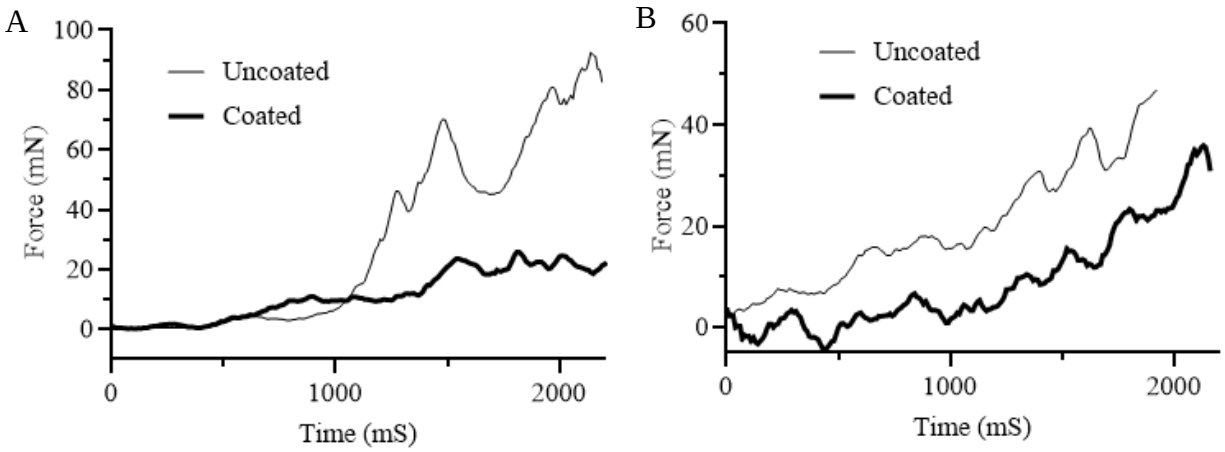

Figure 10. Representative force insertion profiles for uncoated (gray) and SBMA-coated (black) electrodes depicted over the time course of electrode insertion, wherein can be seen the lower maximum force and lower overall force over time for two manufacturers: A) Array Type 1 and B) Array Type 2.

insertion time the measured force for the uncoated implant began to rapidly increase and topped out at around 90 mN. On the other hand, the coated array type I experienced a gradual increase to a maximum force of about 25 mN (Figure 10A). Array type II uncoated implant increased from the beginning and topped out around 45 mN while the coated implant reached about 35 mN (Figure 10B). Array type I experienced a much larger difference between the coated and uncoated implants than array type II.

The maximum force for each implantation was also determined. For both array types, a significant reduction in the maximum insertion force was determined between the uncoated and coated samples (Figure 11A). The work of insertion was calculated using an area under the curve analysis for both array types, comparing uncoated and SBMA-coated implants. A general decrease in the work was observed, though the values did not statistically reflect a significant difference for coated and uncoated (Figure 11B). These data support the qualitative observation that the coated implants were inserted more easily and with less force compared to the uncoated implants.

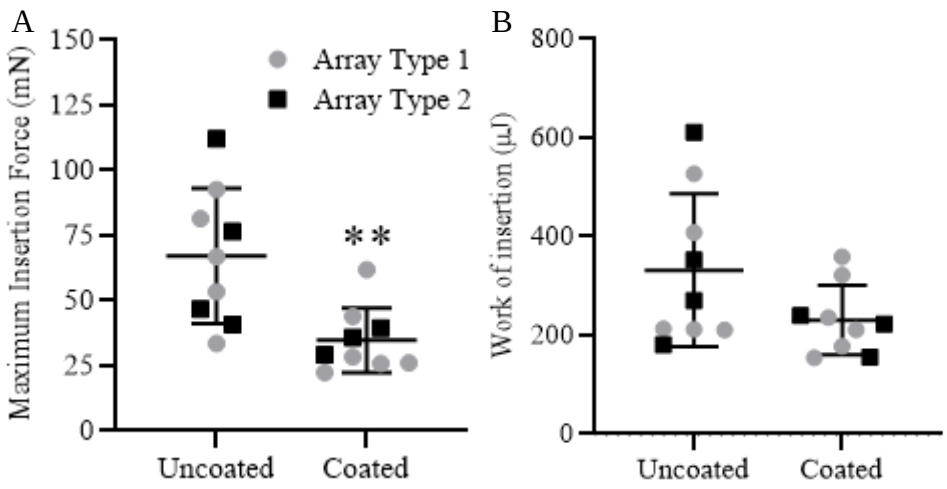

Figure 11. A) The maximum force of insertion during insertions of uncoated (n = 9) and coated (n = 9) human electrode arrays. B) Area under the curve analysis of the force over the average distance of insertion was found for insertion and averaged as a correlative measure of total work of insertion depicted in microjoules (µJ). The maximum force was significantly reduced (** $p < 0.003$) and overall work of insertion tended to be reduced, though this was not significant ($p = 0.19$).

4. Discussion

Hydrogels have been increasingly used for many biomedical applications,[31-35] including coatings to prevent the foreign body response.[36-40] One major drawback of a typical hydrogel is the inherent lack of durability of the polymer system.[41, 42] For a hydrogel to be reliably used for any biological application, but especially for coatings on implants, sufficient mechanical durability must be shown to ensure the hydrogel can remain viable during implantation and for the lifetime of the coated implant.[43] Zwitterionic hydrogels have successfully been shown to prevent the

foreign body response in flat, unperturbed systems. To transition to using them as coatings in actual applications requires enhanced durability. Previous work showed that by using a simultaneous photografting and photopolymerization, zwitterionic hydrogel network systems could reliably be attached to PDMS surfaces.[22] Herein, the same system was subjected to various challenges to test the mechanical durability of the hydrogel coating itself.

Overall, the zwitterionic hydrogels were able to show reliable durability and maintenance of desirable properties, especially lubricity, within certain parameters. The large reduction in coefficient of friction due to the presence of zwitterionic hydrogels has been well established. Herein, the limits to this lubricity were determined. Figure 2 demonstrates that the reduction in friction is well maintained over longer cycles of force. The hydrogel does need some amount of crosslinker to form a successful network system that can withstand the force applied during tribometry, as demonstrated by the value nearly identical to uncoated PDMS (relative value of 1.0) for 0% crosslinker. The sample did appear to become more lubricious overtime; however, this anomaly can likely be explained by the probe tip becoming coated by the uncrosslinked zwitterionic polymer over time leading to a slight decrease in the coefficient of friction given long enough. For the less crosslinked hydrogels, small amounts of coating did sometimes detach and coat the probe, the only large difference qualitatively was observed for the uncrosslinked system. Even with higher amounts of crosslinker, the average value of friction was not significantly different between the first and last forty minutes of testing. As expected, an increase in coefficient of friction is noted as the crosslinker percent increases, but the trend is maintained roughly the same with only 100% crosslinker seeing a significant jump in friction. Similarly, for the various crosslink densities, no significant difference was noted due to increasing speed of the probe tip. Kontorinis et al. reported that for standard CIs, as insertion

rate was increased from 0.17 mm/s to 3.3 mm/s, both mean and maximum force increased following a roughly linear trend.[29] However, the zwitterionic hydrogel appears to negate the force differences due to speed, reducing the trauma experienced due to variations in insertion speed.

Along with speed of the force that is experienced, the weight of force can change for what implants experience. As expected, the increase in normal force applied to the zwitterionic hydrogel coatings led to greater coefficient of friction, shown in Figure 3. While the coefficient of friction did increase, the value was still much less than for uncoated PDMS. CIs should not experience forces nearly as high as 10 N, but as zwitterionic hydrogel coatings are applied to other medical implants, maintaining lubricity with greater normal forces is ideal. At some point, the normal force would be sufficient to disrupt the mechanical integrity of the hydrogel system and the lubricious effects would be lost but that intensity of force is not feasible with the tribometer used in these experiments.

All implants experience forces, whether during insertion or within the body. However, the materials typically used for implants are durable enough that minute changes within the body don't affect the properties. Hydrogels, however, are characterized by large uptake of water, and therefore their properties will largely be determined by the hydration state and surrounding environment. Particularly, zwitterionic hydrogels impart their favorable antifouling effects due to the water-binding capacity of the zwitterionic moieties. In order to maintain the integrity of the film and the desired properties, multiple tests were conducted to understand how changes in moisture content affect the hydrogels and when failure occurs. First, the hydrogels were compared for coefficient of friction in normal state (created with some water present and then immediately swollen to equilibrium) with coatings which were completely dried before

rehydrating and testing. Below 25% crosslinker, the coefficient of friction was basically identical between the two groups, demonstrating that these coatings are stable enough to undergo drying without flaking or other failure that leads to loss of lubricity, shown in Figure 4. As the crosslinker percent increased, more flaking was observed as the coatings dried out, which was then reflected in greatly increased friction values following rehydration. These results provide a good working range of crosslinker percents that are stable in case drying does occur at some point following the coating process. Dried hydrogels were also tested but for nearly every dried sample, the force from the tribometer caused the film to fail (if it hadn't already flaked) and measured approximately the value of uncoated PDMS.

To ensure that the hydrogels remain sufficiently wetted to avoid failure due to drying when possible, the hydrogels were tested for how long they could undergo drying before failure, reflected in Figure 5. As is expected due to higher swelling capacity, the lower crosslinked hydrogel coatings resisted failure longer than higher crosslinked. The films proved to be quite durable and able to retain water for long periods of time, with the least crosslinked films only reaching failure (T90) after 50 minutes for CBMA and 60 minutes for SBMA. All crosslink percentages for either zwitterionic monomer did not even begin to show signs of drying and failure (T10) until 20 minutes. CBMA hydrogels especially showed marked resistance to failure. Likely the propensity of CBMA to bind higher number of water molecules[15] explains why the T10 values at lower crosslink density are higher. The values were comparable to what was measured for PEGMA hydrogels, suggesting that the zwitterionic films are durable and hydrated enough to maintain water as long as more mechanically stable PEG films. For CIs, the possibility of hydrogel coating drying before completion of implantation will not be a concern if the implant is not exposed to air for longer than these measured times. With the initial increase

of coefficient of friction occurring around thirty minutes for most coatings, SBMA and CBMA can confidently be used to coat implants where they may be exposed to air for significant periods of time. For the lower crosslinked films, which have shown better biological effects,[16] the lubricity and water content can be maintained for up to an hour, which is far longer than required for surgery. Because the results were determined by tribometry which exerts a small force, if hydrogel coatings are not disturbed, the time before failure may even be slightly elongated.

The mandrel bend testing (Figure 6) confirmed the stability of the coatings while in hydrated state and the length of time the hydrogels remain wetted even when exposed to ambient air. CIs will experience bending when inserted, so withstanding bending forces is also important. Because none of the zwitterionic hydrogels failed when swollen, the insertion should not cause failure to the film due to bending. The CBMA hydrogels at low crosslink density lasted for well over an hour before failure due to bending in a semi-swollen state, whereas the SBMA film was under an hour. However, both zwitterions showed excellent ability to retain water and resist bending. The hydrated state obviously is what allows a hydrogel to remain viable under bending, as PEGMA films failed the quickest around the time that drying was observed for the samples.

While zwitterionic hydrogels have often been shown to increase lubricity, *in vivo* understanding of lubricity changes is much more limited. Various tissues were tested against the coatings and showed that the decreased friction was translatable. For the various guinea pig tissues tested, coefficient of friction was dramatically reduced relative to uncoated PDMS for SBMA coatings with low crosslinker percent (Figure 7). However, even when the crosslinker percentage was increased, a reduction in friction was still noted. The measurements give confidence that zwitterionic hydrogel coatings will reduce the friction that is experienced during implantation for

CIs and should decrease the trauma experienced by the surrounding tissues. Literature shows that as hydration increases of skin, so does friction. Increase in normal force exerted also led to higher friction.

Additionally, the coating was tested by maintaining the lubricity after *in vivo* placement (Figure 8). Both CBMA and SBMA coatings experienced no significant change in coefficient of friction after implantation into a mouse, both showing a large reduction relative to PDMS. If lubricity is maintained, it can confidently be stated that the hydrogel was still significantly attached and durable following implantation time. The results shown in Figure 9 demonstrate that thin zwitterionic hydrogel coatings above 5% crosslinker were durable for the handling, surgical insertion, incubation, and surgical removal process. These findings support the previous data on showing resistance to bending and abrasion for a hydrated hydrogel. Insertion forces measured during actual CI placement somewhat reflected these trends (Figures 10 and 11). While one array type showed decreased force over time relative to the uncoated implant, the other array type only showed about 25% reduction in experienced force. Any force reduction is beneficial and should reduce trauma and scarring, but the lessened effects due to the hydrogel for array type 2 shows that the experiments measuring lubricity using tribometry may not always effectively translate. While the work of insertion appeared less for coated implants, the difference was not significant. However, the maximum insertion force was significantly reduced for both array types with the coating. The maximum insertion force likely has greater bearing on the extent of trauma experienced, so a decrease suggests that the coated implants would lead to less trauma to the surrounding tissue and inner ear during implantation.

5. Conclusion

In order for photografted zwitterionic hydrogel coatings to be used for CIs or other biomedical implants to impart antifouling, they must be sufficiently durable so the hydrogel remains intact and viable during implantation and for the lifetime of the implant. With a small amount of crosslinker, hydrogel network systems are formed that create durable hydrogels which can impart lubricity to the implant surface, even over long periods of time and after experiencing drying. The zwitterionic hydrogels remain sufficiently wet to maintain their lubricious properties for long periods of time, up to an hour, both when experiencing normal and bending forces. Lubricity was measured for tissues, suggesting good translation to *in vivo* systems, as well as following implantation in mice. Actual insertion of cochlear implants into cochleae showed that abrasive forces can be dramatically reduced when protected by zwitterionic hydrogel coatings. These findings show that the zwitterionic hydrogel coatings developed are durable enough to be used for application to biomedical implants and devices leading to reduction of biofouling and friction.

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