ENGINEERING NOVEL HIGH-RESOLUTION BIOELECTRONIC INTERFACES FROM MXENE NANOMATERIALS

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A DISSERTATION

in

Bioengineering

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2021

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To view a copy of this license, visit https://creativecommons.org/licenses/by-nc-sa/4.0 This work is dedicated to my sisters—Alana, Fallon, & Reina. Brag about me to your friends & future in-laws, yeah?

Acknowledgments

While some may take Isaac Newton's remark in a 1676 letter to his rival Robert Hooke about "standing on the shoulders of Giants" as a sarcastic comment directed at Hooke's less-than-imposing stature, I cannot help but apply these too fitting words to my own situation as I embark on completing this dissertation. I have indeed stood "on the shoulders of Giants" myself, and this is the only way that I have reached this current stage of my professional career. The work presented herein would not have been possible without the support and guidance of many individuals to whom I am greatly indebted.

First, I would like to thank my primary advisors, Drs. Brian Litt and Flavia Vitale. They accepted me into their research groups at the very beginning of this journey, and have ever since seen me through the years of my graduate career. Both of my advisors have given me the freedom to explore the fullest extent of my capabilities and interests as a researcher, yet they have also provided me with incredible guidance to keep me on track in order to reach the present moment. I could not be more grateful for their patience and understanding in mentoring me over these years, and for their consideration and respect for my personal growth as an engineer and a human being, as well.

I also wish to convey my sincerest respect and deepest gratitude to Dr. Nicolette Driscoll. Indeed, I would go so far as to say that the entirety of this dissertation could very well exist as a mere supplement to the immense body of work which Nicki has completed during and since her own tenure as a graduate student but part of me also thinks she would beg to differ on this point. Regardless: Nicki, you have been an amazing mentor to me in your own right, a wonderful lab mate, and an honest friend. Thank you very much for these past few years.

Next, I want to thank everyone whom I have had the genuine pleasure of working with in all of this time. Much of my work here would have been impossible without the help and collaboration of Dr. Andrew G. Richardson. Also, I am indebted to Dr. Nicholas V. Apollo for his guidance and training in how to be a creative engineer who can really "think outside the box." I also cannot thank the research support staff of the CNT enough: Carolyn Wilkinson, Everett Prince, Jacqueline Boccanfuso, and Magda Wernovsky were immeasurably helpful in so many ways, big and small, throughout my entire time in lab. I also wish to single out Patrick J. Mulcahey and Brittany H. Scheid for their particular help in completing large portions of the work presented in this dissertation. And of course, I wish to acknowledge all of the undergraduate students whom I have mentored over the years, and who have assisted me in my research efforts in turn, including Quincy Hendricks, Francesca Cimino, Tessa Posey, Nancy Rodriguez, Gary Eberly, and Natalia Nottingham. Finally, I am very grateful for the entire CNT "family unit," as we all consider it to be; to my lab mates, past and present: you have made all of these years enjoyable, fulfilling, and worthwhile. Certainly the future will be just as filled with adventures and excitement!

I am also grateful for the support I have received from my thesis committee: Drs. Kacy Cullen, Mark Allen, and Yury Gogotsi, I thank you each for your continuous support and thoughtful advice throughout the process of finalizing my doctoral thesis. From Dr. Gogotsi's group, as well, I would like to thank Dr. Mikhail Shekhirev and Kateryna Shevchuk in particular for their help in finalizing a significant aim of this work. And I also wish to thank the entire staff of the Quattrone Nanofabrication Facility and Singh Center for Nanotechnology, without whom I would have been unable to make a single microfabricated device.

Finally, I would thank my family and my very best friends for more than they can comprehend. To Arjun Shankar and Dr. Preya Shah, my siblings-in-spirit: thank you for your continual support over these past few years. And to my parents, to my sisters, to Akshay Nagendra, and to the rest of my family: without your love and support over the course of my entire life to the present moment, this very moment would not have even been possible. When I say that I am here today because of all of you, I fully mean that. This is as much for me, as it is for you.

ABSTRACT ENGINEERING NOVEL HIGH-RESOLUTION BIOELECTRONIC INTERFACES FROM MXENE NANOMATERIALS

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At the interface between Man and Machine are electrode technologies. Using recording electrodes, it is possible to observe and monitor the activity of neurons or nervous tissue, affording us with an understanding of the basic dynamics underlying behavior and disease. By interacting with the nervous system through stimulating electrodes, it is possible to impact brain function, or evoke muscle activation and coordination, paving the way for treatments to severe neurological and neuromuscular disorders. However, despite the exciting promises of electrode technologies, current state-of-the-art platforms feature stiff and high-impedance materials, which are incompatible with soft biological tissue. Additionally, many current technologies suffer from shorter lifetimes than may be desirable for a truly chronic implant or wearable health monitoring device. Recently, there has been a shift in focus towards two-dimensional nanocarbons as alternative materials for superior electrode technologies. This comes as a result of the enhanced flexibility, biocompatibility, and electronic and electrochemical properties that most carbonbased nanomaterials exhibit. In particular, the 2D nanomaterial titanium carbide MXene $(Ti_3C_2T_x)$ has very recently shown great promise for a variety of biomedical applications. However, the long-term stability of $Ti_3C_2T_x$ has not been fully explored, and it is still unknown whether $Ti_3C_2T_x$ can be used for chronic bioelectronic applications. Accordingly, in this thesis, I address and explore the key advantages of $Ti_3C_2T_x$ for biopotential sensing, with a particular emphasis on validating this unique material for chronic recording studies. First, I demonstrate the superior advantages of $Ti_3C_2T_x$ for direct recording of biopotential signals at the skin level in humans. Second, I define the long-term stability of Ti₃C₂T_x MXene in dried film form, and explore modifications in synthesis and film assembly to improve the material's lifetime. Third, I fabricate and validate Ti₃C₂T_x-based epidermal sensors that exhibit comparable recording capabilities to state-of-the-art clinical electrodes, firmly establishing Ti₃C₂T_x electrode technologies for future, chronic experiments. The processing and fabrication methods developed herein translate into mature technologies with unique properties that are comparable to state-of-the-art designs, thereby offering a novel bioelectronic platform with the potential to benefit a variety of fields in both the research and clinical settings.

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Chapter 1. INTRODUCTION

The first standard interface between any bioelectronic platform and the human body will always be composed of electrodes, since these can be used for both recording and modulation of neural and neuromuscular activity (Cogan 2008). However, most electrodes used for interfacing with living systems have traditionally been composed of metallic or silicon-based contacts. These are expensive materials to manufacture, and are also stiff and rigid. Indeed, the Young's moduli of metals and silicon are on the order of E = 1-100 GPa (Hong and Lieber 2019); these levels of rigidity aggravate the foreign body response (FBR) upon their implantation, or may result in poor conformability upon attachment of the electrodes to the skin surface. Furthermore, these materials typically have high impedances ($\sim 1-5 M\Omega$) when miniaturized to accommodate the millisecond and micron scales on which neural and neuromuscular activity occurs (Hong and Lieber 2019). High impedances increase the thermal noise of the electrodes, though, thus degrading their signal-to-noise ratio (SNR) (Scalia, Sperini, and Guidi 2012; Lempka et al. 2011; Huigen, Peper, and Grimbergen 2002), which, in turn, impacts the quality of recordings and may also result in poorer charge injection capabilities for stimulation.

In the past 20 years, however, research into alternative materials and designs has shown exciting promise, largely enabled by soft and biocompatible packaging materials (Y. Liu, Pharr, and Salvatore 2017), and highly conductive two-dimensional (2D) nanomaterials (J. Peng and Snyder 2019; Aflori 2021) (Figure 1.1). In particular, 2D carbon-based nanomaterials—such as carbon nanotubes (CNTs), graphene and its numerous derivatives, and transition metal carbides, nitrides, and carbonitrides (MXenes)—have come to the forefront of bioelectronic materials science research, as they show significant improvements over metals and silicon in terms of biocompatibility, flexibility, and recording and stimulation capacities (Teradal and Jelinek 2017; Szuplewska et al. 2020; Maiti et al. 2019). However, fully realizing these materials in functional bioelectronic interfaces for truly chronic in vivo studies remains a largely unmet need. This is due to a variety of factors. First, ad hoc fabrication processes must be developed in order to leverage the excellent properties of these materials for high performance electrodes. Second, manufacturing processes must be optimized to enable full scale-up, which also minimize time, cost, and effort. Finally, there still remains a basic uncertainty over the true performance stability of these 2D carbon-based materials in the long-term, so that work must be done to define—and, if necessary, expand—the material lifetime.



Figure 1.1 Ashby plot of the yield strength *vs.* Young's (elastic) modulus for common material classes. Many of the materials used as recording or stimulating electrodes in more novel bioelectronic platforms are composed of the strong and more flexible carbon nanomaterials group (upper right corner), while most novel packaging materials are made up of the elastomers group (middle left). The metals and ceramics group (right off-center) still represent a large portion of state-of-the-art electrode technologies. Figure taken from (J. Peng and Snyder 2019).

The primary goal of this thesis is to explore the feasibility of solutionsprocessable titanium carbide MXene ($Ti_3C_2T_x$, or simply MXene) for bioelectronic interfaces, with a particular emphasis on laying the groundwork for establishing $Ti_3C_2T_x$ as a promising material for chronic wearable applications. I begin from a basic materials science understanding of the advantageous structural, molecular, electrical, and electrochemical properties of $Ti_3C_2T_x$, and then move on to develop and optimize processing and fabrication methods to realize bioelectronic interfaces composed of this unique nanomaterial. Additionally, I explore the long-term stability of $Ti_3C_2T_x$ based interfaces, and investigate techniques and hybrid composites for improving the lifetime of finalized devices. In total, I demonstrate the use of MXene-based electrode technologies for a few of the most commonly implemented biopotential recording paradigms, and I highlight the performance of $Ti_3C_2T_x$ as it compares to contemporary bioelectronic interfaces. Specifically, I have organized this thesis into three major sections:

Establishing the optimal materials processing & fabrication techniques to realize $Ti_3C_2T_x$ -based bioelectronic interfaces

In this section, which comprises **Chapter 3** and **Chapter 4**, I systematically investigate the solutions- and materials-processing techniques necessary to reliably create $Ti_3C_2T_x$ MXene dried films. Subsequently, I characterize the properties of these films, and fine-tune my processing methods. With these protocol in place, I then use established microfabrication techniques to incorporate the optimized MXene films into thin, flexible, high-resolution electrode arrays, which I subsequently validate for acute high-density surface electromyography (HDsEMG). By comparing the performance of $Ti_3C_2T_x$ electrodes to bare metal electrodes and clinical standards, I demonstrate that the optimized properties of MXene-based dried films results in improved impedance characteristics and recording capabilities. I subsequently explore the impedance properties of fabricated $Ti_3C_2T_x$ electrodes in further detail, by proposing a variety of equivalent circuit models to describe the electrical interactions of MXene interfacing with two of the standard media represented in the biological sciences: physiologic saline and human skin.

Defining the lifetime of $Ti_3C_2T_x$ dried films & exploring alternative solutions to extend long-term stability

Long-term stable Ti₃C₂T_x films are necessary in order to truly realize chronic biosignals recording technologies. However, Ti₃C₂T_x is known to degrade in both ambient and wet environments, losing many of its excellent properties in the process. The exact nature of MXene's degradation is thought to begin at the exposed edges of the individual $Ti_3C_2T_x$ flakes (Xia et al. 2019), and many strategies have already been explored in the literature to improve the MXene lifetime. However, the vast majority of work done to characterize and investigate the stability of Ti₃C₂T_x has been done for MXene in its solution form only. Thus, it remains to be seen whether any of the strategies employed for solutions are also translatable to $Ti_3C_2T_x$ in a dried film form. Accordingly, in this section of my thesis, comprising **Chapter 5**, I first define the lifetime of my optimally processed MXene dried films, as well as explore alternative $Ti_3C_2T_x$ compositions, with the hope of extending the long-term stability of films that will ultimately be incorporated into mature, fully functional electrode technologies. This section, in turn, provides key insights that guide me in realizing the long-term stable MXene-based bioelectrode technologies that are subsequently described in Section 3.

Developing a chronically stable bioelectronic interface based on the most favorable solutions-processable MXene composites

Finally, in this section of my thesis, comprising **Chapter 6**, I integrate the most promising MXene compositions from Section 2 into mature bioelectronic interfaces, using the microfabrication and benchtop processes which I develop in Section 1. Subsequently, I validate my fabricated electrodes in acute electrocardiography (ECG) studies, comparing the MXene-based arrays to state-of-the-art clinical technologies. I hypothesize that the $Ti_3C_2T_x$ -based electrode technologies will show comparable, if not improved, recording performance compared to clinical standards, paving the way for future studies, which will investigate the benefits of MXene-based bioelectronic interfaces for truly chronic applications.

In the end, this work will outline and establish the entire process flow necessary for realizing a mature $Ti_3C_2T_x$ -based electrode technology, beginning from optimal processing methods at the solutions level, to techniques for characterizing and benchmarking the next generation of bioelectronic interfaces based on MXene nanomaterials. The final products of this thesis will be real-world device technologies, suitable for actual use in long-term physiology and health monitoring studies, in both the research and clinical settings.

Chapter 2. BACKGROUND

Contemporary Electrode Technologies

Electrodes are one of the most common technologies used to investigate the human nervous system and gather information about the processes underlying behavior and disease. This is true of both implantable designs-such as the electrocorticography (ECoG) surface grids and penetrating Utah arrays commonly used to study neural activity in the brain (Boehler et al. 2020; Hong and Lieber 2019)—as well as of skin-based, wearable sensors—such as the arrays and montages electrocardiography electromyography used for (ECG), (EMG), and electroencephalography (EEG) (Heikenfeld et al. 2017; Y. Liu, Pharr, and Salvatore 2017). Recently, electrode technologies have even been developed to aid in studying electrochemical changes that are evident from biofluid secretions of the body, such as sweating or salivation (Heikenfeld et al. 2017; Windmiller and Wang 2013). Finally, electrodes represent the most widely accepted interface incorporated into current brain-machine interface (BMI) platforms (Lebedev and Nicolelis 2006) and prosthetic limb technologies (Ngan, Kapsa, and Choong 2019; Ortiz-Catalan et al. 2012).

Due to the wide variety of biological targets that can be assessed and modulated with electrodes, there is an equally wide variety of technologies currently in use. Nevertheless, the vast majority of contemporary bioelectronic interfaces are composed of similar materials: metals—most commonly platinum (Pt), iridium (Ir), gold (Au), and silver/silver chloride (Ag/AgCl)—or silicon. These materials have good electronic and electrochemical properties, and their widespread use comes as a direct result of the well-established processing and manufacturing techniques that exist for them, courtesy of the semiconductor industry (Hierlemann et al. 2003; Zaouk, Park, and Madoui 2006). Over the last 20 years or more, however, major drawbacks to these materials have come to light, resulting in a paradigm shift in thought and approaches towards electrode technology fabrication and development.

Foremost among the shortcomings of many current platforms and designs is the rigidity of the electrode materials themselves. Metals and silicon have elastic moduli ~6 orders of magnitude larger than the modulus of brain tissue, and ~4 orders of magnitude larger than the modulus of human skin (Y. Liu, Pharr, and Salvatore 2017; Apollo et al. 2020; Hong and Lieber 2019). This mechanical mismatch causes an aggravated foreign body response (FBR) in the case of implantable technologies, resulting in cell death and loss of recorded signals (Apollo et al. 2020). In the case of skin-based sensing, electrode rigidity can lead to poor conformability to the skin surface, which may lead to increased motion artifact (MA) and poor recording quality, hindering meaningful data collection and interpretation (Heikenfeld et al. 2017). Metals and silicon are also difficult to scale down to the sizes that are relevant for physiological recording or stimulation, because as they are miniaturized, there is a concurrent and dramatic increase in their impedance, resulting in lower signal-to-noise ratio (SNR) and poorer charge storage and injection capacities (Cogan 2008). Yet another concern is the long-term stability of metals and silicon as they interface with the human body. Implantable devices often suffer from the chronic FBR that extends beyond the initial implantation, which facilitates material breakdown of both the insulating and conducting components of the device, resulting in significant degradation of signal quality over time (Apollo et al. 2020) (Figure 2.1). Along similar lines, many wearable technologies require adhesives or gels in order to improve the skin conformability and recording capabilities of the device, however, these intermediate elements can dry out over time or become otherwise ineffective, resulting in skin irritation and changes in the device performance (Murphy et al. 2020).



Figure 2.1 Illustration of the foreign body response (FBR) to an implanted electrode array. (A) The acute FBR. In response to damage to blood vessels and neural tissue due to the electrode implantation procedure, astrocytes and microglia are activated. (B) The chronic FBR. Over time, astrocytes and microglia attach themselves to the implant, and fibroblasts also form along the electrode surface, presenting a barrier to recording or stimulation. At the same time, neurons in the region of the implant may die off, as well. Figure adapted from (Apollo et al. 2020).

A number of materials science strategies have been employed to overcome the major drawbacks of metallic and silicon-based electrodes. For example, fairly simple design changes can help improve mechanical flexibility, such as in the case of mesh electrodes (Dai et al. 2018; Yeo et al. 2013) or ultrathin nanoelectronic threads (X. Wei et al. 2018). Meanwhile, electrodeposition of porous coatings (Ferguson, Boldt, and Redish 2009; Meyer et al. 2001) and other forms of surface roughening

(Ivanovskaya et al. 2018; Leber et al. 2017) are common techniques adopted to impart a larger effective surface area to the electrode contact itself. This, in turn, can improve the impedance characteristics of the device by offering more available sites for electrochemical and other processes to occur, thus playing a role in improving the SNR during recording. Replacing metals or silicon entirely with 2D nanocarbons including CNTs (David-Pur et al. 2014; Vitale et al. 2015) carbon fibers (Patel et al. 2015; Yoshida Kozai et al. 2012), or reduced graphene oxide (rGO) (Apollo et al. 2015)—is yet another alternative method which overcomes the shortcomings of current electrode technologies. And indeed, because of their inherently high flexibility and exceptional electrochemical properties, 2D nanocarbons offer many exciting avenues to improve the final electrode's mechanical stability, while also affording the finalized device with improved recording and stimulating characteristics.

The Rise of Titanium Carbide MXene (Ti₃C₂T_x)

A more recent addition to the large family of 2D carbon-based nanomaterials are MXenes: a library of transition metal carbides, nitrides, and carbonitrides that exhibit an advantageous combination of exceptional electronic conductivity, hydrophilicity, and a high capacity for functionalization or surface modification (Naguib et al. 2014; Hart et al. 2019; Khazaei et al. 2017; Anasori and Gogotsi 2019; Maleski et al. 2018). Of the many MXenes that have been synthesized and explored to date, titanium carbide MXene $(Ti_3C_2T_x)$ has been the most extensively characterized. $Ti_3C_2T_x$ is particularly unique because it is colloidally stable in water, and therefore does not require surfactants or other stabilizing agents (Dillon et al. 2016). This makes it highly compatible with solutions-processing techniques, as well as with the other methods and materials commonly used to fabricate biocompatible devices for bioelectronic applications (Driscoll et al. 2018b; 2020; Murphy et al. 2020). In fact, the remarkable electronic, electrochemical, and mechanical properties of $Ti_3C_2T_x$ have already led to its inclusion in a variety of biomedical applications (Szuplewska et al. 2020), including cancer theranostic platforms (G. Liu et al. 2017), flexible microscale neural recording devices (Driscoll et al. 2018a; 2020), and a wide range of skin-based sensors (Cai et al. 2018; Kedambaimoole et al. 2020; Jiagi Zhang et al. 2019).

However, despite its many exciting attributes, $Ti_3C_2T_x$ is known to degrade in both ambient and wet environments. Presently, it is hypothesized that $Ti_3C_2T_x$ becomes titania (TiO_2) in the process of degradation, and subsequently loses many of its excellent properties. The process of MXene's degradation is thought to begin at the exposed edges of the individual 2D $Ti_3C_2T_x$ flakes (Xia et al. 2019), and many strategies have been explored in the literature to improve the MXene lifetime.

Early approaches explored storage conditions of the solution: MXene dispersed in water was stored in argon-sealed vials (C. J. Zhang et al. 2017), or was stored at -80 °C (Chae et al. 2019), in order to slow the rate of degradation. Additional work determined that MXene dispersed in ethanol needed only to be stored at 5 °C to keep for nearly 9 months (Chae et al. 2019). More recently, other groups have explored adding stabilizing agents to the MXene solutions-such as natural antioxidants (Zhao et al. 2019), or polyanionic salts that "cap" the edges of the MXene flakes to prevent oxidation from the edge inward (Natu et al. 2019). Work has also demonstrated that changes to the MXene synthesis process itself can improve the final stability of the solution (T. Mathis et al. 2020). At least two papers have demonstrated that $Ti_3C_2T_x$ dried films can be recovered by annealing them at high temperatures (600-900 °C), and these films show improved resistance against further oxidation post-annealing (Y. Lee et al. 2020; Zhao, Holta, et al. 2020). Except for this final example, however, the vast majority of work done to characterize and investigate the stability of $Ti_3C_2T_x$ has been done for MXene in its solution form alone. Accordingly, it remains to be seen whether any of the strategies employed for MXene solutions are also translatable to $Ti_3C_2T_x$ in a dried film form, which more accurately reflects the form MXene will take when it is incorporated into bioelectronic interfaces.

State-of-the-Art Electrodiagnostics

Currently, electrode technologies are employed in a wide range of different fields, as described above, ranging from electrodiagnostics and clinical health monitoring, to assistive and rehabilitation prostheses, to basic science research into the human nervous system. A common theme connecting many of these applications, though, is the necessity to transduce biopotential signals generated by the human body into meaningful signals that can be collected with standard recording equipment. The ECG, for example, is a simple and clear biopotential to record from the skin surface, and is also one of the hallmarks of clinical diagnostics and monitoring (Mirvis and Goldberger 2012; Al Ghatrif and Lindsay 2012). One major challenge that often presents itself during skin-based recordings, though, is the presence of MA and other noise that can confuse the data collection and interpretation processes (Kearney, Thomas, and McAdams 2007; Cömert, Honkala, and Hyttinen 2013). Dry epidermal electrodes composed of stiff metals or silicon do not conform well to the complex contours of the skin, and are also high impedance, making them more susceptible to MA than the same electrodes featuring an electrolytic gel or conductive paste between the contact and the skin (Puurtinen et al. 2006). However, an investigation of gelled electrode technologies still revealed that the noise and other artifacts present in gelled electrode recordings originates primarily from the skin-gel interface (Huigen, Peper, and Grimbergen 2002). Furthermore, the long-term use of gelled electrodes is prevented by the fact the gels coating the electrode surface dry out over time, increasing the electrode impedance, as well as the susceptibility to MA, diminishing the device's overall performance (Murphy et al. 2020).

Recently, the Vitale lab at the University of Pennsylvania has begun to explore the benefits of $Ti_3C_2T_x$ for skin-based recording applications (Murphy et al. 2020; Driscoll et al. 2021). Given the many remarkable properties of $Ti_3C_2T_x$, and also as a result of the use of thin and flexible elastomeric substrates that are highly skin conformable, the epidermal electrode technologies developed thus far in Dr. Vitale's lab have shown great promise. Indeed, to date, MXene has outperformed both dry and pre-gelled metal-based electrodes in passive surface EMG studies (Murphy et al. 2020) (see **Chapter 3**), and textile matrices infused with $Ti_3C_2T_x$ have performed just as well as state-of-the-art clinical electrodes during EEG, EMG, and ECG recording sessions (Driscoll et al. 2021). Beyond the Vitale lab, other groups have also developed MXene-based epidermal sensors for health monitoring applications, which have largely made use of stress/strain sensing (Cai et al. 2018; Kedambaimoole et al. 2020; Jiaqi Zhang et al. 2019). Altogether, MXene is a promising candidate to replace current electrode technologies for electrodiagnostics at the skin level. Accordingly, one major goal of this thesis is to continue the work already begun investigating $Ti_3C_2T_x$ for skin-based sensing applications, with the hope of presenting a new technology capable of high fidelity, long-term recording at the interface between Man and Machine.

Chapter 3. Materials Processing & Fabrication of $Ti_3C_2T_x$ Interfaces

In Chapter 3, I focus the discussion on the optimal processing and fabrication methods required to realize bioelectronic interfaces based on $Ti_3C_2T_x$ MXene. Specifically, I introduce MXenes, then outline the protocols necessary to repeatably process MXene solutions into dried films using standard solutions-processing techniques. I then characterize these dried films, and furthermore incorporate them into a standard microfabrication process flow. I realize wearable, $Ti_3C_2T_x$ -based electrode arrays for high-density surface electromyography (HDsEMG), and subsequently validate the fabricated HDsEMG arrays for acute human recording. Comparing MXene's recording capabilities to size-matched metal electrodes, as well as to standard clinical technologies, I highlight the benefits of $Ti_3C_2T_x$ compared to traditional materials. The results of this discussion pave the way for $Ti_3C_2T_x$ -based epidermal electrodes to be adapted for direct biopotential signal sensing applications.

Relevant Citations:

(1) Murphy BB, Mulcahey PJ, Driscoll N, Richardson AG, Robbins GT, Apollo NV, Maleski K, Lucas TH, Gogotsi Y, Dillingham T, Vitale F. A Gel-Free $Ti_3C_2T_x$ -Based Electrode Array for High-Density, High-Resolution Surface Electromyography. *Advanced Material Technologies*, 2020, 2000325.

(2) Driscoll N, Maleski K, Richardson AG, Murphy B, Anasori B, Lucas TH, Gogotsi Y, Vitale F. Fabrication of Ti₃C₂ MXene Microelectrode Arrays for *In Vivo* Neural Recording. *Journal of Visualized Experiments*, 2020, e60741.

Introduction to MXenes

MXenes are a family of transition metal carbides, nitrides, and carbonitrides with the general chemical formula $M_{n+1}X_nT_x$, where M is an early transition metal from Groups 3–7 of the periodic table, X is either carbon or nitrogen, n is an integer between 1 and 3, and T_x represents the x total number of possible functionalities that exist at the MXene surface (Anasori and Gogotsi 2019; VahidMohammadi, Rosen, and Gogotsi 2021; Gogotsi and Huang 2021). The most prominent MXene functional groups are typically =0, -F, and -OH, which are left behind as result of the synthesis process required to realize MXene. The precursor state of MXene is called the MAX phase, which is a layered ternary carbide or nitride, in which the $M_{n+1}X_n$ layers are bonded to layers of an A element, from Groups 13-16 of the periodic table (Anasori and Gogotsi 2019). After etching of this A element component, the MAX precursor reaches the MXene phase, and the surface terminations that remain are typically the result of etching the A element with hydrofluoric acid (HF), or a mix of lithium fluoride (LiF) and hydrochloric acid (HCl) (Naguib et al. 2014; Anasori and Gogotsi 2019). As Figure 3.1 shows, MXenes encompass a very large family of nanomaterials, although not all possible compositions have been synthesized to date.



Figure 3.1 All possible MXene structures known or predicted to exist to date. Titanium carbide MXene, $Ti_3C_2T_x$, would fall among the M_3X_2 structure in the second row. Figure taken from (VahidMohammadi, Rosen, and Gogotsi 2021).

Of supreme importance is the fact that all MXenes are hydrophilic, which allows them to be dispersed in water and a variety of other organic solvents, such as ethanol, isopropyl alcohol (IPA), and acetone (Dillon et al. 2016; Anasori and Gogotsi 2019; Naguib et al. 2014; Maleski, Mochalin, and Gogotsi 2017). As a direct result, standard solutions-processing methods may be easily adopted for handling MXene solutions, or for realizing technologies based on these solutions. This is particularly advantageous, because the scalability of solutions-processing techniques makes manufacturing cheaper and higher throughput. The fact that MXenes are water dispersible, in particular, also makes them compatible with the processing requirements for most other materials commonly used for device packaging or encapsulation, such as elastomers, polymers, and epoxies (Driscoll et al. 2018b; 2020; Murphy et al. 2020; Ahn, Jeong, and Kim 2019; Chen, Luo, and Kang 2017).

Now, of the many MXene compositions that have been predicted or synthesized to date, the most extensively characterized and explored is also the first one to have been synthesized back in 2011: titanium carbide MXene, or $Ti_3C_2T_x$. $Ti_3C_2T_x$ exhibits an exceptionally high electronic conductivity (10^4 S cm⁻¹ and above), and may be processed into optically transparent films with a high optoelectronic figure of merit, or made into thicker films with remarkable electronic stability under repeated mechanical deformations (Dillon et al. 2016; Maleski et al. 2018; Hart et al. 2019). $Ti_3C_2T_x$ also exhibits a high psuedocapacitance (Ando et al. 2020): volumetric capacitances upwards of 900 F cm⁻³ have been measured for $Ti_3C_2T_x$ films in certain electrolytes, and $Ti_3C_2T_x$ -based hydrogel film electrodes have reached capacitances up to 1,500 F cm⁻³ (Lukatskaya et al. 2017). $Ti_3C_2T_x$ has furthermore exhibited high gravimetric capacitance in alkaline electrolytes, reaching 140 F g⁻¹ by one report (S. Xu et al. 2017).

As a result of its impressive electronic and electrochemical properties, $Ti_3C_2T_x$ has been most extensively studied for energy storage applications (Anasori, Lukatskava, and Gogotsi 2017; VahidMohammadi, Rosen, and Gogotsi 2021). However, in the various fields of biomedical applications, as well, $Ti_3C_2T_x$ has already been successfully demonstrated for many applications, including as an antibacterial coating (K. Rasool et al. 2017; 2016); in the role of a photothermal treatment (PTT) agent in cancer theranostics (G. Liu et al. 2017); and as the primary sensor in wearable (H. Lin, Chen, and Shi 2018; K. Huang et al. 2018) and implantable (B. Xu et al. 2016; Driscoll et al. 2018a) bioelectronic platforms. The biocompatibility of $Ti_3C_2T_x$ was initially investigated as part of a study that explored MXene-based microelectrode arrays for recording neural activity in vivo in rodent models (Driscoll et al. 2018a; 2020), but since then, other work has further established the high biocompatibility and low cytotoxicity of $Ti_3C_2T_x$ films (Lim et al. 2021; Scheibe et al. 2019). The full range of applications that have seen the adoption of $Ti_3C_2T_x$ since its first synthesis in 2011 is described in part in Figure 3.2, and by a number of recent reports (VahidMohammadi, Rosen, and Gogotsi 2021; Gogotsi and Huang 2021).



Chapter 3: Materials Processing & Fabrication of Ti₃C₂T_x Interfaces

Figure 3.2 Applications of $Ti_3C_2T_x$ and other MXenes to date. While research into MXene is largely dominated by the fields of energy and energy storage, there have been notable advances in the fields of sensors technology, optoelectronics, and Biomedical and Environmental applications, as well, which leverage MXene's many advantageous properties. Figure taken from (VahidMohammadi, Rosen, and Gogotsi 2021).

While $Ti_3C_2T_x$ has shown significant promise over the years, in order to fully leverage the unique properties of this particular MXene, it is necessary to develop reliable, repeatable, and versatile processing and fabrication methods. Furthermore, once fabricated, any fully functioning device incorporating MXene must be benchmarked in order to assess its electrical and electrochemical advantages, as compared to standard metal-based electrodes. This motivates a more thorough investigation into reproducible solutions-processing of $Ti_3C_2T_x$ MXene solutions.

Reproducible Solutions-Processing of Ti₃C₂T_x MXene

In order to establish $Ti_3C_2T_x$ for bioelectronic applications, the very first step was to develop and optimize film casting methods for realizing conductive MXene

dried films. Accordingly, $Ti_3C_2T_x$ MXene was synthesized by Murata Manufacturing Co., Ltd. in Yasu, Japan, using a modified version of the well-established MILD synthesis recipe (Alhabeb et al. 2017). The resulting product was then provided to our research group at the University of Pennsylvania through a joint research agreement (JRA), as a stable colloidal solution of 2D MXene flakes in deionized (DI) water. Typically, we received MXene slurries at or around a concentration of 30 mg mL⁻¹. In order to easily adopt these solutions for standard, high-throughput solutions-processing, though, we first diluted the solutions to ~10 mg mL⁻¹ and explored our ability to create uniform MXene films via two of the most common film casting techniques: spin-coating and spray-coating. One important and rather preliminary finding from this study was that even though the MXene itself is hydrophilic, unless the target substrate is also hydrophilic, processing of MXene solutions into dried films cannot be easily achieved (Figure 3.3). Having both a hydrophilic MXene solution and a hydrophilic substrate will ensure that the final $Ti_3C_2T_x$ film is uniformly distributed across the entire surface area of the substrate.



Figure 3.3 Determination of the optimal solutions-processing technique for realizing $Ti_3C_2T_x$ dried films. (A) Image of a 10 mg mL⁻¹ MXene solution intended for spin-coating on a sample that *was not* treated with an oxygen plasma before spin-coating. The solution does not spread across the entire substrate, and the resultant dried film is non-uniform and does not coat the entire substrate. (B) Image of a 10 mg mL⁻¹ MXene solution intended for spin-coating on a sample that *was* treated for 2 mins. with an oxygen plasma before spin-coating. The resultant solution coats the entire substrate before spinning began, and the resultant film was much more uniform across the surface of the glass substrate. (C) Image of a spin-cast dried film. (D) Image of a spray-cast dried film.

Figure 3.3, panels C and D, make it abundantly clear that spray-coating affords us greater control over the final film uniformity and thickness compared to spincoating. Thus, for all subsequent work, spray-coating was adopted as the main solutions-processing technique to enable more repeatable processing of MXene solutions into dried films. To further ensure the reproducibility of our results, we also finalized an exact spray-coating procedure to adopt for creating MXene dried films, as outline in Table 3-1. To make all spray-cast samples, we first cleaned and activated the surface of our substrates using a Harrick PDC-32G Basic Plasma Cleaner. After our substrates were made more hydrophilic by exposure to an air or oxygen plasma, we then moved the samples to a 150 °C hot plate, and loaded the desired volume and concentration of MXene solution into a Fy-Light SP180 Copper Airbrush, connected to a TC-20 air compressor. To complete the spray-coating procedure, we moved our airbrush back and forth across the entire width of the hot plate, keeping time with a metronome beating out 120 beats per minute (bpm). Following the spray-coating procedure, we baked all MXene films overnight in an 80 °C vacuum oven, at a pressure of ~500–550 mTorr.

Plasma cleaning and activation parameters	305 mTorr, 18 W, 5 mins.
Spraying pressure [MPa]	0.241
Avg. distance between nozzle and substrate [cm]	12-15
Hot plate temperature during spray-coating [°C]	150
Avg. time for one spray [s]	3 (120 bpm, 6 counts)
Avg. liquid volume per minute [mL min-1]	3 (<i>NB:</i> <u>only</u> for 10 mg mL ⁻¹)
Vacuum oven parameters for dried film bake	80 °C, 500–550 mTorr
Time for dried film bake [hrs.]	~15 (overnight)

Table 3-1 Spray-coating parameters to reproducibly achieve $Ti_3C_2T_x$ dried films.

Having established a standardized spray-coating procedure, we thereafter explored the effects of solution concentration on both the final film uniformity, and the final film conductivity (Figure 3.4). From this characterization, we found that highly uniform MXene films were most readily reproducible by spray-coating MXene solutions diluted to a final concentration of ~10 mg mL⁻¹. These films provided us with the optimal processability, and also resulted in dried films with average sheet resistances of $R_s = 55 \pm 5 \Omega \text{ sq}^{-1}$, as measured with a 4-point probe (Loresta handheld AX-MCP-T370 resistivity meter). As these films were ~100nm in thickness, the resultant DC conductivity for our Ti₃C₂T_x dried films was calculated to be $\sigma_{DC} \equiv$ $(R_s \times \tau)^{-1} = (55 \Omega \text{ sq}^{-1} \times 100 \text{ nm})^{-1} \approx 1,800 \text{ S cm}^{-1}$, which is an appreciably high value of conductivity for such a thin film. Accordingly, we adopted 10 mg mL⁻¹ as the target MXene concentration for all further processing.

Next, we devised a simple strategy to reliably create MXene dried films at targeted thicknesses, ranging from ~0.25–3 µm. This thickness range was chosen given that devices based on MXene thin films may require different thicknesses depending on the application. For example, thinner films may be more desirable to improve the conformability of a device to the skin surface (Nawrocki et al. 2018; 2016), whereas thicker films may be more desirable to reduce the overall device impedance (Cogan 2008). Accordingly, we used the total volume of spray-cast MXene solution as the independent variable, and found a linear relationship between the final film thickness and the total spray-cast volume, as expected (Figure 3.4, panel C). A profilometer was used to measure the final film thickness (KLA Tencor P7 2D/3D profilometer with applied force = 2 mg, speed = 100 µm s⁻¹, sampling rate = 500 Hz). The slope of the of line of best fit was 0.43 ± 0.09 µm mL⁻¹ (R^2 = 0.828) over 3 separate samples.



Figure 3.4 Characterization of spray-cast $Ti_3C_2T_x$ films, varying solution concentration and spray-cast volume. (A) Image of spray-cast MXene films at various solution concentrations. The solution concentration increases from left to right. (B) Bar plot of the sheet resistance of the films in panel (A), as measured with a 4-point probe. N = 4 measurements per sample. (C) Average film thickness *vs.* total volume of spray-cast MXene solution for films ranging from ~0.25 µm to ~3 µm in thickness. Points represent averages and errorbars show standard deviations across N = 6 measurements for each of 3 samples.

A Ti₃C₂T_x-based array for HDsEMG

Background: Electrode Technologies for Surface Electromyography (sEMG)

Surface electromyography (sEMG) is the technique of recording muscle activity from the skin surface. Standard technologies for sEMG have been composed of metals such as Au, Ag, or Ag/AgCl, with Ag/AgCl electrodes being the most widely used due to their lower baseline noise interface (Roberts et al. 2016; Roberto Merletti et al. 2009). Most commercially-available Ag/AgCl electrodes are pre-gelled, meaning they feature an electrolytic gel or conductive paste coating the main metal contact. This gel not only forms the interface between the electrode and the skin during recording, but also serves to reduce the interfacial impedance and improve overall signal quality due to its ionic content (Roberto Merletti et al. 2009; Paolo Cattarello and Merletti 2016; Piervirgili, Petracca, and Merletti 2014). Nevertheless, these gels may dry out over time, which results in skin irritation and significant variations in the interfacial impedance (Yapici et al. 2015; Scalisi et al. 2015). A high contact impedance between the electrode and the skin results in lower quality sEMG signals, which may adversely affect muscle activation and coordination studies (D J Hewson, Langeron, and Duche 2003; Yamagami et al. 2018).

To realize flexible sEMG electrodes with a low interfacial impedance, a variety of materials strategies and device architectures have been proposed. In previous reports, very thin layers of metals (usually Au) were deposited onto thin polymeric substrates to serve as electrodes and interconnects, and these ultrathin contacts achieved relatively low impedance values (Lapatki et al. 2004; Yamagami et al. 2018; Nawrocki et al. 2018; Tian et al. 2019). To improve device wearability and skin conformability, sEMG arrays have also been patterned using fractal geometries and microporous structures (Tian et al. 2019). Metal nanowires and nanowebs (E. Lee et al. 2017), as well as metal nanoparticles (MNPs) (Roberts et al. 2016; Matsuhisa et al. 2015; Khan et al. 2016; Scalisi et al. 2015), have also been explored. MNP-based HDsEMG arrays have attracted particular attention because they can be fabricated using fast, additive inkjet printing (IIP), which is a high-throughput process with low materials waste (Roberts et al. 2016); however, the MNP inks must be sintered after their deposition, requiring optimization of a thermal treatment process that is compatible with the target substrate material. Since many of the substrates commonly used in wearable electronics have low glass transition temperatures (T_q) — typically, $T_q < 150^{\circ}$ C (Roberts et al. 2016; Matsuhisa et al. 2015) — this optimization is highly non-trivial. Furthermore, once dried, the MNP films often have lower surface densities than the same metal films in bulk form, resulting in lower electrical conductivity (Roberts et al. 2016). Other materials that have been explored

for decreasing the contact impedance and improving overall device flexibility include rGO (Yapici et al. 2015; Lou et al. 2016) and CNTs (Jung et al. 2012; J. H. Kim et al. 2018). These materials have been typically integrated into flexible polyester (Lou et al. 2016) or nylon (Yapici et al. 2015) textile matrices, or alternatively into plastic molds (Jung et al. 2012; J. H. Kim et al. 2018). However, such configurations often require complex and time-consuming fabrication processes in order to ensure sufficient volume distribution of the conductive carbon filler within the plastic, fabric, or polymeric target matrix (Yao, Swetha, and Zhu 2018).

Beyond metallic and carbon-based materials, other materials that have been proposed for reducing the skin-electrode impedance include: conductive polymers such as poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS), which can be deposited onto the electrode surface (Roberts et al. 2016); liquid metal inks which can be directly painted onto the skin surface (Yu, Zhang, and Liu 2013); and ionic liquid gels at the interface between the electrode and the skin, which do not dry out at room temperature like most commonly used gels (Leleux et al. 2014). However, while any of these or the other materials strategies described above may be beneficial in reducing the skin-electrode interface impedance, there remains currently no gel-free HDsEMG electrode technology that readily achieves both a low interfacial impedance, and excellent skin conformability.

To address this unmet need, then, we investigated low-impedance, highsensitivity, gel-free HDsEMG arrays based on $Ti_3C_2T_x$ MXene, encapsulated in thin layers of flexible parylene-C.

Device Fabrication & Characterization

The Vitale Lab at the University of Pennsylvania has pioneered $Ti_3C_2T_x$ MXene for bioelectronic applications, having recently demonstrated *ad hoc* fabrication flows for producing neural (Driscoll et al. 2018b; 2020) and epidermal (Murphy et al. 2020) recording arrays. These fabrication protocols allow us to make high-density arrays of MXene contacts with Au interconnects, embedded in thin parylene-C packaging with total device thickness of ~8–10 µm (Figure 3.5). Such arrays are highly flexible and in the case of our epidermal sensors, in particular—skin conformable. The precise patterning of the $Ti_3C_2T_x$ contacts and general fabrication protocol outlined in our previous work has been made possible by the development of a "dry lift-off" technique (Driscoll et al. 2020; 2018b; Murphy et al. 2020), which my colleague adapted from a previously reported method for micropatterning PEDOT:PSS films (Sessolo et al. 2013). This technique involves coating the entire processing wafer with a layer of cleaning solution (2% Micro90), followed by deposition of a thin (~3-µmthick) "sacrificial" layer of parylene-C. Electrode contacts can be patterned into this "sacrificial" layer using standard photolithography and reactive ion etching (RIE) techniques, and subsequently, our MXene dried films may be spray-cast into the etched features using the techniques described in Table 3-1. The "sacrificial" parylene layer is then manually removed, leaving behind $Ti_3C_2T_x$ dried films only in the contacts that were patterned via photolithography and RIE. It is worth noting that this fabrication technique is compatible with any solutions-processable material, so that the same protocol can be adopted for alternative compositions besides MXene, such as PEDOT:PSS or rGO.



Figure 3.5 Development of HDsEMG arrays based on $Ti_3C_2T_x$ MXene. (A–F) Flow chart of the fabrication process for realizing multichannel $Ti_3C_2T_x$ -based high-density arrays for sEMG. (G) Light microscopy image of a completed MXene HDsEMG array, with an inset of a single channel. (H) Demonstration of the flexibility and skin conformability of completed MXene HDsEMG arrays. Adapted from (Murphy et al. 2020).

Previous studies in the Vitale lab first established $Ti_3C_2T_x$ MXene for neural recordings in rodents on the microscale (Driscoll et al. 2018b; 2020). Building on this work, I fabricated 16-channel arrays of 1.6 mm x 1.6 mm sEMG electrodes for human recording (Murphy et al. 2020) (Figure 3.5). The resulting electrodes were flexible and highly skin conformable, encouraging us for their use in surface electromyography (sEMG) applications. The fact that we were able to fabricate an array with 16 distinct channels also promised to improve recording resolution, given the recent interest in the literature surrounding higher density surface recording arrays (Martinez-Valdes et al. 2016; 2017; P Cattarello, Merletti, and Petracca 2017;

Roberto Merletti, Holobar, and Farina 2008; R. Merletti, Rainoldi, and Farina 2001; Lapatki et al. 2004; Roberts et al. 2016).

A critically important property for recording devices is the impedance of the device contacts (Franks et al. 2005; Boehler et al. 2020). This property depends on both the geometric surface area (GSA), and the inherent material properties of the contact. A later discussion in Chapter 4 will also reveal the important role that the medium plays on the total impedance, as well. In any event, following fabrication, I first conducted electrochemical impedance spectroscopy (EIS) on completed HDsEMG devices in 1X phosphate buffered saline (PBS; Quality Biological, pH 7.4), in order to compare MXene electrodes to size-matched Au electrodes (Figure 3.6). On the millimeter scale, I found that MXene had an average 1 kHz impedance of 301 ± 56 Ω , which was comparable to the impedance of the Au electrodes (399 ± 38 Ω). At lower frequencies, however, MXene outperformed Au: at 10 Hz, for example, MXene's impedance was 0.46 \pm 0.16 k Ω , which was >40x lower than the 10 Hz impedance of the Au electrodes $(20 \pm 3.4 \text{ k}\Omega)$ (Murphy et al. 2020). This significant reduction in the impedance is to be expected, though, based on previous reports which have highlighted the rough surface morphology and inherently high capacitive performance of $Ti_3C_2T_x$ as affording it with enhanced electrochemical capacity (H. Lin, Chen, and Shi 2018; Hong Ng et al. 2017; B. Xu et al. 2016; Driscoll et al. 2018b). The rough surface morphology of the MXene films, in particular, should result in a larger effective surface area, which in turn facilitates enhanced ion interactions, contributing to the remarkably low impedance observed.



Figure 3.6 Characterization of $Ti_3C_2T_x$ and Au HDsEMG arrays in PBS. (A, B) Light microscopy images of the (A) MXene and (B) Au HDsEMGs. (C, D) Bode plots of the impedance (C) magnitude and (D) phase comparing electrode types. Points represent means, shaded areas show the standard deviations. $N_{MXene} = N_{Au} = 16$ channels. Taken from the Supplementary Information to (Murphy et al. 2020).

Beyond the impedance in PBS, there is also the matter of how the MXene electrodes compare to metal electrodes and other standard clinical electrodes when interfacing with human skin. Accordingly, in a follow-up study, we also explored "cutaneous EIS" (Roberts et al. 2016; Leleux et al. 2014; Murphy et al. 2020), and measured the impedance of MXene HDsEMG arrays on the forearms of healthy human subjects. The MXene arrays had vastly improved skin impedance compared to size-matched Au HDsEMGs, as well as the larger, gelled, and clinically used Ag/AgCl electrodes (Figure 3.7). At the 10 Hz reference frequency for epidermal recordings (American National Standards Institute 2020), the MXene electrodes had impedances of 0.55 k Ω cm² when normalized by the GSA (0.0256 cm²), compared to 2.9 k Ω cm² for same-size Au, and 169 k Ω cm² for Natus Ag/AgCl contacts (GSA = 0.8 cm²). The Covidien (4.3 × 10³ k Ω cm², GSA = 3.14 cm²), 3M (490 k Ω cm², GSA = 10.1 cm²), and ConMed (570 k Ω cm², GSA = 5.1 cm²) electrodes had much higher cutaneous impedance values than the smaller and gel-free MXene electrodes. We ascribed the

lower skin impedance to MXene's intrinsically high capacitance (Ando et al. 2020), as well as to the rough surface morphology of the films. The mechanical properties of the HDsEMG devices may have also played a role, as well, given that the thinness of our parylene-based devices also improves the overall skin conformability (see, again, Figure 3.5).



Figure 3.7 Electrochemical impedance at the skin-electrode interface. (A) Bode plot of the cutaneous impedance modulus for $Ti_3C_2T_x$ and Au HDsEMG arrays compared with Natus pregelled Ag/AgCl disk electrodes. Data points represent means; the shaded regions show standard deviations. $N_{MXene} = N_{Au} = 16$ contacts, and $N_{Natus} = 6$ contacts. (B) Bode plot of the cutaneous impedance phase for the same electrodes as in (A). (C) Scatter plot of the areanormalized 10 Hz impedance moduli for (from left to right): 16-channel $Ti_3C_2T_x$ and Au HDsEMG arrays, Natus disposable disk electrodes, Covidien H124SG EMG pre-gelled Ag/AgCl electrodes. 3M Red Dot adhesive patches, and ConMed Cleartrace 1700-030 commercial electrodes. Data points represent individual channels for the HDsEMG arrays (N = 8 channels for each array). For Natus and Covidien, points represent individual electrodes (N = 6 separate contacts). Solid black lines denote means. Values for the 3M and Cleartrace contacts were obtained from product datasheets. Adapted from (Murphy et al. 2020).
Validation of $Ti_3C_2T_x$ -based HDsEMG arrays for high-fidelity muscular cartography

Encouraged by our impedance analysis in PBS and on human skin, we subsequently assessed the performance of our $Ti_3C_2T_x$ arrays for high-fidelity muscle activity recording in healthy human subjects.

Muscle activity may be in one of two varieties: it may be either evoked through electrical stimulation (Roberto Merletti et al. 2009), or it may occur naturally through voluntary contraction and relaxation of a given muscle group (R. Merletti, Rainoldi, and Farina 2001; Roberto Merletti et al. 2009; Roberto Merletti, Holobar, and Farina 2008). In our validation study, then, we focused primarily on voluntary muscle activation. First, we measured voluntary muscle contractions of the whole hand over the *flexor digitorum superficialis* (FDS). We compared the recordings on our MXene arrays to size-matched Au arrays, as well as to monopolar gelled Ag/AgCl clinical electrodes from Natus, which are the standard electrodes used in clinical sEMG recordings (Figure 3.8). Following this, we next studied the *thenar eminence* (TE) muscle group of the thumb, and observed muscle activation and spread over the course of force loading (*i.e.*, voluntary pinching) trials (Figure 3.9).



Figure 3.8 Baseline sEMG recording with the HDsEMG arrays and a Natus monopolar contact. (A) Experimental setup for a standard voluntary contraction recording experiment. The $Ti_3C_2T_x$ array is placed over the FDS, while Natus electrodes are placed on the elbow (not shown) and at the wrist, as ground and reference, respectively. (B) Close-up image of the MXene array, showing its excellent skin conformability. (C) Examples of the sEMG signal recorded from the FDS during a typical voluntary contraction experiment. The rms envelope of the sEMG signal is overlaid in black for each trace. Signals were recorded separately for the Ti₃C₂T_x and Au arrays, and the Natus electrodes, but data was time- and amplitude-matched offline for ease of viewing. (D) Average power spectral density of the baseline sEMG recordings on all channels of the $Ti_3C_2T_x$ and Au arrays, as well as a single Natus contact, for one subject. Note that a 60 Hz notch filter was applied to the data for all contacts. (E) The average rms envelope of the sEMG signal during the first contractions shown in (C), comparing the magnitudes of the rms signals recorded on each of the three electrode types. A value was computed for the SNR for each electrode, using root-mean-square (rms) values in the 'Baseline' and 'MVC' Regimes shaded in yellow and purple, respectively. Figure adapted from (Murphy et al. 2020).

To record from the FDS, we asked our subjects to make a fist and hold it, then slowly relax. Each such task lasted for ~ 5 s, with ~ 10 s of rest in between. During this time, the subject's sEMG was continuously recorded with a 128-channel Intan Stimulation/Recording Controller, at a sampling rate of 20 kHz. Following recording with all electrode types, the data was notch-filtered offline at 60 Hz, followed by bandpass filtering between 85–400 Hz (Tian et al. 2019). The root-mean-square (rms) envelope of the filtered signal was then calculated using a moving window 200 ms in width, with an overlap of 25 ms (Roberts et al. 2016; Tian et al. 2019). To full

quantify the recording performance among the different electrode types that we explored, we defined our SNR metric according to the equations:

$$SNR_{tot} [dB] \equiv \frac{1}{N} \sum_{n=1}^{N} \sum_{i=1}^{16} (snr_n^i)$$
 (1)

$$snr_n^i \equiv 20 \log_{10} \left[\frac{\max(\mathrm{rms}_{\mathrm{MVC}}) \text{ on electrode } i \text{ in epoch } n}{\max(\mathrm{rms}_{\mathrm{baseline}}) \text{ on electrode } i \text{ in epoch } n} \right]$$
 (2).

In Equation(1), *N* is the total number of voluntary contraction epochs considered in the analysis, where a single epoch was defined as the 5 s of an individual contraction ± 3 s (Figure 3.8, panel E). Equations(1,2) thus allowed us to compute SNRs for all 16 channels of the Ti₃C₂T_x and Au HDsEMG arrays, as well as for the monopolar Natus electrodes, over the same number of voluntary contraction tasks. Looking at the sEMG signal collected during these exercises, then, we computed an average SNR for the MXene array of 39 ± 16 dB over 10 separate voluntary contractions, which was higher than the SNR for the Au HDsEMG array (13 ± 7 dB) and for the pre-gelled Ag/AgCl electrodes (9 ± 5 dB) over the same number of contractions. The trend of higher SNR on the MXene-based electrodes held true for all subjects from whom we recorded voluntary contractions over the FDS (Table 3-2).

Table 3-2 SNRs for each electrode type, for sEMG recordings over the FDS. N_{tasks} is the number of completed contraction tasks for each subject. The SNRs given for the Ti₃C₂T_x and Au HDsEMG arrays are the averages of the epochal SNRs across all 16 channels in the arrays for all of the N_{tasks} , following from Equation(1). The SNRs given for the Natus contacts are the averages of the epochal SNRs calculated for a single electrode, over all of the completed N_{tasks} .

		SNR [dB]			
SUBJ###	Ntasks	Ti ₃ C ₂ T _x HDsEMG	Au HDsEMG	Natus	
001	10	39.23 ± 16.25	12.54 ± 7.35	8.72 ± 4.69	
002	12	24.21 ± 1.67	19.13 ± 2.74	10.99 ± 3.63	
003	5	19.33 ± 4.23	16.57 ± 1.99	—	

The next step in our validation study was to demonstrate the functionality of the our $Ti_3C_2T_x$ HDsEMG arrays for muscular cartography studies, by tracking muscle activation during voluntary force exertion tasks. In these experiments, the electrodes were placed over each subject's TE muscle group, while a reference electrode was placed on the first metacarpal bone of each subject's thumb, and a ground electrode was placed on the back of each subject's hand (Figure 3.9). Subjects were then asked to hold a load cell between their thumb and index finger, and to subsequently pinch

the load cell at different force levels while their sEMG was continuously recorded. The action of pinching between the thumb and forefinger implicates the *abductor pollicis brevis* (APB) and *opponens pollicis* (OP) muscle branches of the TE muscle group, and represents a standard example of the motion known as "opposition" (Kozin et al. 1999; NAPIER 1952). Accordingly, opposition was recorded at each channel of the Ti₃C₂T_x HDsEMG array, while the Intan system was also able to pick up the magnitude of exerted force at the load cell, as well (Figure 3.9, panel B).



Figure 3.9 High-resolution mapping of muscle activation in the TE with the $Ti_3C_2T_x$ HDsEMG array. (A) Picture of the experimental setup. The $Ti_3C_2T_x$ array is placed over the TE muscle group of the thumb, and the subject is asked to pinch a force loading cell between their thumb and forefinger at varying levels of exerted force. (B) Example of HDsEMG and force data recorded during successive load cell trials in a single experiment, for one subject. The top trace in red tracks the force intensity recorded at the load cell, while the traces in blue below are the recorded sEMG for each channel in the $Ti_3C_2T_x$ array. The rms envelopes fir each channel are overlaid on the sEMG data, shown in black. (C–F) Maps of the rms amplitude generated during the epoch highlighted in green in (B). Maps were generated during the (C) pinch grip, (D) loading start, (E) maximum loading, and (F) relaxation phases of the force loading task. Locations of the channels of the MXene array are overlaid on the heatmaps, with the channel numbering shown in (C). Adapted from (Murphy et al. 2020).

To streamline interpretation of the collected data, we generated heatmaps representative of the rms envelope of the sEMG signals collected with our highdensity $Ti_3C_2T_x$ arrays (Figure 3.9, panels C–F). This allowed us to see very clear spatial patterns of muscle activation over time, which in turn provided us with insights into the underlying muscle activity during each force exertion task. Initially, there was little activation of any particular branch of the TE, as the muscle group remained in a relaxed phase (Figure 3.9, panel C). However, we observed clear activation of the APB subgroup of the TE after >50% of the maximal contraction force, F_{max} , had been exerted on the loading cell (Figure 3.9, panel D). Once F_{max} had been reached, there was also the recruitment of the OP subbranch of the TE group (Figure 3.9, panel E). Finally, during the relaxation phase of the pinching trial, we saw the deactivation of all implicated branches of the TE group (Figure 3.9, panel F). Besides the APB and OP, there is also another muscle branch belonging to the TE group, called the *flexor pollicis brevis* (FPB). However, FPB is not implicated in opposition of the thumb and index finger, as are the APB and OP branches (Kozin et al. 1999; NAPIER 1952). Since the upper-righthand corner of the rms heatmaps saw little change in magnitude during the entirety of the force loading experiments, we concluded that, from an anatomical standpoint, this region of the Ti₃C₂T_x array must have been lying over FPB.

As a final analysis in this validation study, we also correlated the force of the subject's pinching grip, F, to the recorded sEMG signal. This is a sensible analysis given that sEMG intensity, I_{EMG} , is linearly related to the contraction force following from the equation (Jacquelin Perry and Bekey 1981):

$$\log I_{EMG} = k \cdot \log F \tag{3}.$$

With this relationship, we could use the slope of the line, k, as a way to measure the electrode sensitivity, and compare across materials, allowing us to directly compare MXene to Au and Ag/AgCl. Accordingly, we asked one subject to complete force loading tasks with all three electrode types placed over their TE muscle group. Following data acquisition, we plotted the average rms signal of the subject's sEMG for each trial vs. the maximum force they exerted in that trial, and we fit the results with a line of best fit (Figure 3.10). From this analysis, we found that the MXene-based electrodes had force loading sensitivities (*i.e.*, the slope of the line) of $k = 49.61 \text{ mV}_{\text{rms}}$ N^{-1} cm⁻² when normalized by the GSA. This level of sensitivity was ~10x higher than the same-size Au electrodes ($k = 4.69 \text{ mV}_{\text{rms}} \text{ N}^{-1} \text{ cm}^{-2}$), and ~50x higher than the larger Natus electrodes ($k = 1.59 \text{ mV}_{\text{rms}} \text{ N}^{-1} \text{ cm}^{-2}$). Thus, we found further support for the fact that our MXene HDsEMG arrays were better suited for resolving changing force levels than either the dry Au electrodes or the pre-gelled Ag/AgCl contacts. This result further supported our findings, as well, from the SNR analysis over each subject's FDS, emphasizing once more that $Ti_3C_2T_x$ has the capacity to endow wearable monitoring platforms with higher sensitivity than either Au or pre-gelled Ag/AgCl.



Figure 3.10 Correlation of sEMG signals and loading force. Scatter plot of the average rms signal from sEMG recording *vs.* the maximum exerted force in each epoch. Points represent individual pinches in a single recording experiment. Lines were fitted to the data on a log-log scale, for each of the electrode types, to characterize the sensitivity of the electrode material at resolving changing levels of force exertion. Sensitivity values were normalized by the electrode GSA to allow for accurate comparison among the different materials and electrode designs. Adapted from the Supplementary Information to (Murphy et al. 2020).

Outlook

Altogether, our validation study established $Ti_3C_2T_x$ -based HDsEMG arrays as a useful tool for highly sensitive, highly specific muscle cartography. Due to the high density and small sizes of the electrodes in our arrays, we were able to precisely discriminate between various regions of muscle activity with millimeter-scale resolution, as demonstrated by the force loading results over the TE muscle group (Figure 3.9). It is worth noting that state-of-the-art, monopolar sEMG contacts, such as the Natus Ag/AgCl electrodes, are too large and too coarse to localize muscle activity at this resolution, nor can they clearly resolve changing spatial patterns of recruitment and deactivation among individual muscles within a group over time. Thus, our validation study demonstrated, for the first time, the use of $Ti_3C_2T_x$ MXene for sEMG acquisition in human subjects, with significant improvements over current, metal-based clinical standards. This study also represented the first direct biopotential signals study to use $Ti_3C_2T_x$ MXene on human subjects, as opposed to the epidermal strain/stress sensing that has been previously reported in the literature (Cai et al. 2018; Kedambaimoole et al. 2020; Jiaqi Zhang et al. 2019). Future directions may seek to realize even higher-density HDsEMG arrays based on $Ti_3C_2T_x$ MXene. Indeed, even in the Vitale lab, we have already explored the fabrication of a 24-channel MXene array, following the protocols detailed above. Figure 3.11 shows one such fully fabricated array, which could be used for muscle cartography studies in larger muscle groups than the TE, such as the *biceps brachii* or quadriceps.



Figure 3.11 An alternative $Ti_3C_2T_x$ HDsEMG array with higher channel count and different mapping configuration (24 channels in an 8 x 3 grid). The separate branches at the device backend were made to be compatible with two separate, 18-channel Intan head-stages, to allow for simultaneous recording from all 24 channels at one time. The completed 24-channel $Ti_3C_2T_x$ array in the image at right sits atop a custom-cut piece of $EcoFlex^{TM}$, which may be used to improve adhesion of the electrode to the skin. Although we found that EcoFlex was not necessary for good skin conformability, due to the thinness of completed devices, nevertheless, it could be beneficial for use as a kind of supporting, "sticky" adhesive layer for longer-term studies.

Another area of future interest may be to apply the $Ti_3C_2T_x$ HDsEMG arrays for recoding during evoked potential (EP) studies. EP recording experiments are particularly useful for studying discharge rates and muscle fiber conduction velocities (Reaz and Hussain 2006), and in a clinical setting, EP sEMG may be used to modulate muscle activation and coordination (Drost et al. 2006; Martinez-Valdes et al. 2016; 2017), to investigate neural control of fine motor movement (Vigotsky et al. 2018; Tao et al. 2012), or to define peripheral nerve and muscle fiber conduction behavior and interaction (Farina et al. 2019; Troiano et al. 2008). Muscle activation mapping via EP sEMG has also been demonstrated as a useful tool for localizing the neuromuscular junction (NMJ), which is a common target in chemodenervation therapies to treat muscle spasticity (Chang et al. 2013; Simon and Yelnik 2010). In this paradigm, the target muscle complex is purposefully stimulated, and the EP response is observed with an sEMG array. By calculating the latency of the EP response to stimulation across each channel of the array, it is possible to localize the

area of shortest latency, which can be taken to be closest to the zone of actual muscle innervation. In other words, EP latency may be treated as a proxy for spatial localization of the innervation zone. As mapping of the activation and coordination of large muscle groups—and thus accurately localizing the NMJ—would necessarily require electrode arrays with large area coverage and higher channel counts (Farina et al. 2008), the $Ti_3C_2T_x$ HDsEMG arrays proposed in this chapter—as well as HDsEMG technologies in general (Roberto Merletti, Holobar, and Farina 2008; Martinez-Valdes et al. 2016)—are well-suited technologies to meet these requirements.

In fact, we have already run a few preliminary studies investigating EP recording with the $Ti_3C_2T_x$ HDsEMG arrays, as Figure 3.12 illustrates. In our initial study, we were excited to be able to corroborate the results seen in our voluntary force contractions studies (Figure 3.9), as we saw shorter latencies for the APB and OP branches of the TE muscle group implicated in opposition between the thumb and forefinger, when we stimulated the median nerve which innervates these same regions of the TE muscle group (Figure 3.12, panel C).



Figure 3.12 Evoked potential recording with the $Ti_3C_2T_x$ HDsEMG array. (A) Picture of the experimental setup, involving placing the $Ti_3C_2T_x$ array over the TE muscle group, and stimulating the median nerve with a handheld stimulator. (B) Close-up image of the $Ti_3C_2T_x$ array on the skin, with the approximate summary region of the APB and OP muscle groups highlighted in orange. (C) Heat map of the average onset latency (in milliseconds) of the evoked responses to stimulation, as recorded with the $Ti_3C_2T_x$ HDsEMG array. Warmer colors represent longer latencies, and cooler colors represent shorter latencies. The average Eps recorded at each channel of the array are represented by the traces in white, grey, and black. The divisions in the lower left corner of the map correspond to the average EP traces, and represent 10 mV along the *y*-axis and 5 ms along the *x*-axis.

Indeed, the utility of EP muscle activation mapping with HDsEMG has previously been demonstrated for single motor unit analysis in facial musculature (Lapatki et al. 2004), as well as for localizing the NMJ activation area in human biceps (G. Rasool et al. 2017; Roberts et al. 2016). HDsEMG technologies have even been used in EP studies approximating the propagation of motor unit activity in the *vastus medialis* and *vastus lateralis* as isometric knee extensions are completed (Martinez-Valdes et al. 2016; 2017). Accordingly, we believe our MXene-based HDsEMG arrays could be beneficial for further EP studies along similar lines. Once again, we would highlight that the true advantages of our arrays over current technologies are due to the inclusion of $Ti_3C_2T_x$ MXene in high-density, and in thin packaging, which allowed us

- 1. to realize a highly skin-conformable device, resulting in lower interfacial impedance and, thus, improved signal recording quality;
- 2. to observe millimeter-activity over millisecond timescales, increasing the overall resolution of our recording study; and
- 3. to offer a gel-free electrode technology which boasts superior performance compared to current, largely gelled, state-of-the-art technologies.

Altogether, then, the $Ti_3C_2T_x$ MXene HDsEMG arrays may prove to be very useful tools for a variety of studies, with applications in a wide range of fields, such as sports medicine and monitoring, physical medicine and rehabilitation, and prosthetics.

Chapter 4. Equivalent Circuit Models for Bioelectronic Interfaces

In Chapter 4, I develop a library of equivalent circuit models to help describe the behavior of $Ti_3C_2T_x$ -based electrodes interfacing with the most common media used in the biological sciences. First, I introduce and motivate the need for equivalent circuit models to help understand the performance of fabricated electrodes. Second, I propose an equivalent circuit model for MXene electrodes interfacing with physiologic saline. Finally, I propose equivalent circuit models to describe both clinical electrodes and $Ti_3C_2T_x$ -based interfaces interacting with the complex and multifaceted medium of human skin. This chapter emphasizes the role that modelling can have in understanding the interactions between recording electrodes and the target media. Furthermore, I discuss how circuit models may help not only for designing better technologies, but also for exploring alternative interventions at the medium level, as well, with the overall goal of improving recording performance.

Relevant Citations:

(1) Murphy BB, Mulcahey PJ, Driscoll N, Richardson AG, Robbins GT, Apollo NV, Maleski K, Lucas TH, Gogotsi Y, Dillingham T, Vitale F. A Gel-Free $Ti_3C_2T_x$ -Based Electrode Array for High-Density, High-Resolution Surface Electromyography. *Advanced Material Technologies*, 2020, 2000325.

(2) Murphy BB, Scheid BH, Hendricks Q, Apollo NV, Litt B, Vitale F. Time evolution of the skin-electrode interface impedance under different skin treatments. *Sensors*, 2021, 21:5210.

Equivalent Circuit Modelling: Theory & Best Practices

Equivalent circuit models can be invaluable tools for understanding—and sometimes predicting—the interactions between fabricated electrodes and media such as physiologic saline (Franks et al. 2005; Hirschorn et al. 2010; Boehler et al. 2020) or the surface of human skin (Heikenfeld et al. 2017; Beckmann et al. 2010). Often, such models are developed in order to simplify calculations and analysis of more complex physical or electrical systems (Johnson 2003). This is the case, for example, with the widespread Thévenin circuit, which is used to reduce electrical circuitry with loading resistances down to a simpler circuit featuring only a single voltage source with a series resistor (Haley 1983). The Norton equivalent circuit does something similar, though with a current source, instead of a voltage source (Johnson 2003).

In its most basic form, an equivalent circuit model should be composed of only linear, passive elements: basic resistors, capacitors, and (though rarely) inductors. This arises from the fact that all circuit behavior stems originally from Ohm's Law, Kirchhoff's Laws, and the superposition theorem (Johnson 2003), and each of these only involves the most basic electronic components. One equivalent circuit model that uses only passive linear elements is the simple Randles cell (Figure 4.1), which is also perhaps the most commonly used equivalent circuit model in the biological sciences (Randles 1947; Franks et al. 2005). The Randles cell consists of a solution (or spreading) resistance (R_s) in series with a parallel circuit comprising a double-layer capacitance (C_{dl}) and a charge-transfer resistance (R_{ct}). The Randles cell most often represents an electrode submerged in an ionic solution, where R_s is used to describe ionic currents passing through the aqueous medium, while C_{dl} describes the charging of the electric double-layer at the electrode surface, and R_{ct} is included to describe any Faradaic reactions that might occur in parallel with the charging of the double-layer.



Figure 4.1 The simple Randles cell. A solution resistance (R_s) is used to describe ionic currents flowing through the electrolyte and interacting with the electrode. The electrode is described by a parallel RC circuit, comprising a charge-transfer resistance (R_{ct}) and a double-layer capacitance (C_{dl}).

From the Randles cell configuration, it is possible to calculate the total impedance, Z_{tot} , at the electrode-saline interface, which follows from the equation,

$$Z_{tot} = Z_{R_s} + \left(\frac{1}{Z_{R_{ct}}} + \frac{1}{Z_{C_{dl}}}\right)^{-1} = R_s + \frac{R_{ct}}{1 + j\omega R_{ct}C_{dl}}$$
(1),

where *j* is the imaginary unit, and $\omega = 2\pi f$ is the angular frequency in rad s⁻¹, with the frequency, *f*, in Hz. It is evident from Equation(1) that the second term becomes negligible at high frequency values, and so the overall impedance is dominated by the first term, or the solution resistance. At lower frequencies, however, the second term—describing the actual electrode behavior, and dependent on the frequency through ω —dominates the impedance response.

While the Randles cell is very commonly applied in EIS in order to interpret the total impedance spectra of electrodes in aqueous media and model their frequency response according to Equation(1), there are yet some phenomena that cannot be perfectly described with simple combinations of resistors and capacitors alone. For example, if the rate of Faradaic reactions is diffusion-controlled, a simple R_{ct} may not be enough to capture this information (Boehler et al. 2020). Especially when exploring equivalent circuit models for porous, rough, or irregular electrode surfaces interfacing with biological tissue, issues may arise from the fact that there are no simple circuit elements that accurately describe the complex behavior of the electrode surface or the biological tissue. Accordingly, approximations may be made using non-ideal components, such as the constant phase element (CPE) or the Warburg impedance (itself a special case of the CPE) (Boehler et al. 2020; Mcadams and Jossinet 1996; Hirschorn et al. 2010). Introducing these elements increases the overall model complexity, however, making physical interpretation of the model a touch more difficult. Still, such elements have been shown to help with modelling of specific physical or chemical phenomena, particularly in corrosion studies of metallic films in aqueous salt and acid solutions (J. Pan, Thierry, and Leygraf 1996; Nady, El-Rabiei, and Samy 2017; Jayaraj et al. 2019).

There are certain strategies and "best practices" that may be employed to fully ensure one's equivalent circuit model is fundamentally sound, as well. One such practice is to limit the number of equivalent circuit components to the bare minimum necessary to describe the behavior observed in the total impedance spectra. Furthermore, by introducing logical bounds for each complex circuit element's value, based either on measurements or on theory (Franks et al. 2005; Heikenfeld et al. 2017), one can also keep their model more physically sensible and accurate. By applying these best practices during model composition, it becomes possible to create more robust, more generalizable equivalent circuit models, which may be applicable for a wider variety of conditions.

An Equivalent Circuit Model for Ti₃C₂T_x in aqueous media

To better understand how $Ti_3C_2T_x$ films interact with aqueous media, we developed and applied an equivalent circuit model to the impedance data collected in PBS with the MXene HDsEMG arrays from Chapter 3. We also fabricated larger area electrodes (1.5 mm, 3 mm, and 1 cm diameter disk-shaped electrodes) in order to explore the robustness of our model at capturing the electrode behavior. We used the *EChem Analyst* software package (v7.8) made publicly available through Gamry Instruments, Inc. to build our equivalent circuit model, and based our initial designs for the circuit on the simple Randles cell. However, given that $Ti_3C_2T_x$ is known to exhibit pseudocapacitive behavior (Ando et al. 2020), we decided to adopt a CPE in our model in place of the *C*_{dl} (inset of Figure 4.2, panel A).



Figure 4.2 Modeling the impedance of $Ti_3C_2T_x$ electrodes in 1X PBS. (A) Bode plot of the impedance modulus of a 1.5 mm diameter disk-shaped MXene electrode in 1X PBS. Inset shows the modified Randles cell used as the equivalent circuit model to fit the impedance data. (B) Bode plot of the impedance phase for the same electrode as in (A). (C) Bar plot of the C_{dl} for various electrode types measured in 1X PBS. Note that the $Ti_3C_2T_x$ -based electrodes have higher C_{dl} than the purely metallic electrodes. This is attributed to the rough surface morphology of the MXene films.

CPEs are often employed when modelling "imperfect" capacitors. Porous electrodes, permeable coatings, and roughened surfaces are a few representative examples that may result in "imperfect" capacitive behavior, as the surface morphology of the electrode has significant effect on the charging rates of the electric double-layer (Boehler et al. 2020; Hirschorn et al. 2010; Ivanovskaya et al. 2018). The rough edges and coarse morphology of dried films of $Ti_3C_2T_x$ makes them likely

candidates to be described by the CPE. Certainly, as well, this morphology plays a key role in MXene's excellent capacitive behavior (Anasori, Lukatskaya, and Gogotsi 2017; Kong et al. 2018). Now, the CPE has an impedance, Z_{CPE} , described by the equation (Boehler et al. 2020; Hirschorn et al. 2010):

$$Z_{CPE} = \frac{1}{Y_0(j\omega)^{\alpha}}$$
(2),

where Y_{θ} is the admittance of the CPE, and $\alpha \in [0,1]$ is the CPE constant. The CPE constant is essentially a measure of how resistive or capacitive the CPE behaves: a value of $\alpha = 1$ describes an ideal capacitor, while a value of $\alpha = 0$ describes a pure resistor. Accordingly, the CPE is a circuit element that is allowed to vary between purely Faradaic and purely non-Faradaic reactions, and exists so as to model the frequency dependence of such a non-ideal capacitor (Hirschorn et al. 2010). As a final point, the effective double-layer capacitance can be computed from the CPE parameters and the solution resistance, following form the equation (Boehler et al. 2020; Hirschorn et al. 2010):

$$C_{dl} \equiv \frac{(R_s Y_0)^{1/\alpha}}{R_s} \tag{3}$$

With knowledge of the CPE thus established, we modeled the total impedance of MXene-based electrodes interfacing with 1X PBS at difference sizes. Exemplary fits are shown in Figure 4.2 for the 1.5 mm electrodes. As can be seen, the modified Randles cell appropriately fits the impedance spectra, and accurately tracks the frequency response of the MXene electrodes. On average, the values of the goodness of fit from a Pearson's χ^2 test comparing the fitted data to the experimental data ranged from $5 \times 10^{-5} - 3 \times 10^{-2}$, where the degrees of freedom, *df*, was always 4 (*i.e.*, the total number of fitting parameters: $df_1 = R_s$, $df_2 = R_{ct}$, $df_3 = Y_0$, and $df_4 = \alpha$). These values of goodness of fit demonstrate that our modified Randles cell is a robust equivalent circuit model, which can accurately describe the behavior of Ti₃C₂T_x-based electrodes interfacing with physiologic saline.

Further support of our equivalent circuit model came from calculations of the C_{dl} values from impedance fitting, following Equation(3). By looking across a variety of MXene electrodes, we were able to observe expected trends in the data (Figure 4.2, panel C). Comparing Ti₃C₂T_x electrodes to metal electrodes composed of Pt, Au, or Ag, we found that MXene had higher values of C_{dl} than the metallic electrodes. This was an expected outcome, and may be attributed to the high electronic conductivity and rougher surfaces of the MXene electrodes in comparison to the smooth surfaces of the metal electrodes (Driscoll et al. 2018b). Comparing only the MXene electrodes, we

further found that the higher film uniformity and higher MXene density, as achieved with spray-coating, resulted in a much larger double-layer capacitances for the HDsEMG arrays, compared to the same arrays made via spin-coating. When we fabricated larger-area electrodes, too, we saw the expected trend in the C_{dl} values: namely, that larger surface areas resulted in higher capacitances. This result is to be expected given that the capacitance is generally proportional to the electrode area, following from the equation for a parallel-plate capacitor from introductory physics:

$$C = \varepsilon \varepsilon_0 \frac{A}{d} \tag{4},$$

where *C* is the capacitance, ε is the material permittivity, ε_0 is the permittivity of free space, *A* is the area of the parallel-plates, and *d* is the separation between them.

Table 4-1 Summary of the fitting results for MXene and metal electrodes in 1X PBS. The equivalent circuit parameters are normalized by the geometric surface area so as to allow easier comparison between materials. Values of the CPE constant α were restricted to $\alpha \in [0.80, 1]$ for the metallic electrodes, since it was hypothesized that metals would behave as more pure capacitors compared to Ti₃C₂T_x-based electrodes. All circuit element values are reported as avg. \pm std. dev. from values of fitting across N = 3 separate impedance spectra for each electrode type.

Material GSA [cm ²]		<i>R</i> s [Ω cm ²]	<i>R_{ct}</i> [kΩ cm ²]	<i>C_{dl}</i> [µF ст ⁻²]	α [unitless]	χ ² (×10 ²)
Pt	Pt 0.0415 3.67 ± 0.98 9.82 ±		9.82 ± 2.02	43.08 ± 5.32	0.97 ± 0.02	1.12 ± 0.13
Au	0.0256	5.97 ± 0.66	339.12 ± 31.37	31.37 ± 3.39	0.96 ± 0.01	0.88 ± 0.02
	0.0707	4.65 ± 1.01	121.30 ± 47.93	36.93 ± 2.94	0.94 ± 0.03	0.03 ± 0.00
	0.0177	3.42 ± 0.14	252.35 ± 54.15	29.94 ± 3.18	0.93 ± 0.02	0.02 ± 0.00
Ag	0.62	4.51 ± 0.76	1.31 ± 0.83	16.13 ± 0.73	0.92 ± 0.04	3.43 ± 0.48
Spin-cast MXene	0.0256	7.89 ± 1.13	24.56 ± 4.94	3.17 ± 0.56	0.85 ± 0.01	0.23 ± 0.12
Spray-cast MXene	0.0256	5.82 ± 0.06	215.77 ± 96.12	623.99 ± 46.99	0.83 ± 0.08	0.68 ± 0.09
	0.785	7.13 ± 0.98	496.14 ± 24.70	631.80 ± 47.50	0.89 ± 0.02	0.84 ± 0.12
	0.0707	4.24 ± 0.05	15.38 ± 5.43	(2.1 ± 0.2) 10 ³	0.84 ± 0.04	0.05 ± 0.01
	0.0177	2.14 ± 0.13	2.52 ± 0.32	$(2.0 \pm 0.6) 10^3$	0.76 ± 0.12	0.04 ± 0.00

In a little more detail, Table 4-1 provides a summary of the results from fitting the impedance data of various electrodes in 1X PBS. One noteworthy aspect of this

exploration is the fact that all electrodes exhibited similar values of R_s when normalized by the electrode area, within the range of 2–8 Ω cm². The solution resistance is, in fact, one of the few parameters within the Randles cell that can be theoretically derived, as it depends only on the resistivity of the medium and the electrode geometry (Franks et al. 2005). For square electrodes, the theoretical value of R_s is determined by the equation

$$R_s = \frac{\rho \ln 4}{\pi l} \tag{5},$$

where l is the side-length of the electrode; and for disk-shaped electrodes, the equation is

$$R_s = \frac{\rho}{4r} \tag{6},$$

where *r* is the electrode radius. In both Equations(5,6), ρ is the resistivity of the medium, which, for 1X PBS at room temperature, is $\rho = 72 \Omega$ cm (Franks et al. 2005). Given that all the electrodes we explored were millimeter-scale in size, our surface areas were within the range of 0.01 cm² < GSA < 1 cm². Thus, it is reasonable that all electrodes should have similar values of R_s .

Table 4-1 also clearly highlights the improved capacitive behavior of $Ti_3C_2T_x$ MXene compared to bare metals: while Pt, Au, and Ag exhibit C_{dl} on the order of only a few tens of μ F cm⁻², the spray-cast MXene-based electrodes exhibited 1–2 orders of magnitude greater capacitance. This is to be expected based on previously published literature highlighting MXene's enhanced ability for energy storage (Ando et al. 2020; Lukatskaya et al. 2017; S. Xu et al. 2017; Anasori, Lukatskaya, and Gogotsi 2017). Values of the CPE constant, α , also highlight that $Ti_3C_2T_x$ does not, in fact, behave as a perfect capacitor in aqueous media, since $\alpha \in [0.75, 0.90]$ across all MXene electrodes. Nevertheless, by including the CPE in our modified Randles cell, we were able to track changes in the MXene dried films behavior just as clearly.

In conclusion, given the accuracy of the fitting procedure, and further supported by the appearance of expected trends in data calculated after the fitting process was completed, we have concluded that the modified Randles cell featuring a CPE in place of the C_{dl} is an appropriate model to describe $Ti_3C_2T_x$ electrodes interfacing with an aqueous medium. Future studies may look into applying this equivalent circuit to aid in the design of novel $Ti_3C_2T_x$ electrodes for *in vivo* applications. This equivalent model may also provide useful insights for biochemical sensing applications using MXene electrodes, as knowledge of the electrode's behavior during diffusion-controlled reactions can have an impact on the ability of that electrode to sense certain analytic species *in vitro*.

An Equivalent Circuit Model for skin-based recording

Unlike electrolytic solutions, human skin is a significantly more complex recording medium. In fact, skin is a natural barrier to direct charge transfer, as it is highly resistive (Heikenfeld et al. 2017). The multilayered epidermis—and particularly the stratum corneum, the uppermost epidermal layer—is a highly complex barrier with electrical, chemical, and mechanical properties that depend on the subject's sex, age, diet, sweat and activity levels, and even environmental conditions such as the temperature and humidity, among many others (Matsukawa et al. 2020; Heikenfeld et al. 2017; Paolo Cattarello and Merletti 2016; White, Orazem, and Bunge 2013; F. Lu et al. 2018; Andrews, Lee, and Prausnitz 2011; Yamamoto and Yamamoto 1976).

As described in Chapter 2, the majority of skin-based recording electrodes especially those most commonly used in a clinical setting—are composed of metallic contacts such as Pt, Au, or Ag/AgCl (Roberto Merletti et al. 2009). Given the highly resistive nature of human skin, though, conductive gels, adhesives, or pastes are typically necessary between the skin and the electrode, in order to lower the interfacial impedance and thereby improve signal quality (G. Li, Wang, and Duan 2017; Yamagami et al. 2018; H. L. Peng et al. 2016; Roberto Merletti et al. 2009; Puurtinen et al. 2006). However, prior studies have revealed that the noise present in gelled electrode technologies originates primarily from the very same skin-gel interface (Huigen, Peper, and Grimbergen 2002), and although additional factors such as the electrode surface area and the amount of pressure applied to the contact can also cause slight variations in the impedance or the recording quality (Mihajlovic and Grundlehner 2012; G. Li, Wang, and Duan 2017), once more it boils down to the skin's inherent properties, rather than the electrode properties, that have the greatest impact on the interfacial impedance and its stability over time (Heikenfeld et al. 2017; White, Orazem, and Bunge 2013).

To mitigate some of the issue posed by the epidermis, skin treatments are often applied before electrode placement. The most common such intervention is abrasion of the skin with sandpaper tape, which removes dead skin, oils, dirt, and other such debris from the skin surface and uppermost epidermal layers (David J. Hewson et al. 2003; Ferree et al. 2001; Paolo Cattarello and Merletti 2016). However, this same abrasion can also cause skin irritation and discomfort for the subject; furthermore, the initial effects of abrasive skin treatments may not persist over extended periods of time, as some studies have shown that the skin impedance will naturally decrease once the skin rehydrates with sweat (Baek et al. 2008; Pylatiuk et al. 2009; H. L. Peng et al. 2016; Huigen, Peper, and Grimbergen 2002; Matsukawa et al. 2020; David J. Hewson et al. 2003). The recovery of the stratum corneum after abrasion has also been well-documented (Andrews, Lee, and Prausnitz 2011; Yamamoto and Yamamoto 1976), and it only takes \sim 12–24 hrs. before the skin has fully recovered after abrasive skin treatments are applied.

Given that maintaining a low-impedance skin-electrode interface is absolutely necessary to facilitate clean and meaningful recording of skin-based biopotentials (Puurtinen et al. 2006: Radüntz 2018: Paolo Cattarello and Merletti 2016: Piervirgili. Petracca, and Merletti 2014; Huigen, Peper, and Grimbergen 2002), pursuing an equivalent circuit model to describe and track changes in the interfacial impedance between human skin and electrode technologies is a valuable exercise. Already, a variety of equivalent circuit models have been proposed in literature (Oliveira et al. 2014; C. T. Lin et al. 2011; Beckmann et al. 2010; H. L. Peng et al. 2016; David J. Hewson et al. 2003; Heikenfeld et al. 2017; Mihajlovic and Grundlehner 2012), and among these models, a few distinguish between phenomena occurring at the electrode level from phenomena happening at the skin level. Such a dichotomized circuit model could be particularly useful for guiding electrode designs, or might be valuable in informing approaches to alternative skin treatments, as well. Nevertheless, there remain very few systematic approaches proposed in the literature to effectively evaluate the effects of different skin treatments on equivalent circuit parameters, nor how such parameters may change over time (David J. Hewson et al. 2003; Oliveira et al. 2014). As such, we developed our own methodology for exploring the electrochemical impedance of the complete skin-electrode interface over time, using standard clinical pre-gelled electrodes as our validation technology. After collecting impedance data, we fitted the collected data to an equivalent circuit model in order to illustrate the effects of various skin treatments on the equivalent circuit parameters over time.

Study Methodology & Experimental Setup

More specifically, we explored four skin conditions. We chose (i) mechanical abrasion, (ii) chemical exfoliation, and (iii) microporation as our primary skin treatments, and compared these to a (iv) no treatment control. We monitored the skin-electrode impedance under these skin conditions over a full 24-hour period in a cohort of 14 healthy human subjects. Fitting the experimental impedance data with our proposed model (Figure 4.3), we extracted equivalent circuit parameters, and then developed and tested hypotheses to explain differences in the impedance response at specific timepoints. We clearly detailed how these changes in the overall impedance arose from variations in the skin- or the electrode-level elements.



Figure 4.3 Schematic of the equivalent circuit model used for fitting skin-electrode impedance data for gelled electrodes. At the electrode level (right three elements): R_{ct} is the charge-transfer resistance, C_{dl} is the double-layer capacitance, and R_{gel} is the resistance of the gelled electrolyte coating the electrode surface. At the skin level (left three elements), R_{epi} and C_{epi} are the epidermal resistance and capacitance, respectively, and R_{sub} is the resistance of the dermis and subcutaneous fat layers.

Experimentally, we first cleaned the skin of each subject's upper arms using an alcohol prep pad. Next, we applied the skin treatments. Again, the main interventions that we evaluated were: (i) mechanical abrasion with 3M TracePrepTM abrasive tape (AT); (ii) skin exfoliation with Neutrogena® Acne Face Wash, featuring 2% salicylic acid (SA); and (iii) microporation of the skin using the AdminPatch® 0900 microneedle array device from AdminMed nanoBioSciences LLC (µNA). Abrasive tape was selected because it is the most widely used intervention in a clinical setting. It is also known to remove significant portions of the stratum corneum, thus allowing for easier charge-transfer between the more conductive underlying tissue layers, and the electrode contact (Heikenfeld et al. 2017; F. Lu et al. 2018; Yamamoto and Yamamoto 1976). We similarly chose SA because we expected it to be able to chemically exfoliate the stratum corneum in a less damaging or irritating fashion than medical tape (Decker and Graber 2012; Arif 2015). We chose the FDA-approved µNA device because it is commonly used to deliver drugs and vaccines to the dermis layer of the skin (Cormier et al. 2004; Nguyen et al. 2020; Ilić et al. 2018). It is composed of 85 stainless steel microneedles, all within a 1 cm² circular area; each needle is 800 μm tall, allowing them to penetrate the stratum corneum and viable epidermis layers, without damaging the blood capillaries and pain receptors located deeper in the dermis (D. Zhang, Das, and Rielly 2014). The µNA device typically forms aqueous micropores in the skin surface that are $\approx 200 \ \mu m$ in diameter (D. Zhang, Das, and Rielly 2014), and so we hypothesized these micropores would allow for readier release of sweat and oils from the deeper skin layers to the surface, which is known to improve impedance properties at the skin-electrode interface (Huigen, Peper, and Grimbergen 2002; Matsukawa et al. 2020).

Each skin treatment was applied to a skin region approximately 5 cm² in area, and then pre-gelled CleartraceTM 1700-O30 Diagnostic ECG electrodes from ConMed Corporation were placed in a row down the length of the subject's arm over each treatment region, respectively. A total of four separate skin conditions were thus explored for each subject, and replicated on both arms. The level of "harshness" of the skin conditions increased from NT (the gentlest), to μ NA, SA, and AT (the harshest). The spatial order in which we applied the skin treatments to the length of each subject's upper arms was randomized from subject to subject, in order to eliminate potential effects from the arm region.

Following electrode placement, the skin impedance was measured on both arms of each subject at three timepoints: (i) after a 5 minute equilibration period following electrode placement (t_0), (ii) 8 hours after placement (t_{mid}), and (iii) 24 hours after the initial electrode placement (t_f). Before each impedance measurement, two ConMed electrodes were placed on the elbow and the deltoid muscle as counter and reference electrodes, respectively. The skin impedance was measured via "cutaneous EIS," using a 10 mV_{rms} sinusoidal input driving voltage on a Gamry Instruments Interface 1010E potentiostat/galvanostat/ZRA, with a 1–10⁵ Hz frequency sweep (Roberts et al. 2016; Leleux et al. 2014; Murphy et al. 2020). To secure and protect the electrodes from the subject's unconscious everyday movements that could have displaced or detached the contacts, we wrapped a self-adhering non-woven bandage around each subject's upper arms; this wrap was removed for each impedance measurement, and was replaced afterwards.

The equivalent circuit model that we developed for all impedance spectra fitting is shown in Figure 4.3. It was built in *EChem Analyst*, and describes a metalbased electrode with a gel electrolyte coating, interfacing with the epidermal and subcutaneous layers of the skin (Heikenfeld et al. 2017; Beckmann et al. 2010). This model allows for more precise evaluation and comparison of effects from the electrode-level *vs* skin-level components. The total impedance of this circuit is

$$Z_{tot} = R_{sub} + \left[\frac{R_{epi}}{1 + j\omega R_{epi}C_{epi}}\right] + R_{gel} + \left[\frac{R_{ct}}{1 + j\omega R_{ct}C_{dl}}\right] \quad (7).$$

The elements belonging to the clinical grade electrode comprise: (i) R_{gel} , the gelled electrolyte resistance, (ii) R_{ct} , the charge-transfer resistance and (iii) C_{dl} , the double-layer capacitance. The representative skin elements comprise: (i) the subcutaneous resistance, R_{sub} , (ii) the epidermal resistance, R_{epi} , and (iii) the epidermal capacitance, C_{epi} .

Given that there are six total degrees of freedom in this equivalent circuit model, we suspected that we would overfit the experimental data, or else have physically implausible values, if we did not restrict out parameter spaces. Accordingly, each circuit element was assigned specific bounds, chosen according to values reported in literature (Heikenfeld et al. 2017; Paolo Cattarello and Merletti 2016), and based on considerations of the total impedance magnitude as given in Equation(7). We employed the same parameter bounds for each model parameter across all subjects, all treatments, and all timepoints. We also normalized all parameter values by the electrode surface area (5.1 cm²) in order to attain expected values and ranges for our equivalent circuit model's components that could be applied to a variety of gelled electrode technologies. Thus, we sought to employ as generalizable an approach as possible in this study.

Once we had collected the impedance data and fitted the results with our equivalent circuit model, we also ran statistical analyses in order to define the significance of our experimental results at each level. First, impedance values for a given treatment and timepoint were averaged across both arms for each subject, and then Bartlett's test was performed to ensure equal variance across the groupings of skin conditions. If the null hypothesis of Bartlett's test was rejected, we log-transformed the data to account for a log-normal distribution. We also excluded subjects with data points lying 1.5 interquartile ranges outside the extreme quartiles from analysis for that given timepoint. After this, a one-way repeated measures analysis of variance (ANOVA) test was performed in order to determine whether there was a significant difference between skin treatments at each timepoint. We finished analysis with a pairwise Tukey-Kramer *post hoc* test.

The effects of skin condition on the values of the equivalent circuit model were also analyzed, though using three paired rank-sum tests, between the harshest skin treatment group (the AT condition) and the three milder groups (NT, SA, and μ NA) respectively. We set the significance level at p = 0.0167 after a Bonferroni correction, in order to account for multiple comparisons between skin conditions. Lastly, using *R* and the specific 'lme4' package (Bates et al. 2015), we performed a linear mixed-effects analysis on the equivalent circuit parameters at each timepoint, in order to understand the interaction of skin treatment and time on the skin- and electrode-level components, respectively. As fixed effects we had the intercepts for each subject. By inspecting the residuals for any obvious deviations from homoscedasticity or normality, we again log-transformed the parameter values if Bartlett's test failed. At the final step, *p*-values were obtained by likelihood ratio testing (LRT) via ANOVA of the linear mixed effects model including the treatment-time interaction term, against a linear mixed effects model that excluded this term. If the interaction was not found

to be not significant for a given treatment-time condition, then we still assessed the main effect of time on the equivalent circuit parameters. Otherwise, the simple effect of time for each treatment was independently tested for significance.

Total Impedance Results

Based on values of goodness of fit from a Pearson's χ^2 test with df = 6 degrees of freedom, we determined that the equivalent circuit model described by Equation(7) and shown in Figure 4.3 provided a consistent fit to the experimentally collected impedance data. Values of the goodness of fit existed between $\sim 2 \times 10^{-3}$ and $\sim 5 \times 10^{-2}$ for all subjects, skin treatments, and timepoints (Table 4-2).

Table 4-2 Values of the goodness of fit from fitting the skin impedance for each skin treatment, at t_0 , t_{mid} , and t_f with the proposed equivalent circuit model. Values are reported as avg. \pm std. dev. across N = 14 subjects.

Skin Condition	Timepoint	Model Fit, χ^2 (×10 ²)		
	t_0	3.75 ± 0.71		
No Treatment	t_{mid}	2.18 ± 1.85		
	t_f	3.09 ± 0.84		
	t_0	2.97 ± 0.75		
Abrasive Tape	t_{mid}	0.27 ± 0.08		
	t_f	0.36 ± 0.18		
	t_0	4.72 ± 0.97		
Salicylic Acid	t_{mid}	0.97 ± 0.05		
	t_f	1.15 ± 0.49		
	t_0	4.15 ± 1.40		
µNeedle Array	t_{mid}	2.62 ± 1.34		
	t_f	1.22 ± 0.40		

Representative Bode plots for the impedance under each treatment condition are given for one subject at t_0 in Figure 4.4. We found that the impedance magnitudes across treatment conditions diverged noticeably at frequencies below 5 kHz. The average cutoff frequency (*i.e.*, the frequency at which the phase shift = -45° (Boehler et al. 2020)) was 4.00 ± 2.23 kHz for the AT condition, which was higher than the average cutoff frequencies of the SA (800 ± 32 Hz), μ NA (397 ± 132 Hz), and NT (320 ± 56 Hz) skin conditions.



Figure 4.4 Summary of the total impedance results. (A,B) Representative Bode plots of the impedance (A) modulus and (B) phase for a single subject at t_0 , for all skin treatments. Points represent experimental data while solid black lines denote the curves from fitting the data with the equivalent circuit model in Figure 4.3. (C–E) Values of the 10 Hz for all subjects, for all skin treatments, at the (C) initial (t_0 ; ANOVA F(14,3) = 68.02, $p < 1.5 \times 10^{-15}$), (D) middle ($t_{mid} \sim 8$ hr.; ANOVA F(10,3) = 19.31, $p < 5.0 \times 10^{-4}$), and (E) final ($t_f \sim 24$ hr.; ANOVA F(10,3) = 19.17, $p < 7.5 \times 10^{-7}$) timepoints. Significance levels: (**) denotes p < 0.01 and (***) denotes p < 0.001.

Comparison of the average impedance magnitude at the 10 Hz reference frequency (American National Standards Institute 2020) over time showed that AT resulted in a significantly lower impedance than all other skin treatments over the full 24 hours of the study. At t_0 , the NT condition had the highest impedance (188.7 ± 163.3 kΩ), while the AT condition had significantly lower impedance (1.67 ± 0.47 kΩ, p < 0.001). The SA and μ NA treatments had approximately equivalent impedances at t_0 , (80.0 ± 77.5 kΩ and 74.9 ± 21.3 kΩ, respectively). At t_{mid} , or roughly 8 hours after initial placement of the electrodes, the impedance of the NT, SA and μ NA treatments decreased considerably (NT = 29.7 ± 20.2 kΩ, SA = 43.2 ± 23.4 kΩ, μ NA = 22.0 ± 15.4 kΩ), but the AT impedance remained essentially unchanged and still significantly lower than all other cases (1.76 ± 0.77 kΩ, p < 0.001). Finally, at the 24-hour timepoint, t_j , the average impedance for all skin conditions was < 20 kΩ, although the difference between AT and all other skin conditions was still found to be significant (p < 0.01). Notably, the impedance difference between the NT, SA, and μ NA treatments was not found to be significant at any of the timepoints.

It is worth noting that the trends seen in the overall impedance as it changes

over time are expected, given the various treatment types. The milder treatments— NT, SA, and μ NA—showed decreases in their 10 Hz impedance from the t_0 to t_f timepoints (Figure 4.4, panels C–E), which is consistent with the hypothesis that natural skin hydration decreases the interfacial impedance due to the presence of ions in human sweat (Matsukawa et al. 2020). Additionally, there was a slight increase in the impedance of the skin under the AT treatment over 24 hours. While the change was minimal over time, it may still be explained from a biological standpoint. After abrasive damage, the stratum corneum naturally recovers within 12 hours (Andrews, Lee, and Prausnitz 2011), and after 24 hours, the uppermost layers of the epidermis are largely repaired. Indeed, the stratum corneum has a natural cycle of self-repair that it completes in order to maintain its normal function as the outermost skin layer and environmental barrier (Rosso et al. 2011).

From Equation(7), it is understandable that at high frequencies, the total impedance is dominated by the subcutaneous and gelled electrolyte resistances (R_{sub}) and R_{qel}), whereas the total impedance becomes the sum of all resistive components at lower frequencies, when the epidermal and electrode charge-transfer resistances, R_{epi} and R_{ct} , respectively, are added to R_{sub} and R_{gel} . In effect, while more frequencyindependent effects dominate the impedance behavior in the high and low frequency regimes, capacitive attenuation must occur between these extremes. In the frequency ranges relevant for recording biopotentials such as the electroencephalogram (EEG, 0.1-100 Hz), EMG (25-500 Hz), or ECG (0.5-150 Hz) (Yazicioglu, Hoof, and Puers 2009), the impedance already shows a predominantly resistive response: however, due to the capacitive attenuation of the skin, in the case of the milder conditions (i.e., NT, SA, μ NA) the impedance is higher than in the case of AT. This result indicates that the capacitive attenuation from the skin is less relevant as the skin treatment becomes harsher. In conclusion, the impedance response becomes progressively more resistive with harsher skin treatments, shifting towards lower magnitude values and higher cutoff frequencies.

Model Fitting Results

Table 4-3 provides values of each of the equivalent circuit model parameters at each of the timepoints, for all conditions, averaged across all subjects. Of particular note is that the epidermal components (R_{epi} and C_{epi}) were significantly lower for the AT condition compared to all other conditions at t_0 , while the subcutaneous resistance, R_{sub} , remained uniform across treatment types. This suggests that the interfacial impedance is most largely impacted by the superficial epidermal layers, while the deeper skin layers contribute little to the overall impedance barrier presented by human skin (Garmirian, Chin, and Rutkove 2009; L. Li et al. 2016; Heikenfeld et al. 2017). Since the stratum corneum is a hydrophobic insulating layer, it is the main barrier to signal transduction between the skin and the electrodes (Matsukawa et al. 2020; Heikenfeld et al. 2017; F. Lu et al. 2018; Yamamoto and Yamamoto 1976), and requires removal in order to lower the skin's intrinsic impedance properties.

Table 4-3 Equivalent circuit elements, their boundaries, and average fitted values for each skin treatment, at t_{0} , t_{mid} , and t_{f} . Values are reported as avg. \pm std. dev. across N = 14 subjects, and normalized by electrode surface area in order to be more generalizable.

		<i>R_{sub}</i> [kΩ cm ²]	<i>R_{epi}</i> [kΩ cm²]	C _{epi} [nF cm ⁻²]	R _{gel} [kΩ cm²]	R_{ct} [k Ω cm ²]	<i>C</i> _{dl} [nF cm ⁻²]
Lov Upp Parai Bour	ver- oer meter nds	0.05 - 5.00	0.10 - 500	0.10 - 500	0.05 - 5.00	0.5 - 5.0×10 ³	1.0 - 1.0×10 ⁵
NT	t ₀	0.71 ± 0.16	86.38 ± 29.43	242.62 ± 148.41	0.87 ± 0.46	$(1.31 \pm 1.27) \times 10^3$	5.38 ± 2.19
	t _{mid}	0.74 ± 0.19	47.07 ± 33.09	27.98 ± 26.86	1.07 ± 0.90	147.93 ± 101.27	63.42 ± 25.62
	t_f	0.71 ± 0.20	40.90 ± 30.70	34.70 ± 19.11	0.72 ± 0.42	66.54 ± 31.96	36.29 ± 15.24
АТ	t ₀	0.70 ± 0.20	6.61 ± 5.02	4.95 ± 3.73	0.59 ± 0.13	2.90 ± 2.39	7.00 ± 2.90
	t _{mid}	0.72 ± 0.18	1.80 ± 1.45	8.49 ± 2.46	0.59 ± 0.11	3.30 ± 2.66	10.50 ± 6.56
	t _f	0.67 ± 0.22	2.27 ± 1.39	28.28 ± 20.65	0.76 ± 0.48	1.26 ± 0.49	12.23 ± 4.27
SA	t_{0}	0.79 ± 0.14	69.43 ± 30.09	97.63 ± 76.73	0.65 ± 0.14	675.80 ± 501.10	9.97 ± 5.40
	t _{mid}	0.77 ± 0.18	48.39 ± 32.86	47.81 ± 27.09	1.17 ± 1.02	17.67 ± 14.97	9.88 ± 9.54
	t _f	0.77 ± 0.16	35.97 ± 33.36	44.74 ± 31.84	1.36 ± 1.18	12.76 ± 9.71	7.70 ± 3.40
μΝΑ	t_{0}	0.81 ± 0.14	81.56 ± 31.34	29.46 ± 22.32	0.67 ± 0.17	807.42 ± 394.97	5.05 ± 2.17
	t _{mid}	0.74 ± 0.18	46.50 ± 35.41	24.59 ± 21.80	1.19 ± 1.09	92.16 ± 60.74	9.50 ± 5.52
	t _f	0.71 ± 0.16	35.36 ± 32.01	33.65 ± 28.34	0.91 ± 0.67	72.29 ± 30.89	5.38 ± 2.19

At the electrode level, the charge-transfer resistance, R_{ct} , was also significantly lower under the AT condition compared to the other treatments at t_0 , whereas the double-layer capacitance, C_{dl} , was not significantly different among the different skin conditions. A lower value of R_{ct} in the case of AT may be explained by the fact that in non-polarizable, faradaic electrodes—such as the Ag/AgCl electrodes used for this study—charge-transfer characteristics are typically dominated by the R_{ct} branch of the circuit (Roberto Merletti et al. 2009; H. L. Peng et al. 2016; Yamagami et al. 2018). Accordingly, interventions at the skin-level should have less effect on C_{dl} than on R_{ct} . Another important item to note is that R_{gel} is comparable across all skin conditions; this is a sensible result given that all the electrodes used in this study were the same commercially manufactured ConMed contacts.

Exploring the time evolution of our model parameters revealed further interesting trends about the skin impedance and its change over time. Figure 4.5

shows the values of each model parameter to each time point, while Table 4-4 summarizes the results of our LRT analysis. At the skin level, there was no significant variation in *R_{sub}* over 24 hours, confirming the ability of our model to accurately track the invariant characteristics of the subdermal layers of human skin. At the epidermal level, however, all treatments showed an overall \sim 50% decrease in their values of R_{epi} over time, which was likely the result of hydration of the skin via release of sweat under the electrodes. While R_{epi} decreased, C_{epi} equilibrated over time. The slight increase seen in the *C*_{evi} results for the AT treatment over time are likely related to changes in the dielectric constant of the stratum corneum as it repaired itself (Yamamoto and Yamamoto 1976; Andrews, Lee, and Prausnitz 2011). The findings of a time-only interaction for R_{epi}, but a time-treatment interaction for C_{epi}, indicates that sweating affects both the capacitive attenuation of the interfacial impedance, and also the faradaic reactions at the skin level. From this, we were able to conclude that the main effects on different skin treatments over time are changes in the epidermal capacitance, while abrasion of the superficial layers allows the skin to retain a predominantly faradaic behavior throughout at least the first 24 hours of contact between the skin and electrode.



Figure 4.5 Time evolution of the fitted circuit model parameters for pre-gelled clinical electrodes over 24 hours. Bars represent means, errorbars are standard deviations (N = 14 subjects); results are normalized by the electrode GSA. t_0 – initial timepoint, t_{mid} – study midpoint, 8 hrs. after skin treatment application, t_f – final timepoint, 24 hrs. after skin treatment application. Significance levels: (*) denotes p < 0.05, (***) denotes p < 0.01 (values reported Table 4-4).

At the electrode level, R_{gel} was largely invariant over time; it was also

unaffected by treatment type. While it is known that gelled electrodes can dry out over extended periods of time, we determined that 24 hours may not be nearly long enough to observe this drying phenomenon (Yapici et al. 2015). Nevertheless, we observed other changes at the electrode-level. R_{ct} dramatically decreased for the three milder skin treatments (NT, SA, and μ NA) between t_0 and t_{mid} , and equilibrated by t_{f_i} whereas the values of C_{dl} remained essentially unchanged over the full 24-hour period. Comparatively, electrodes over skin treated with AT saw an overall decrease of R_{ct} concurrent with and a slight increase in C_{dl} by t_{f} . These changes were found to be significant from a time-treatment interaction standpoint, as well (Table 4-4), highlighting that changes in the electrode-level parameter values also depends on the type of skin treatment used before electrode placement. The variability of R_{ct} in particular, across all treatment types, underscored for us the point that the majority of charge-transfer through gelled electrodes, even over time, occurs through this specific branch of the model, more so than through the C_{dl} parameter. Again, though, this was to be expected due to the non-polarizable nature of gelled Ag/AgCl electrodes (Paolo Cattarello and Merletti 2016).

Element	Interaction Effect $\chi^2(df=6)$	Main Effect F(<i>df</i> = 2) (<i>p</i> -value)	Simple Effect F(<i>df</i> = 2) (<i>p</i> -value)			
	(<i>p</i> -value)		NT	AT	SA	μΝΑ
Rsub	3.0 (0.81)	0.9 (0.4)				
Repi	8.8 (0.19)	16.8 (2.7e-7)				
Cepi	34.2 (6.13×10-6)		9.5 (4.6×10-4)	7.0 (0.003)	0.9 (0.4)	2.1 (0.1)
Rgel	6.0 (0.43)	0.8 (0.4)				
R _{ct}	58.0 (1.16×10 ⁻¹⁰)		30.2 (1.4×10 ⁻⁸)	3.7 (0.03)	21.2 (5.9×10 ⁻⁷)	3.1 (0.06)
Cal	58.0 (1.15×10-8)		1.0 (0.4)	10.0 (3.9×10 ⁻⁴)	1.2 (0.32)	1.9 (0.16)

Table 4-4 Results of likelihood ratio testing (LRT) of equivalent circuit model parameters for pre-gelled electrode impedance. The significance level was set to p = 0.05.

Study Conclusions

Altogether, this study clearly highlighted that the impedance of the skinelectrode interface is largely controlled by the epidermis. As such, only mechanical abrasion of the stratum corneum appeared to effectively reduce R_{epi} and C_{epi} . Alternative skin interventions that likewise remove or decrease the epidermal insulating barrier may also offer benefits to the interfacial impedance, such as controlled laser microporation (Giannos 2014) or electroporation (F. Lu et al. 2018). In addition to this finding,, though, our analysis also revealed that while the initial skin conditions have an important effect on the interfacial impedance in the short-term, over time the skin impedance tends to equilibrate. Thus, by the final timepoint, even though skin treated with AT still had the lowest impedance compared to the other treatments, all skin conditions had total impedance moduli < 20 k Ω at 10 Hz. The time evolution of the skin-electrode response was likely driven by natural skin hydration, and resulted in all skin conditions adjusting to reach equilibrium within the explored 24-hour timeframe.

Finally, this study was important foundational work to establish our proposed equivalent circuit model as a useful tool for evaluating the skin-electrode impedance at both acute and chronic timepoints. The experimental framework of this study was based on an equivalent circuit model with separate branches to account for the skin and the electrode, which allowed us to investigate the contributions of each specific level to the total impedance response. We also applied this same model to impedance data collected under different skin interventions, and at different timepoints, and were able to gain an improved understanding of the skin-electrode interface as a result of this exercise. Particularly the ability to discriminate between the contributions of the skin or the electrode to the overall impedance may help in the development of alternative skin treatment interventions. Furthermore, with the ever increasing interest in gel-free electrodes (Roberts et al. 2016; Hoffmann and Ruff 2007; Murphy et al. 2020) and also given recent innovations in gelled electrode technologies (Sawangjai et al. 2020; LaMonte 2021), we found this study provided a rigorous understanding of the factors affecting the skin-electrode impedance, which might be useful in order to enable improved design of high-fidelity biosensors for future wearable applications.

An Equivalent Circuit Model for Ti₃C₂T_x-based epidermal sensors

The effect of skin treatment on Ti₃C₂T_x skin impedance

With our knowledge of human skin broadened by our investigation into the nature of the interfacial impedance of pre-gelled electrodes, we next began to investigate the full skin impedance properties of $Ti_3C_2T_x$ -based epidermal sensors. Having developed protocols to realize such sensors, as described in Chapter 3, we subsequently fabricated arrays incorporating $Ti_3C_2T_x$ as the primary sensing contact. Following fabrication, we decided to explore the cutaneous impedance of the $Ti_3C_2T_x$ arrays under different skin conditions. For this test, we looked at three specific interventions: (i) no treatment (NT), in which the skin was simply cleaned with an alcohol prep pad; (ii) abrasive tape (AT), in which the skin was mechanically abraded after cleaning, using 3M TracePrepTM abrasive tape; and (iii) a saline swabbed case

(PBS), in which, after skin cleaning, a cotton swab was soaked in 1X PBS and then dabbed across the skin surface immediately before electrode placement. PBS was chosen as a novel third skin treatment, given our knowledge that natural skin hydration via sweating and oil release serves to lower the interfacial impedance. Since sweat is a form of ionic solution, we thus hypothesized that using an alternative ionic solution—such as physiologic saline—would show a similar effect of lowering the impedance. Figure 4.6 shows the results of impedance measurements under these different interventions.



Figure 4.6 The effect of skin treatments on the impedance of $Ti_3C_2T_x$ HDsEMG arrays. (A) Bode plot of the impedance modulus under the different treatments tested. Points represent averages, and shaded areas represent standard deviations, across N = 8 channels from one array, for each treatment. (B) Phone camera images of the skin directly under the array after application of each skin treatment. Adapted from the Supplementary Information in (Murphy et al. 2020).

We discovered that application of PBS prior to placement of the $Ti_3C_2T_x$ arrays resulted in a dramatic lowering of the skin impedance values compared to NT. Furthermore, we discovered that the frequency response of the impedance under PBS was similar to the frequency response under the harsher AT treatment. At the 10 Hz reference frequency, the impedance values under each treatment were: 32.86 ± 3.93 M Ω under NT; 45.32 ± 7.42 k Ω with AT; and 36.33 ± 16.05 k Ω with the PBS skin treatment. Indeed, the PBS treatment seemed to have even a slightly lower impedance on average than the AT treatment, suggesting that gel-free $Ti_3C_2T_x$ HDsEMG arrays do not require abrasive skin treatment in order to achieve comparably low cutaneous impedances for acute recording studies. This was encouraging, as it suggested that our arrays could be used with minimal discomfort to the subject.

An equivalent circuit model for dry electrode technologies

Unlike the gelled electrodes used for the study outlined in the previous section, the Ti₃C₂T_x and Au electrodes that we fabricated for the HDsEMG study were dry electrode technologies. And although the PBS skin treatment allows for us to propose a sort of pseudo-electrolytic interface existing between the skin and the electrode, we hypothesized that a pure R_{gel} connecting the skin and electrode levels would be insufficient to capture all the true activity that goes on at the recording interface over time. Accordingly, we developed and here propose a separate equivalent circuit model for dry electrode technologies (Figure 4.7), which models the skin-electrode interface as a parallel RC circuit, containing R_{int} and C_{int} elements (Heikenfeld et al. 2017; Beckmann et al. 2010). Furthermore, given our knowledge of MXene's pseudocapacitive behavior, and based on our equivalent circuit modelling of Ti₃C₂T_x interfacing with aqueous media in the first section of this chapter, we decided to replace the C_{dl} component of the electrode branch with a CPE.



Figure 4.7 Schematic of the equivalent circuit model used for fitting skin-electrode impedance data for dry $Ti_3C_2T_x$ electrodes. At the skin level (left three elements), R_{epi} and C_{epi} are the epidermal resistance and capacitance, respectively, and R_{sub} is the resistance of the dermis and subcutaneous fat layers. At the interface between the skin and the electrode are the interfacial resistance and capacitance, R_{int} and C_{int} , and at the electrode level, a modified Randles cell is used to model the behavior of the MXene film.

The total equivalent impedance of the proposed circuit model is given by

$$Z_{tot} = R_{sub} + \left[\frac{R_{epi}}{1 + j\omega R_{epi}C_{epi}}\right] + \left[\frac{R_{int}}{1 + j\omega R_{int}C_{int}}\right] + \left[\frac{R_{ct}}{1 + Y_0(j\omega)^{\alpha}R_{ct}}\right]$$
(8),

where, according to Equation(2), Y_0 is the admittance, and $\alpha \in [0,1]$ is the constant, of the CPE. R_{int} and C_{int} here are used to model the complex behavior of the dry electrode-skin surface interface, where PBS may be applied, or where sweat and oils exist. As a final point, in this proposed equivalent circuit model, we deduced that the double-layer capacitance of the Ti₃C₂T_x films could be calculable with a modified

version of Equation(3), which replaces the solution resistance, R_s , with the subcutaneous resistance, R_{sub} , instead:

$$C_{al}^{\text{cutaneous}} \equiv \frac{(R_{sub}Y_0)^{1/\alpha}}{R_{sub}}$$
(9).

With this equation, then, and with our proposed model built, we explored fitting of the total impedance for a variety of electrodes, as we had done for $Ti_3C_2T_x$ -based electrodes in saline. In particular, to validate our model, we decided to compare the parameter values of the Au and $Ti_3C_2T_x$ HDsEMG arrays to the same parameters in clinical gelled electrode impedance measurements.

Similar to the case of the pre-gelled electrodes from the previous section, we restricted the bounds of the parameter values in the dry electrodes equivalent circuit model, in order to avoid physically implausible values for the various components. We gave the same parameter bounds to all components shared between the dry and gelled epidermal electrode models (*i.e.*, R_{sub} , R_{epi} , C_{epi} , and R_{ct}), as described in Table 4-3, and for the R_{int} and C_{int} components of the dry epidermal model, we restricted the bounds to lie between 1–10 k Ω cm² and 1–90 nF cm⁻², respectively (Murphy et al. 2020). The CPE for the MXene films was initialized with values from Table 4-1.

Comparing Ti₃C₂T_x MXene to other epidermal electrode technologies

In total, we explored five separate skin-based electrode interfaces. The clinical grade electrodes that were explored included 0.8 cm² Natus electrodes, and 5.1 cm² ConMed contacts. Meanwhile, for dry electrodes, we had Au HDsEMG contacts, and Ti₃C₂T_x electrodes made with both spin-cast and spray-cast MXene films. All dry fabricated electrodes had surface areas of 0.0256 cm². For our impedance measurements, the skin of the forearm was first cleaned with an alcohol prep pad, and then the PBS skin treatment was applied to the skin before placement of the electrodes. All impedance measurements were done between $1-10^5$ Hz, with a Gamry Reference 600 potentiostat/galvanostat/ZRA and a 10 mV_{rms} driving voltage. The reference and counter electrodes—placed at the wrist and the pit of the elbow, respectively—were ConMed pre-gelled Ag/AgCl electrodes. Following impedance measurements, we were careful to model the gelled electrodes data with the gelled electrodes equivalent circuit model proposed in the previous section (Figure 4.3), and only modelled the Au and MXene impedance data with the new, dry electrode technologies model (Figure 4.7).



Chapter 4: Equivalent Circuit Models for Bioelectronic Interfaces

Figure 4.8 Summary of equivalent circuit modelling of the skin-electrode impedance for five different epidermal electrode interfaces. (A–E) Bode plots of the impedance modulus (left) and phase (right) for (A) a monopolar Natus Ag/AgCl electrode, (B) a single ConMed Ag/AgCl contact, (C) a microfabricated Au HDsEMG electrode, (D) a microfabricated $Ti_3C_2T_x$ HDsEMG contact made from a spin-cast MXene film, and (E) a microfabricated $Ti_3C_2T_x$ HDsEMG contact made from a spray-cast MXene film. (F) Bar plot of the GSA-normalized double-layer capacitance of each electrode type.

The results of fitting once again demonstrated the enhanced capacitive behavior of Ti₃C₂T_x MXene when in contact with human skin. By fitting the impedance spectra with our proposed equivalent circuit model of the full skin-electrode interface, and calculating capacitance values with Equation(9), we discovered that Ti₃C₂T_x exhibited higher C_{dl} values on human skin compared to pre-gelled Ag/AgCl electrodes, as well as size-matched Au contacts. Spray-cast MXene films boasted $C_{dl} = 1.57 \pm 0.58$ µF cm⁻² ($\chi^2(df = 8) = (6.24 \pm 1.39) \times 10^{-3}$), which was roughly 2x the values of C_{dl} for Au HDsEMG arrays (724.32 ± 192.40 nF cm⁻²; $\chi^2(df = 8) = (2.34 \pm 0.40) \times 10^{-2}$), ~20x greater than the C_{dl} of pre-gelled Natus Ag/AgCl contacts (69.52 ± 16.18 nF cm⁻²; $\chi^2(df = 6) = (8.03 \pm 1.15) \times 10^{-3}$) (Murphy et al. 2020), and >100x greater than the C_{dl} of the much larger area ConMed Ag/AgCl contacts (10.32 ± 9.00 nF cm⁻²; $\chi^2(df = 6) = (6.13 \pm 0.98) \times 10^{-3}$).

The spin-cast Ti₃C₂T_x films had $C_{dl} = 310.67 \pm 227.26$ nF cm⁻² ($\chi^2(df = 8) = (3.15 \pm 1.46) \times 10^{-3}$), which highlights once again that the processing conditions clearly affect the properties of MXene thin films. Likely because they are less uniform

films than the spray-cast $Ti_3C_2T_x$ films, the spin-cast MXene films performed even worse than the Au electrodes. Nevertheless, it is still worth noting that superior impedance behavior was observed for all microfabricated electrodes compared to the pre-gelled clinical electrodes. This is particularly noteworthy since the $Ti_3C_2T_x$ and Au HDsEMG arrays had no need for the conductive gels or adhesives which are critically required in order to achieve suitable skin–electrode impedances for all state-of-theart, prepackaged clinical technologies. This further serves to emphasize the benefits of the processing and fabrication steps outlined in Chapter 3, for realizing highdensity, highly skin-conformable, and high-fidelity electrode arrays for epidermal applications (Murphy et al. 2020).

Conclusion & Outlook

In this chapter, we explored a variety of equivalent circuit models to assist in understanding the behavior of our fabricated Ti₃C₂T_x-based bioelectronic interfaces as they interact with various media. In particular, we established a modified Randles cell to describe MXene electrodes interfacing with aqueous media. This circuit model may be useful for understanding interactions during *in vivo* experiments, when MXene-based technologies will need to interface with cerebrospinal fluid (CSF). For epidermal applications, we explored equivalent circuit models for both state-of-the-art bioelectronics—such as the Natus and ConMed pre-gelled Ag/AgCl electrodes—as well as dry skin-based sensors—such as our own microfabricated HDsEMG arrays. This fitting exploration has resulted in useful tools which may come in handy not only for future design of wearable electrode platforms themselves, but also when deciding on which type of skin treatment to apply before electrode placement.

Chapter 5. Exploring the lifetime of Ti₃C₂T_x dried films

While $Ti_3C_2T_x$ has many exciting and promising properties, it is also known to be relatively unstable in the long-term, both in ambient and wet environments. As such, previous work has been completed in order to improve the lifetime of $Ti_3C_2T_x$. However, the vast majority of this work has been done for MXene only in its solution form. As such, in Chapter 5, I will first define the lifetime of $Ti_3C_2T_x$ MXene in a dried film form, which more accurately reflects the nature of MXene as it will be incorporated into functional bioelectronic technologies. Next, I will explore the most promising approaches to improve the lifetime of MXene dried films, and benchmark the properties of modified $Ti_3C_2T_x$ films featuring stabilizing additive agents. This, in turn, will provide key insights into the nature of MXene's lifetime, which will guide me in realizing longer-term stable $Ti_3C_2T_x$ -based electrode technologies.

Relevant Citations:

(1) Shekhirev M, Murphy BB, Shevchuk Y, Shankar S, Cimino F, Vitale F, Gogotsi Y. Assessing the long-term stability of $Ti_3C_2T_x$ MXene solutions and spray-cast dried films featuring passivating additives. *In Preparation*.

Previous Work

Describing Ti₃C₂T_x's Degradation

Due to its ease of processability, its inherently high electronic conductivity, and its valuable intrinsic electrochemical properties, $Ti_3C_2T_x$ has been extensively studied for a wide variety of applications since its original synthesis in 2011. However, $Ti_3C_2T_x$ is known to degrade over time in ambient environments, which deteriorates it's excellent properties and poses serious concerns for its long-term use. Of course, when maintained as a "slurry" (corresponding to concentrations typically exceeding ~15 mg mL⁻¹), $Ti_3C_2T_x$ may be stable for many months or more (Iqbal et al. 2021; Zhao, Vashisth, et al. 2020; Bhat et al. 2021), but such high concentrations preclude the use of most solutions-processing techniques, as we have already explored in Chapter 3. Accordingly, the mechanisms of $Ti_3C_2T_x$'s degradation at processable concentrations has been previously assessed, and a variety of strategies have been proposed to improve the lifetime of $Ti_3C_2T_x$ solutions and films (Iqbal et al. 2021).

The major mechanism of MXene degradation is believed to be through oxidation and formation of titania (TiO_2) (Iqbal et al. 2021). This phenomenon has been observed in ambient environments (i.e., at room temperature) near atomic defects in the basal planes of individual flakes, as well as at the edges of the flakes (Xia et al. 2019). However, work has also shown that $Ti_3C_2T_x$ degrades more severely in water-based environments, driven largely by hydrolysis reactions resulting in the formation of TiO₂ alongside other complex molecules, such as CO₂, CH₄, and titanium hydroxide (Mashtalir et al. 2014; S. Huang and Mochalin 2019; 2020). Water molecules are also known to infiltrate the space between individual MXene nanosheets, resulting in an overall decrease in performance (Z. Lu et al. 2019; L. Ding et al. 2020). Harsh oxidizing agents—such as hydrogen peroxide (H_2O_2) —also cause similar swelling of the MXene sheets, in addition to formation of TiO₂ on the flake surface (Ahmed et al. 2016). TiO₂ formation rates are known to increase with increasing temperature (Lotfi et al. 2018). Finally, Ti₃C₂T_x is susceptible to deterioration under exposure to ultraviolet (UV) light, likely a result of MXene's intrinsically strong absorbance in the 250–300 nm wavelength range (Habib et al. 2019), and the strong photocatalytic properties of TiO_2 once it has formed (Mashtalir et al. 2014).

Combatting Ti₃C₂T_x's Degradation

To combat MXene's degradation, a variety of strategies have been previously explored. The first way to address the issue may involve adjustments to the MXene synthesis process itself: changing the concentration of the acidic etchant solution during MAX phase processing can improve the resulting MXene's solution stability (Zhao, Vashisth, et al. 2020), and the inclusion of excess aluminum in the MAX precursor can also result in significant improvements in the shelf-life of MXene nanosheets as a result of higher carbon stoichiometry and decreased oxygen activity (T. S. Mathis et al. 2021). Beyond synthesis, however, storage conditions are the most important factor affecting MXene's lifetime. Solutions stored in argon-sealed vials, for example, are known to be more stable, even at room temperature, than solutions stored in air alone (C. J. Zhang et al. 2017). Low temperatures are also known to slow the oxidation process (Chae et al. 2019), making colder environments more favorable for solution storage than room temperature environments. Furthermore, if solutions are frozen, this can significantly extend their shelf-life, even beyond one year (Jizhen Zhang et al. 2020). Adjusting the pH of the MXene solution to be more acidic can also improve the solution shelf-life (Zhao, Vashisth, et al. 2020). The dispersion agent also has a large impact on stability: organic solvents such as ethanol (Chae et al. 2019), isopropanol (S. Huang and Mochalin 2019), or acetone and acetonitrile (Habib et al. 2019) have been shown to slow degradation of MXene solutions relative to water. Indeed, Maleski et al. conducted a systematic study of the colloidal stability of Ti₃C₂T_x dispersions in eleven separate organic solvents compared to water (Maleski, Mochalin, and Gogotsi 2017), and found several options with the potential for longterm stability. The most favorable agents that this study identified were N,Ndimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), propylene carbonate (PC), and ethanol.

Along similar lines, a large number of publications have more recently demonstrated the improved stability of MXene dispersions incorporating a wide variety of antioxidant additives. Zhao *et al.*, for example, explored suspending $Ti_3C_2T_x$ in sodium ascorbate solutions, and hypothesized that association of *l*-ascorbate anions at the edges of the MXene nanosheets prevented oxidation from the edges of the flakes inward (Zhao et al. 2019). Similarly, using *l*-ascorbic acid—a known and commonly used reduction agent, better known as vitamin C—it becomes possible to not only improve the initial electrical properties of MXene, but also improve its resistance to oxidation (Zhao et al. 2019; Limbu et al. 2020). Other antioxidant additives that have been explored include: tannic acid (Zhao et al. 2019); sodium phosphate, oxalate, and citrate (Wu et al. 2020); and sodium polyphosphates, borates, and silicates, which "cap" the edges of individual $Ti_3C_2T_x$ nanosheets to protect them from moisture ingress, similar to *l*-ascorbate (Natu et al. 2019).

It is worth noting that in the additives literature described briefly above, the majority of studies have only lasted for 3–4 weeks (21–28 days) (Limbu et al. 2020; Zhao et al. 2019; Natu et al. 2019), although one study extended out to 80 days (Wu et al. 2020). Another important consideration is that the concentrations of the
explored solutions have not been consistent from report to report. Among the different publications, the concentration of $Ti_3C_2T_x$ solution has ranged from 6.1×10^{-3} mg mL⁻¹ to 15 mg mL⁻¹, and the explored library of additives have had concentrations ranging from 5 mM to 500 mM. Only one report washed the combined $Ti_3C_2T_x$ and additive solution, as well (Natu et al. 2019): all other reports have simply mixed the $Ti_3C_2T_x$ and additive solutions together, without any centrifugation or washing steps.

Alternatively, thermal treatments may be explored for processed films or MXene membranes: Ti₃C₂T_x can be induced to self-crosslink via thermal treatment at 180 °C, for example, resulting in stronger Ti–O–Ti bonds that reject common ions found in aqueous environments (Z. Lu et al. 2019). Annealing of Ti₃C₂T_x at 600 °C can result in the oxidation of only the surface and edges of the individual nanoflakes, providing a passivation layer against moisture ingress in the film (Zhao, Holta, et al. 2020). Finally, annealing of MXene films in an H₂ environment at 900 °C can not only help to recover film properties in the event of oxidation, but also results in greater stability against oxidation after the annealing step, as well (Y. Lee et al. 2020). However, while thermal treatments may be interesting to explore for free-standing MXene films, the higher temperatures necessary to achieve oxidation stability are largely incompatible with the types of materials that are commonly used for device packaging and encapsulation—such as elastomers, polymers, and epoxies—which typically have $T_g < 200$ °C (Ahn, Jeong, and Kim 2019; Chen, Luo, and Kang 2017).

Common Methods of Observation & Characterization

In many of the reports detailed above, the most common techniques used to monitor the condition of the MXene over time was simple visual inspection of the stored solutions (Iqbal et al. 2021; Maleski, Mochalin, and Gogotsi 2017; S. Huang and Mochalin 2019; Zhao et al. 2019; Chae et al. 2019; Limbu et al. 2020). MXene solutions have been considered to be degrading once there is an observed color change, concurrent with a decrease in general colloidal stability (Figure 5.1). Scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), and dynamic light scattering (DLS) have all supported this conclusion (Chae et al. 2019). However, at least one study has suggested that color and colloidal stability are not perfectly reliable as the primary indicators of degradation, and that the DC conductivity (σ_{DC}) of processed films should be used instead (Habib et al. 2019). Another useful method to monitor colloidal stability over time is absorption spectroscopy in the UV and visible ranges (UV-Vis), since the characteristic absorption peak of $Ti_3C_2T_x$ occurs in the 700–800 nm range (Alhabeb et al. 2017; D. Kim et al. 2019; T. S. Mathis et al. 2021). For solutions that are processed into dried films, X-ray diffraction (XRD) is an excellent technique to not only reveal the existence of impurities or other additives,

but also to monitor changes in the crystalline structure of the film over time (Alhabeb et al. 2017; Zhao et al. 2019; Natu et al. 2019). Finally, Raman spectroscopy may also be useful for assessing changes in molecular vibrational modes over time, which may be related to larger structural changes in the $Ti_3C_2T_x$ nanosheets (Sarycheva and Gogotsi 2020; T. S. Mathis et al. 2021).



Figure 5.1 Degradation of a 10 mg mL⁻¹ Ti₃C₂T_x MXene solution over time. This solution was aged under ambient conditions: it was stored in a laboratory cabinet at room temperature and atmospheric pressure, out of direct sunlight. The first signs of colloidal instability appeared in this higher concentration solution around ~1 month, whereas a marked color change began to occur after ~4 months. Between the 4- and 5-month timepoints, the solution took on a lighter greyish hue, and by the ~6th month of aging, the solution was a milky white color, which is usually taken as a sign that the solution contains fully oxidized TiO₂ particles.

Additives Study

Experimental Methods & Solution Results

Given the abundance of previous work exploring additives to improve the lifetime of $Ti_3C_2T_x$ as described above, in our own first study, we incorporated many promising additive candidates into our MXene solution preparation, and subsequently assessed the benefits of these additives for the long-term stability of $Ti_3C_2T_x$ solutions and dried films. In particular, we first assessed the colloidal stability of MXene solutions featuring additives over time, using UV/Vis as our primary characterization measurement. We compared the results to untreated MXene solutions aged under the same conditions. We explored sodium ascorbate (NaAsc), *l*-ascorbic acid (VC), polyphosphate salt (Poly), sodium citrate (Na₃Cit), citric acid

(H₃Cit), oxalic acid (Oxal), lactic acid (Lact), dimethyl sulfide (HD), and sodium dodecyl sulfate (SDS) as the primary anionic additives for this initial investigation.

Ti₃C₂T_x MXene was synthesized following the modified MAX phase synthesis process outlined by Mathis *et al.* (T. S. Mathis et al. 2021) Subsequently, MXene at a concentration of ~0.05 mg mL⁻¹ was dispersed in 5 mM aqueous solutions of each additive. To realize the phosphate salt-treated MXene solutions, the untreated MXene at ~0.05 mg mL⁻¹ was mixed with both 100 mM and 5 mM aqueous solutions of Poly, to explore the effect of the salt concentration on the solution stability over time. This decision was made based on previously published literature, which has highlighted the importance of the polyanionic salt concentration when it comes to the subsequent modified MXene's aqueous stability (Natu et al. 2019).

Once the solutions were made, their UV/Vis was taken, and the absorbance at 750 nm, A_{750} , was calculated from the transmittance data, following from the equation

$$A_{\lambda} = \log_{10}\left(\frac{I_0}{I_{\lambda}}\right) = -\log_{10}\left(\frac{\%T_{\lambda}}{100\%}\right) \tag{1},$$

where I_0 and I_{λ} are the incident and transmitted light wave intensities, respectively, and $\% T_{\lambda}$ is the transmittance at wavelength λ . Following the first absorbance measurement, the solutions were stored in glass vials with caps, and left in room temperature air, out of direct sunlight. Their absorbance was measured periodically over a period of ~3 months.



Figure 5.2 Solution stability of Ti₃C₂T_x MXene with various additives, over 3+ months of aging. Figure courtesy of Dr. Mikhail Shekhirev.

We discovered that, among the additives we explored, VC, NaAsc, and Na₃Cit seemed the most promising candidates to improve the lifetime of $Ti_3C_2T_x$ solutions (Figure 5.2). We also discovered that untreated MXene is still colloidally stable out to ~40 days, but afterwards, it quickly degrades. Depending on the additive type, as well, we saw differences in the starting values of the absorbance, which highlights that $Ti_3C_2T_x$ interacts differently with different aqueous solutions and additive types. Of note, too, was the finding that the 100 mM Poly-MXene dispersion had much lower absorbance than the 5 mM Poly-MXene dispersion, although this is to be expected given that the former dispersion had 20x the amount of polyanionic salts. Surprisingly, however, the 5 mM Poly-MXene solution degraded more significantly over time than the 100 mM Poly-MXene solution, as becomes clear after ~40 days.

Dried Films Study: Experimental Methods & Results at to

Encouraged by the solution study results, we next decided to move on to exploring dried films stability. Before moving to this stage of the project, however, we first decided to revisit the polyanionic salts literature (Natu et al. 2019), in order to better design our processing methods to incorporate this particular class of additive.

Key to the success of the phosphate salt-treated MXene solutions stability is the fact that the polyanionic aqueous solutions and the untreated MXene solutions are washed together, and not merely mixed (Natu et al. 2019). Accordingly, we explored washing of the Poly solutions with the untreated MXene solutions. We also decided to include sodium hexametaphosphate (Hex, Na₆[PO₃]₆) as yet another polyanionic salt to compare with Poly, and to compare against the other additives in this study. We then developed a rather simple process for realizing the phosphate salt-treated Ti₃C₂T_x solutions (Figure 5.3): 10 mM aqueous solutions were made from each salt, and these were next added to untreated MXene solutions at concentrations of ~ 10 mg mL⁻¹. The solutions were mixed in a 1:10 volume ratio of MXene to salt solution. Afterwards, the combined solutions were vigorously shaken by hand, and then the vials were left to sit for 3 hrs. at room temperature, out of direct sunlight. During this time, $Ti_3C_2T_x$ aggregated to the bottom of the vial, and it is hypothesized that the polyanionic salts "cap" the edges of the individual MXene flakes during this sedimentation process, thus providing the individual 2D flakes with a barrier against oxidation and moisture ingress between the MXene layers (Natu et al. 2019). After many rounds of centrifugation and washing (5000*g* for 120 s, 3 rounds of 3000*g* for 240 s; decanting the supernatant and redispersing the slurry in DI water in between each step), the final, phosphate salt-treated MXene slurries were finally redispersed in DI water to final concentrations around ~ 10 mg mL⁻¹, as determined by gravimetry.



Figure 5.3 Development of the phosphate salt-treated $Ti_3C_2T_x$ solutions. (A) The solutions before mixing: ~10 mg mL⁻¹ untreated MXene, and 10 mM aqueous solutions of Hex and Poly salts. (B) The Hex-MXene and Poly-MXene solutions after vigorous hand shaking. (C) The Hex-MXene and Poly-MXene solutions after sedimentation occurred over ~3 hrs. (D) The solutions after centrifugation and washing.

Having optimized the process to obtain phosphate salt-treated $Ti_3C_2T_x$ solutions, we then started the dried films study. $Ti_3C_2T_x$ MXene was again synthesized following the modified MAX phase synthesis process outlined by Mathis *et al.* (T. S. Mathis et al. 2021). Subsequently, MXene at a concentration of ~1 mg mL⁻¹ was dispersed in 100 mM solutions of NaAsc, Na₃Cit, and VC, given that these were the most promising candidates from the solutions stability study. The phosphate salt-treated MXene solutions were made as outlined above, and were redispersed to a final concentration of ~1 mg mL⁻¹ in DI water. Films were made from the final MXene solutions by spray-coating, following the procedures outlined in Chapter 3. Following spray-coating, all films were dried overnight (~15 hrs.) in a vacuum oven at ~500

mTorr and 80 °C. Two sets of control films were made from the starting, untreated MXene solution: one set using 10 mg mL⁻¹ MXene, and the other using MXene at 1 mg mL⁻¹. The goal of this was to explore how slight changes in the processing parameters could affect the final film stability. All samples made from the additive solutions were made using solutions at 1 mg mL⁻¹ concentration. All spray-cast films were roughly 0.5–1.0 μ m in thickness, as determined by profilometry (KLA Tencor P7 2D profilometer).

After baking the films, we measured their initial conductivity, and also completed XRD, Raman, and SEM of the samples (Figure 5.4). Conductivity measurements were completed with a Loresta handheld AX-MCP-T370 resistivity meter (Nittoseiko Analytech Co., Ltd.), and using the equation

$$\sigma_{DC} = \frac{1}{R_s \tau} \tag{2}$$

where σ_{DC} is the DC conductivity in S cm⁻¹, R_s is the sheet resistance in Ω sq⁻¹, and τ is the film thickness. An InVia confocal Raman microscope (Renishaw plc) was used with $\lambda = 785$ nm, 5% power (~0.15 mW), and a 20x objective for Raman measurements. Spectra were measured with a 600 lines/mm grating and 30 s of exposure. XRD was conducted using a Rigaku MiniFlex benchtop X-ray diffractometer (Rigaku Co. Ltd.) with a Cu K α ($\lambda = 0.1542$ nm) source. Spectra were acquired at 40 kV voltage and 15 mA current, for $2\theta = 3^{\circ}-60^{\circ}$, at a rate of 7° min⁻¹, with a step of 0.02°. A Zeiss Supra 50VP Field Emission SEM (Zeiss Group) with a 3 kV accelerating voltage was used for SEM imaging.



Figure 5.4 Summary of the $Ti_3C_2T_x$ and additives dried film results at t_0 . (A) Phone camera images of all samples considered in the dried films portion of this study. (B–D) SEM of (B) untreated $Ti_3C_2T_x$, (C) Hex-MXene, and (D) VC-MXene dried films. Scale bars, 2 µm. (E) The starting DC conductivity for all dried film types. (F) XRD spectra for all dried film types at the initial timepoint. Note that there is a single trace for the untreated MXene films, as the concentration difference between the 10 mg mL⁻¹ and 1 mg mL⁻¹ samples had little impact on the crystallographic properties of the resulting films at t_0 . (G) Raman spectra of all dried film types at the initial timepoint. Again, there is a single trace for untreated MXene as the concentration difference between the samples had little impact on the vibrational properties of the films at t_0 .

At t_0 , we observed that films made from 10 mg mL⁻¹ Ti₃C₂T_x had slightly higher conductivity (10.56 ± 0.91 kS cm⁻¹) than films made using 1 mg mL⁻¹ MXene (9.84 ± 0.62 kS cm⁻¹). The Poly-MXene and Hex-MXene films had higher starting conductivities than the other additive films (7.37 ± 0.03 kS cm⁻¹ and 7.01 ± 0.02 kS cm⁻¹, respectively), which was likely because the concentration of the additives in the phosphate salt solutions was 1/10th of the concentration of the other films. It is also possible that the washing step involved in making the Poly- and Hex-MXene solutions reduced the overall additive content by the final step. The remaining dried films had decreasing conductivities depending on the additive, from NaAsc-MXene (2.21 ± 0.01 kS cm⁻¹) to VC-MXene (1.55 ± 0.03 kS cm⁻¹) to Na₃Cit-MXene (1.11 ± 0.01 kS cm⁻¹).

The decrease in dried film conductivity due to the presence of additives in the MXene solution may be the result of increased spacing between the MXene nanosheets. This is supported by XRD (Figure 5.4, panel F), wherein the characteristic (002) MXene peaks were shifted to lower values of 2θ for the additive films, compared to untreated MXene. In the absence of additives, the (002) peak occurs at

 $(7.10 \pm 0.01)^\circ$, resulting in a *d*-spacing value of 12.43 ± 0.02 Å for untreated MXene by the Bragg equation. This is in good agreement with expected values of the interlayer spacing of pristine Ti₃C₂T_x from literature (Tang et al. 2019). The interlayer spacing increased for all additive samples, though not by more than ~5 Å. Given that the average size of a water molecule is ~3 Å, it is possible that some moisture remained in the dried additive films after spray-coating; this hypothesis is supported by previous literature (Chae et al. 2019; Zhao, Holta, et al. 2020; Mashtalir et al. 2014). Another possibility is that the additive molecules themselves caused the increase in *d*-spacing, after they adsorbed to the edges of the MXene flakes; similarly, there is precedent for this hypothesis in the literature (Wu et al. 2020; Zhao et al. 2019). The Hex-MXene samples had the closest *d*-spacing values to pristine MXene (13.94 ± 0.19 Å), while the NaAsc-MXene samples had the highest *d*-spacing values of all film types (16.25 ± 0.10 Å). Table 5-1 provides a full summary of the t_0 results.

Raman spectroscopy (Figure 5.4, panel G) revealed only minor differences between the untreated MXene films and the films containing additives. Perhaps most noticeably, the Hex-MXene samples had lower intensity than all other samples, however, the characteristic MXene peaks were still located at the appropriate wavenumbers. Bands associated with in-plane and out-of-plane vibrations of Ti, C, and surface functional groups fell between 204–208 cm⁻¹ for all samples, and the $A_{1g}(C)$ peak occurred between 713–729 cm⁻¹. The locations of these vibrational regions are in good agreement with expectations from literature (Sarycheva and Gogotsi 2020). Refer to Table 5-1 for a full summary of the t_0 results.

Table 5-1 Summary of the major film properties at t_0 , for all dried film types. Values of thickness, τ , and conductivity, σ_0 , are reported as avg. \pm std. dev. across N = 4 samples. Values of the (002) peak location and *d*-spacing from XRD are reported as avg. \pm std. dev. across N = 3 spectra. Finally, values of the A_{1g} peak locations from Raman are reported as avg. \pm std. dev. across N = 2 spectra.

Solution	C _{additives} (mM)	C _{solution} (mg mL ⁻¹)	τ (μm)	σ_0 (kS cm ⁻¹)	(002) peak location (°)	d-spacing (Å)	A _{1g} (Ti, C, O) location (cm ⁻¹)	A _{1g} (C) location (cm ⁻¹)
Untreated MXene		10	0.76 ± 0.13	10.56 ± 0.90	(7.10 ± 0.01)°	12.43 ± 0.02	204 ± 2.30	727 ± 1.75
Untreated MXene	_		1.13 ± 0.82	9.84 ± 0.62				
NaAsc-MXene	100	1	0.78 ± 0.04	2.21 ± 0.01	(5.46 ± 0.06)°	16.25 ± 0.10	208 ± 0.64	725 ± 0.47
Na ₃ Cit-MXene			0.57 ± 0.06	1.11 ± 0.01	(5.45 ± 0.02)°	16.22 ± 0.04	207 ± 1.01	729 ± 0.05
VC-MXene			1.14 ± 0.09	1.55 ± 0.03	(6.16 ± 0.03)°	14.29 ± 0.09	204 ± 1.70	725 ± 0.35
Hex-MXene	10		1.07 ± 0.13	7.01 ± 0.02	(6.64 ± 0.06)°	13.94 ± 0.19	204 ± 0.73	713 ± 1.56
Poly-MXene			0.61 ± 0.11	7.19 ± 0.02	(5.54 ± 0.05)°	15.86 ± 0.16	207 ± 1.10	728 ± 1.84

Dried film results after aging for 12 weeks in air and PBS

Next, the films were aged for 12 weeks, with one set of samples aging in room temperature air, and another set of samples submerged in room temperature PBS (Quality Biologic, pH 7.4). The film conductivity was monitored over time by four-point probe measurements every 2 weeks. Following conductivity measurements at the final timepoint, XRD, Raman, and SEM were also completed. Then, the films that had aged in PBS were baked overnight (~15 hrs.) at 80 °C and atmospheric pressure, and the following day their σ_{DC} and XRD and Raman spectra were again measured, to determine if there were any changes to the film properties as a result of the bake step.



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Figure 5.5 Summary of the $Ti_3C_2T_x$ and additives dried film results at t_{f_i} after aging 12 weeks in air and in PBS. (A,B) Raw conductivity results for samples aged (A) in air, and (B) in PBS. (C,D) Bar plot comparing values of the DC conductivity for all dried films at t_0 and t_{f_i} after aging (C) in air and (D) in PBS. Percentages above the values at t_f represent averages of the total conductivity loss, $\Delta \sigma$, for each film type.

Over time, the DC conductivity of the dried films decreased (Figure 5.5). To characterize the conductivity degradation, we defined the total conductivity loss between the initial and final timepoints as

$$\Delta\sigma[\%] \equiv \left(1 - \frac{\sigma_f}{\sigma_0}\right) \times 100\% \tag{3}$$

where σ_f is the final conductivity, σ_0 is the initial film conductivity, and the total loss, $\Delta \sigma$, is expressed as a percentage. The term $\Delta \sigma$ is hereafter referred to interchangeably as the total conductivity loss, or the total degradation percentage.

By the above definition, then, we were able to quantify the total conductivity losses for each film type, under each aging condition. We observed >30% total conductivity loss for all films aged in air (Figure 5.5, panel C), and >80% loss for all films aged in PBS (Figure 5.5, panel D). Specifically, untreated MXene degraded by >60% in air, and >85% in PBS. Interestingly, the untreated MXene films made from solutions at 1 mg mL⁻¹ had slightly less total degradation percentages ($\Delta \sigma = 66\%$ in air, 86% in PBS) than films made using 10 mg mL⁻¹ solutions ($\Delta \sigma$ = 70% in air, 88% in PBS). This suggests that lower concentration solutions of MXene result in slightly more stable dried films, which likely arises from a higher uniformity of the film morphology in the case of the lower concentration films. Lower concentration MXene inks also tend to have a higher content of single-layer $Ti_3C_2T_x$ flakes, as opposed to the multi-layer $Ti_3C_2T_x$ nanosheets found in higher concentration inks and slurries, which would certainly impact the final film properties (Zhao, Vashisth, et al. 2020). The observed conductivity results and hypotheses expressed here are supported by previous work, which has highlighted the importance of $Ti_3C_2T_x$'s unique rheological properties for film processing considerations (Akuzum et al. 2018).

The VC-MXene and Hex-MXene samples performed better than untreated MXene under both aging conditions: VC-MXene demonstrated $\Delta \sigma = 33\%$ in air and $\Delta \sigma$ = 80% in PBS, while Hex-MXene had $\Delta \sigma$ = 58% in air and $\Delta \sigma$ = 80% in PBS. However, despite these improvements in total degradation percentage, the untreated MXene samples still retained the higher absolute values of conductivity at the final timepoint. In air, the 1 mg mL⁻¹ untreated MXene samples had σ_f = 3.24 ± 0.62 kS cm⁻¹, which was greater than the final conductivity of either VC-MXene $(1.05 \pm 0.10 \text{ kS cm}^{-1})$ or Hex-MXene (2.92 \pm 0.67 kS cm⁻¹). Similarly, even after losing >85% of their starting conductivity after aging in PBS for 12 weeks, the 1 mg mL⁻¹ untreated MXene dried films still had $\sigma_f = 1.56 \pm 0.25$ kS cm⁻¹, which was again greater than either VC-MXene $(0.30 \pm 0.03 \text{ kS cm}^{-1})$ or Hex-MXene $(1.41 \pm 0.16 \text{ kS cm}^{-1})$ aged in PBS. Thus, although the Hex- and VC-MXene solutions showed improvements in their relative stability against degradation over time, their absolute conductivity was still lower than untreated MXene at all timepoints. All other dried films featuring additives had greater losses of their conductivity than untreated MXene. The values of absolute conductivity are summarized in Table 5-2.

Table 5-2 Summary of the major film properties at t_{f_i} for all dried film types. Also included are values of the conductivity for the PBS samples that were baked overnight following t_{f} . All values of conductivity are reported as avg. \pm std. dev. across N = 4 samples. Asterisks (*) denote that the final timepoints for the NaAsc-MXene and Na₃Cit-MXene samples aged in PBS were different from the final timepoints of all other films.

	σ_{DC} (kS cm ⁻¹)						
Solution	At t_0	At t _f aged in air	At <i>t_f</i> aged in PBS	Post-bake			
10 mg/mL Untreated MXene	10.56 ± 0.90	3.33 ± 0.17	1.24 ± 0.07	2.76 ± 0.50			
1 mg/mL Untreated MXene	9.84 ± 0.62	3.24 ± 0.62	1.56 ± 0.25	2.25 ± 0.87			
NaAsc-MXene	2.21 ± 0.01	0.78 ± 0.06	(5.57 ± 0.74) 10 ^{-2*}	—			
Na3Cit-MXene	1.11 ± 0.01	0.27 ± 0.04	0.075 ± 0.004*	—			
VC-MXene	1.55 ± 0.03	1.05 ± 0.10	0.30 ± 0.03	2.01 ± 0.44			
Hex-MXene	7.01 ± 0.02	2.92 ± 0.67	1.41 ± 0.16	2.66 ± 0.06			
Poly-MXene	7.19 ± 0.02	1.61 ± 0.03	0.042 ± 0.001	0.37 ± 0.01			

Looking at normalized plots of σ/σ_0 (Figure 5.6), it becomes clear that the degradation in conductivity for all film types was more gradual under ambient aging conditions (Figure 5.6, panel A) than in the wet aging environment (Figure 5.6, panel B). In air, the majority of samples saw the most change in their DC conductivity between the initial timepoint and ~4–6 weeks afterwards. By contrast, samples stored in PBS saw the most dramatic decreases in their conductivity over the course of the first 2 weeks. This finding clearly emphasizes that the presence of moisture—particularly water—has the greatest impact on the degradation rate of MXene dried films.



Figure 5.6 Trends in the $Ti_3C_2T_x$ and additives dried film conductivity at t_{f} . (A,B) Normalized conductivity change (σ/σ_0) for samples aged (A) in air, and (B) in PBS. (C,D) Phone camera images of the samples at t_f having aged (C) in air, and (D) in PBS.

Among the additive samples aged in PBS, a few trends are particularly noteworthy. After just 4 weeks in PBS, the Hex-MXene samples appeared to have reached a steady state, and their degradation slowed considerably. The VC-MXene samples reached a similar steady state after week 6. The Na₃Cit-MXene films failed after the 4-week timepoint, and the NaAsc-MXene samples did not survive beyond week 6. In fact, these two film types completely dissolved in the PBS environment, making all further measurements impossible (Figure 5.6, panel D). Finally, it is again worth highlighting that in the plots of σ/σ_0 , we see clearly that untreated MXene at 10 mg mL⁻¹ degraded more drastically than samples made from 1 mg mL⁻¹ Ti₃C₂T_x. This is true both in air and in PBS, although the differences in aging behavior are especially noticeable in PBS. Once again, this underscores that processing parameters—here, the concentration of the spray-coating solution—have a significant impact on the final film stability.

Recoverability of dried film properties with baking

Following conductivity and spectroscopic measurements at the final timepoint, the samples that had been stored in PBS were baked at 80 $^{\circ}$ C and atmospheric

pressure overnight (~15 hrs.), and the following day their conductivity and XRD and Raman spectra were recollected. The purpose of this step was to fully define the impact of moisture ingress on film degradation, and to explore whether dried film properties could be "recovered" following aging. This step was conducted for the untreated MXene samples, as well as for the Hex-MXene, Poly-MXene, and VC-MXene films. Since the NaAsc-MXene and Na₃Cit-MXene samples disintegrated into the PBS solution before the final timepoint, they were deemed unrecoverable.

Figure 5.7 shows results for the 1 mg mL⁻¹ untreated MXene samples. Again, between t_0 and t_f the conductivity degraded, with larger losses seen for samples aged in the PBS environment compared to samples aged in air. Concurrent with the loss of conductivity, there was a marked shift of the (002) peak to lower values of 2θ , as seen in XRD. From Bragg's equation, the *d*-spacing values for the films at t_f in air and in PBS were 13.05 ± 0.03 Å and 15.05 ± 0.02 Å, respectively. The increase in *d*-spacing for the PBS aged samples in particular is consistent with water ingress into the film. Raman spectroscopy revealed shifts towards higher wavenumbers for the A_{1g} vibrational modes of the films. Shifts to higher wavenumber are typically associated with fewer vibrational restrictions, and thus more free movement of that specific mode (Sarvcheva and Gogotsi 2020). In this case, the peak shifts were larger for films aged in PBS than for films aged in air, which is consistent with the idea that the individual MXene flakes should have had more freedom to move when surrounded by an aqueous medium, than when stored in air. Indeed, SEM revealed only slight changes in the film morphology between a "fresh" film and a film aged for 12 weeks in air, whereas the samples aged in PBS showed more evident signs of damage.



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Figure 5.7 Summary of the untreated 1 mg mL⁻¹ Ti₃C₂T_x dried film results at t_{f} . (A) Bar plot of the DC conductivity for the samples at each timepoint. Note the increase in conductivity post-bake. This is likely the result of moisture being baked out of the film. (B) XRD of the untreated MXene samples at each timepoint. The dotted red line tracks the peak shifts of the (002) peak for each condition, and the *d*-spacing is provided based on the Bragg equation. (C) Raman spectra of the samples at each timepoint. The locations of the major MXene A_{1g} peaks are provided at each condition. (D–F) Scanning electron micrographs (SEM) of (D) fresh, (E) 12 weeks aged in air, and (F) 12 weeks aged in PBS samples. Scale bars, 2 µm.

Baking the untreated MXene films resulted in recovery of some of their properties, though. Post-bake, the (002) peak shifted to higher values of 2θ , and the *d*-spacing lowered to 12.98 ± 0.12 Å. Accordingly, it appears that the bake step evaporated some of the water content that had infiltrated or been trapped in the film. The resulting baked films also had average final conductivity of 2.25 ± 0.87 kS cm⁻¹, which is ~1.5x higher than the σ_f of the same films after they had aged in PBS (1.56 ± 0.25 kS cm⁻¹). Curiously, baking the films resulted in the A_{1g} vibrational modes of the Raman spectra shifting to even higher wavenumbers. It is worth noting, however, that a broad peak was observed in the Raman spectra, located around ~1370 cm⁻¹, though this peak was only observed for the samples that had aged in PBS (Figure 5.8). Since this peak disappeared from the same films after baking, it is likely that it corresponds to water or other moisture trapped in the film. A similar broad peak in the higher wavenumbers region has been previously observed for MXenes treated with polyphosphate salts, and was also hypothesized to be due to water trapped in the films (Natu et al. 2019).



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Figure 5.8 Extended Raman spectra of the $Ti_3C_2T_x$ and additive dried films at t_f and postbaking. Raman spectra for (A) 1 mg mL⁻¹ untreated $Ti_3C_2T_x$. (B) Hex-MXene, (C) VC-MXene, and (D) Poly-MXene samples. Note the broad and high intensity peak for most samples centered around ~1360 cm⁻¹, which only appears for the films aged in PBS.

The trends observed in the conductivity and XRD and Raman spectra for the untreated MXene samples were mostly observed for the other samples that lasted to the final timepoint in PBS and were subsequently baked, as well. Interestingly, though, the Hex-MXene samples that were aged in PBS lacked a prominent and broad Raman peak in the higher wavenumber region (Figure 5.8). However, this may support the hypothesis that Hex salts were effective in "capping" the edges of the individual $Ti_3C_2T_x$ nanosheets to turn away moisture. Increases in the interlayer spacing were still observed for Hex-MXene samples from XRD, as were peak shifts to higher wavenumbers in the relevant Raman range of 100–800 cm⁻¹. Nevertheless, the *d*-spacing could be lowered, and the conductivity was recoverable to a degree, with a bake step (Figure 5.9, panels A–C, and Table 5-2).

The VC-MXene films had large increases in their *d*-spacing value, consistent with moisture ingress into the films, but this interlayer spacing was lowered—and the conductivity increased considerably, as well—with a bake step. The intensity of the Raman A_{1g} (Ti, C, O) peak was much less for the VC-MXene films aged in PBS, than in the case of samples aged in air. Concurrent with this decrease in the A_{1g} peak intensity was an increase in the intensity of the E_g in-plane vibrational modes, centered around ~360 cm⁻¹. In the case of films submerged in a wet environment, however, it is sensible to assume that the surface functional groups of the MXene films—which are described by the E_g modes—are more active than the out-of-plane groups implicated in coupling between the individual MXene layers (Sarycheva and Gogotsi 2020). The VC-MXene results are summarized in Figure 5.9, panels D–F, and Table 5-2.

The Poly-MXene films showed very significant decreases in their conductivity with aging in PBS, though a bake step afterwards resulted in a large recovery of the film properties. Similar to the VC-MXene samples, the Raman A_{1g} peaks were less pronounced after aging, and the E_g peaks became more prominent, indicating that the surface functional groups are more implicated in degradation of the film stability than coupling between MXene layers. Refer to Figure 5.9, panels G–I, and Table 5-2, for a summary of the Poly-MXene results.

Finally, as expected, the 10 mg mL⁻¹ untreated MXene samples lost more of their initial conductivity, and had much larger increases in their *d*-spacing values, than the 1 mg mL⁻¹ untreated MXene samples. Trends seen in the Raman spectra for these films were also consistent with the trends seen for the 1 mg mL⁻¹ Ti₃C₂T_x samples. The results for these films are included in Figure 5.9, panels J–L, and Table 5-2.



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Figure 5.9 Summary of the DC conductivity, XRD, and Raman results for $Ti_3C_2T_x$ and additive dried films at t_f and post-baking. (A–C) Results for Hex-MXene films. (D–F) Results for VC-MXene films. (G–I) Poly-MXene results. (J–L) Results for the 10 mg mL⁻¹ untreated MXene films. Refer to Table 5-2 for actual values of the DC conductivity under each condition, corresponding to panels (A,D,G,J).

Study Conclusions & Outlook

This study benchmarked the stability of $Ti_3C_2T_x$, both in a solution form and after being processed into dried films that are aged in both ambient and wet environments. Furthermore, we explored the impact that passivating, largely antioxidant agents have on the stability of $Ti_3C_2T_x$ when they are included in the solutions-processing steps as additives. We reached three major conclusions based on this work:

- 1. Untreated solutions of $Ti_3C_2T_x$ are stable for ~1 month in DI water when stored in ambient conditions, out of sunlight. Beyond this time, they degrade quickly. When processed into dried films, untreated $Ti_3C_2T_x$ is more stable in ambient conditions than in wet environments, but nevertheless will lose >60% of its initial conductivity over the course of 3 months of aging. The processing conditions are of primary importance for improving the dried film stability: films made from lower concentration solutions (~1 mg mL⁻¹) appeared to be more stable over time than films made from higher concentrations (~10 mg mL⁻¹), likely as a result of the film uniformity and the percentage of singlelayer nanosheets compared to multi-layered nanosheets.
- 2. Certain additives—here, NaAsc, Na₃Cit, and VC—lend improved stability to $Ti_3C_2T_x$ MXene solutions, likely as a result of the antioxidant properties of the additives themselves. When processed into dried films, however, any film featuring additives will have a lower starting conductivity than a film made from purely untreated $Ti_3C_2T_x$.
- 3. Certain additives—here, VC and Hex—appeared to improve the *relative* stability of the dried film conductivity when such films are aged in air or in wet environments (compare values of $\Delta \sigma$ to untreated MXene). Likely, this is the result of these agents interacting as proxies between the environment and the Ti₃C₂T_x portions of the film, preventing moisture ingress into the film.

Indeed, our work here also established a fourth major conclusion: that moisture ingress is the primary mechanism driving the degradation of $Ti_3C_2T_x$ dried films. In other words, as water (or other liquids or vapor) invades the individual MXene layers, it causes separation of the flakes, resulting in losses of the film properties. However, moisture ingress is a reversible phenomenon, as we demonstrated by baking the films post-aging. Accordingly, the dried film properties are recoverable to a certain degree, even after very significant degradation, as seen especially for the VC-MXene and Poly-MXene dried films.

Importantly, this work emphasized that the film processing techniques are of great importance when it comes to the long-term stability of $Ti_3C_2T_x$ -based biotechnologies. Not only the rheological properties of the starting MXene solutions, but also the film casting techniques themselves, should be thoroughly explored and optimized in future work, in order to improve upon the preliminary study completed here. Nevertheless, our initial investigation does provide a good foundation for further explorations into long-term stable $Ti_3C_2T_x$ solutions and dried films. Furthermore, we have isolated a few promising candidates (Hex and VC) that may be worth further optimization and exploration for MXene-based bioelectronic interfaces.

Layered Films Study

Background: reduced graphene oxide (rGO) for biomedical applications

Instead of adjusting the initial $Ti_3C_2T_x$ solution, another option to improve the stability of the final dried film may be to coat the $Ti_3C_2T_x$ with a protective layer that is resistant to oxidation or moisture ingress. In order to realize this structure so as to not lose out on the excellent properties of the $Ti_3C_2T_x$ layer of the film, however, it is most desirable to have the protective coating layer be somewhat conductive itself. Given the excellent stability of reduced graphene oxide (rGO) against oxidation (Fernandez-Merino et al. 2013; H. Ding et al. 2015; Jiali Zhang et al. 2010), and also given the abundant literature establishing its various uses in biomedicine, we decided to consider rGO-MXene composites as a potential improvement over untreated MXene, as well.

To date, electrochemical gas sensors (Zhu et al. 2010; X. Huang et al. 2012), highly sensitive multi-channel electrode arrays (F. Li et al. 2011; Ng et al. 2015), and penetrating electrodes for recording neural activity *in vivo* (Apollo et al. 2015) have all been presented, which are based on rGO. A large body of literature also exists demonstrating rGO for the detection of a range of biologically-relevant chemical species (Fang et al. 2017; W. Li et al. 2011; Yang et al. 2015; Shao et al. 2010). For many of these applications, the rGO films were first produced by creating dried, often vacuum-filtered, films of graphene oxide (GO), followed by a reduction step. GO is hydrophilic and highly stable as an aqueous dispersion, making it highly compatible with common solutions-processing techniques such as drop-casting, spin-coating, and spray-coating. It is also simple to vacuum filter GO suspensions into micron- or nanometer-thick films. However, as GO is itself an electrical insulator due to an abundance of oxygen moieties in its structure, an additional reduction step is necessary after film assembly, in order to restore the electrical conductivity (Alazmi et al. 2016).

Towards this end, exposure to hydrazine or hydrazine hydrate in vapor form (W. Li et al. 2011; Eda, Fanchini, and Chhowalla 2008; Stankovich et al. 2007), as well as high temperature annealing (T > 1,000 °C) (Yilin Wang et al. 2018), are the most popular methods to form rGO dried films from the literature. However, these may pose serious health concerns to the user (Jiali Zhang et al. 2010; H. Ding et al. 2015), and are destructive to the other components of many bioelectronic devices, as well. For example, the T_g of the polymers typically used for device encapsulation and passivation, such as parylene-C, polyimide, SU8, and polyethylene terephthalate (PET), lie between ~120 °C and ~300 °C (Matsuhisa et al. 2015; Harder et al. 2002; Chung and Park 2013; Yu-hua Wang et al. 2016), making high temperature annealing unfeasible. It has been shown, as well, that hydrazine vapor causes creep and long-term degradation in a variety of polymers (Dine-Hart, Parker, and Wright 1971; Goel, Möller, and Weichold 2009).

Alternatively, GO may be reduced while still in the liquid phase, and conductive rGO films may be subsequently attained through solutions-processing of the reduced suspension. This methodology has largely been achieved by the use of strong acids, including hydrochloric (Ning et al. 2014) and hydriodic (Zheng et al. 2012) acids, as well as hydrazine (Pham et al. 2010) and hydrazine-ammonia mixtures (D. Li et al. 2008). Recently, l-ascorbic acid (Vitamin C, VC), was also established as a viable and more biocompatible reducing agent for aqueous GO dispersions (Jiali Zhang et al. 2010; H. Ding et al. 2015; Fernandez-Merino et al. 2013; Dua et al. 2010). However, in every case of liquid-phase reduction, the resulting rGO suspension is not colloidally stable in the long-term (N. Wei, Lv, and Xu 2014; H. Liu, Wang, and Zhang 2015) and thus, surfactants like NMP or DMF are required to allow further solutions-based processing (Park and Ruoff 2009). Both of these surfactants are cytotoxic, though, and while there is one currently available and less cytotoxic alternative, called Cyrene, its biocompatibility has not been fully characterized (Salavagione et al. 2017; K. Pan et al. 2018). Furthermore, it has been reported that the optimal colloidal stability of rGO dispersions is only achieved with NMP, DMF, or Cyrene at high viscosities (K. Pan et al. 2018), which may preclude the ability to use some conventional spray- or spin-coating equipment.

The vitamin C reduction method

What is needed, then, is a scalable, easy-to-implement method for realizing rGO thin films, which is also safe for the user, and non-destructive to the other materials involved in the fabrication process. Towards this end, we first investigated spray-coating combined mixtures of GO and VC (GO-VC) onto preheated substrates, followed by a final heating step to complete the reduction reaction and obtain conductive rGO films (Figure 5.10). Unlike hydrazine and other strong acids, at room

temperature, VC only reduces GO after 48 hours of continuous stirring of the solution (Jiali Zhang et al. 2010; H. Ding et al. 2015): thus, the reaction kinetics of VC are slow enough at room temperature to allow for direct mixing of the solution with GO, without concerns over the solution's colloidal stability (Jiali Zhang et al. 2010; Fernandez-Merino et al. 2013). After spray-coating of the mixture onto heated substrates, the resulting dried films can be quickly and safely reduced into conductive rGO films, simply by continuing to expose the sample to heat. In order for the entire process to be compatible with the vast majority of polymeric substrates described above, though, we chose to explore 150 °C for the reducing temperature of our GO-VC dried films, and found that 15 minutes of heating was sufficient time to reduce GO-VC into conductive rGO films. The use of GO-VC offers a safer, faster, and less destructive alternative to conventional chemical and thermal reduction methods.



Figure 5.10 Overview of the vitamin C reduction method to realize conductive rGO films. (A) The substrate is cleaned and placed on a hot plate at 150 °C for 5 minutes. (B) Equal parts of GO and VC are mixed together and manually spray-cast onto the preheated substrate, resulting in a dried GO-VC film. (C) Baking the GO-VC film for an additional 15 minutes at 150 °C restores the film conductivity, and results in the final, reduced rGO film. Phone camera images are provided for reference at each step, and demonstrate the clear color change associated with the reduction of GO into rGO between (B) and (C).

Figure 5.11 summarizes the characterization we conducted on our highquality, VC-reduced rGO thin films. SEM revealed that the individual rGO sheets resulting from VC reduction were wrinkled or folded, which will likely lead to an enhancement of the electrochemical surface area. Atomic force microscopy (AFM) further revealed that the flake size and distribution between the GO-VC films before and after reduction was similar, demonstrating that reduction via VC is nondestructive to the primitive graphitic lattice. GO and rGO have been previously studied using Raman spectroscopy (Stankovich et al. 2007; Kudin et al. 2008; Kaniyoor and Ramaprabhu 2012; Ferrari 2007), and the distinctions between their lattice structures can been determined using characteristics of the graphitic D and G peaks, which are related to breathing modes of the hexagonal carbon lattice, and highfrequency sp² carbon vibrational modes, respectively (Ferrari 2007). The D band is especially sensitive to edge defects and changes in domain size (Stankovich et al. 2007), and thus, the $I_{\rm p}/I_{\rm G}$ ratio is a useful measure for distinguishing between GO and rGO films (Kudin et al. 2008; Kaniyoor and Ramaprabhu 2012; Ferrari 2007). In the case of GO, overlapping graphitic flakes appear as defects in the Raman spectra, and so there is an initially pronounced D band, resulting in an I_D/I_G ratio near, but still less than, unity. In our spectrum for GO-VC, the ratio is 0.854. Upon reduction of a pristine GO film, the oxygen moieties populating selected regions of the six-atom carbon lattice are stripped away, resulting in smaller-sized domains in which sp² vibrations may occur (Stankovich et al. 2007). This, in turn, results in an increase in the intensity of the D band, and so the I_D/I_G ratio should rise to a value of, or above, unity. In our spectra for the rGO film, we see this rise as the I_D/I_G ratio becomes 1.013. Thus, the Raman spectra also provide evidence that the GO is completely reduced following exposure to VC and heating.



Figure 5.11 Structural and electronic characterization of the GO-VC and rGO thin films on glass. (A) SEM of an rGO-VC dry film on glass. Scale bar, 200 nm. (B,C) Atomic force micrographs of GO-VC (B) before and (C) after reduction via heating for 15 minutes at 150 °C. An individual flake is highlighted in each instance. (D) Raman spectra of GO-VC and rGO-VC films on borosilicate glass. Each spectrum represents the mean of 6 individually collected spectra. The spectrum of the substrate is also provided for reference. The characteristic D and G bands are labeled accordingly, and the I_D/I_G ratio is provided for each spectrum. (E) Sheet resistances of rGO-VC dry films on glass, as a function of vC used during spray-coating. (F) Sheet resistances of rGO dry films on glass, as a function of time of exposure of the film to 150 °C.

To investigate the effect of processing parameters on the final electrical properties of the rGO films, we characterized the influence of VC concentration and substrate heating time on the final dried film's sheet resistance. We found that the sheet resistance showed an inverse dependence on the VC concentration, while in the case of heating time, there was apparent saturation of the sheet resistance after 15 minutes of heating at 150 °C. These results suggested that VC at any concentration will reduce GO when the two are mixed and exposed to heat together as a dried film. However, the higher the concentration of VC, the shorter the amount of time it should take to reduce the GO-VC dry film in the presence of heat.

The minimum sheet resistance value we obtained through the mixture spraycoating method was $354.7 \pm 20.52 \text{ k}\Omega \text{ sq}^{-1}$, when 50 mM VC was mixed with GO and the spray-cast films were allowed 15 minutes to bake after spray-coating (N = 8measurements across 4 samples). Given an average film thickness of 2.20 ± 0.19 nm (determined using AFM), the average film conductivity was determined to be $12.81 \pm$ 1.75 S cm^{-1} for these samples. Previous work has reported GO solutions reduced with hydrazine and vacuum filtered into thin films as having conductivities ranging from ~0.5 to a few 10s of S cm⁻¹ (Stankovich et al. 2007; D. Li et al. 2008). Conductivities for the rGO dry films realized from the VC reduction method are thus higher than the reported values for some hydrazine-based reduction methods, and are also within the expected range of values for films reduced using VC (Jiali Zhang et al. 2010; Fernandez-Merino et al. 2013; Dua et al. 2010).

Performance of rGO/Ti₃C₂T_x layered films in ambient and wet environments

With the VC reduction method thusly well-established, we next sought to develop rGO-MXene composites, and investigate their stability against degradation in both ambient and wet aging environments. rGO-MXene composites were accordingly realized using highly concentrated (>5 mg mL⁻¹) GO purchased from Graphene Supermarket and mixed with 50 mM VC in a 1:1 volume ratio. A bottom layer of ~10 mg mL⁻¹ Ti₃C₂T_x was spray-coated onto glass substrates, and then 2x the volume of the GO-VC mixture was sprayed overtop of the MXene. The final layered films were subsequently baked for 15 mins. at 150 °C after spray-coating to ensure the complete reduction of GO in the films into rGO. Then, the films were baked overnight (>12 hrs.) at ~500 mTorr and 80 °C, to ensure further evaporation of any moisture in the films prior to aging. The layered rGO/MXene films were ~6µm in final thickness, and were aged in the same ambient and wet conditions as the untreated MXene films from the previous section. For this aging study, a control film was also made from untreated Ti₃C₂T_x—also at 10 mg mL⁻¹—which was aged in the same conditions for the same amount of time.



Figure 5.12 DC Conductivity of the rGO/Ti₃C₂T_x thin films on glass substrates while aging in air and PBS. (A,B) DC conductivity (σ_{DC}) of the rGO/MXene films aged (A) in air for 54 days, and (B) in room temperature PBS for 47 days. (C,D) Normalized conductivity changes (σ/σ_0) for the rGO/MXene films aged in (C) air for 54 days, and (D) PBS for 47 days.

The layered rGO/MXene films had starting conductivities of 4.8 ± 1.2 kS cm⁻¹, which is within the same range as the untreated Ti₃C₂T_x films made for this study (7.6 ± 0.3 kS cm⁻¹). Excitingly, though, we found that the rGO/MXene layered films were slightly more stable than the untreated MXene films after aging. Using Equation(3), we calculated the total percent conductivity loss to be $\Delta \sigma = (81 \pm 3)\%$ for the rGO/MXene films aged in air for 54 days, comparable to $\Delta \sigma = (82 \pm 6)\%$ for the untreated Ti₃C₂T_x films. In PBS, though, the rGO/MXene layered films outperformed the untreated MXene films, and had similar total degradation percentages to the Hex-MXene additive films: the rGO/MXene films demonstrated $\Delta \sigma = (80 \pm 4)\%$ after 47 days in PBS, which represents a ~10% improvement in the relative film stability over untreated MXene films ($\Delta \sigma = (91 \pm 8)\%$ after 47 days in PBS). These results strongly suggested that rGO could be used as a protective coating layer for MXene films, in order to improve the lifetime of the final dried film. Thus, we had developed two strategies to combat MXene's degradation: one involving adjustments to the Ti₃C₂T_x solutions themselves, and one involving protective coatings.

Accelerated Aging of Ti₃C₂T_x macroscale electrodes

Accelerated Aging Background & Experimental Methods

The principle behind accelerated aging experiments is that measurements done under harsher conditions over shorter periods of time can be used to determine long-term effects of exposure under much more mild conditions (Hukins, Mahomed, and Kukureka 2008; ASTM International 2017). Accelerated aging may be useful for estimating the ambient condition shelf-life of a product, for example, or for determining the lifespan of a product under aggravated conditions. For implantable electrodes, measuring the impedance *in vitro* at elevated temperatures is one such real-world example of an accelerated aging study. This is precisely the sort of experiment we sought to conduct next, in order to compare the stability of the Hex-MXene and rGO/MXene layered composites for their potential incorporation in chronic *in vivo* neural interfaces.

For this particular study, Hex-MXene and rGO/MXene films were made on glass substrates following the protocols outlined in the previous sections. Untreated $Ti_3C_2T_x$ films were also made as controls. All samples were encapsulated with thin polyimide tape (Kapton), and electrode areas were defined over these films using precut Kapton masks with defined GSAs of ~0.1 cm² (Figure 5.13). A total of N = 3 electrodes were made for each type of solution.

For the accelerated aging experiment itself, we determined to use 73 $^{\circ}$ C as the aging temperature of the PBS bath, and 1 month as the aging time, which translates to almost 12 months of aging at 37 $^{\circ}$ C (body temperature). These parameters were chosen based on the equation (Hukins, Mahomed, and Kukureka 2008; ASTM International 2017)

$$f = 2^{\Delta T/10} = 2^{(T - T_{ref})/10}$$
(4),

where *f* is the aging rate (in months), and $\Delta T = (T - T_{ref})$ is the temperature difference (in °C) between the accelerated aging temperature, *T*, and the target or reference temperature, *T_{ref}*.

Once made, our electrodes were submerged in a large beaker of PBS, which was covered with Parafilm "M" laboratory film to slow the rate of solution evaporation. The covered beaker was placed in an oven at the target temperature of 73 °C and atmospheric pressure (Figure 5.13). The impedance of the electrodes was our characterization parameter, and was first measured after the PBS solution had

reached the target temperature; subsequent measurements were then made every \sim 3 days over the course of the 1 month of aging. An aqueous Ag/AgCl electrode was used as the reference electrode for all measurements (Sigma Aldrich; 3M KCl), and a graphite carbon rod was used as the counter electrode (Bio-Rad Laboratories, Inc.).



Figure 5.13 Setup of the accelerated aging study. (A–C) Light microscopy images of the (A) untreated $Ti_3C_2T_x$, (B) Hex-MXene, and (C) rGO/MXene layered films made for this study. Scale bars, 2 mm. (D) Image of the electrodes in their aging beaker of PBS, covered by Parafilm "M." (B) Image of the experimental set up, with the electrodes in their beaker of PBS, placed in an oven held at 73 °C.

Accelerated aging experiments are the subject of some criticism because thermodynamic processes occur very differently at higher *vs.* lower temperatures, and thus the accelerated and natural aging processes are mutually exclusive (X. Zou, Uesaka, and Gurnagul 1996; Bansa 2002). Nonetheless, the experimental design we chose to implement for this study was only used for a preliminary assessment of the relative stability of our modified MXene solutions, as compared to unmodified MXene controls aged under the exact same conditions. Future studies may choose to replicate a similar comparison under stricter accelerated aging conditions.

Indeed, the major limitations of the experimental setup explored herein lie in the degree to which we could control the temperature, volume, and concentration of the aging solution. The temperature of the solution was determined by the oven temperature, which, although set to 73 °C, may have fluctuated by $\pm 2-3$ °C. Additionally, the volume of PBS changed over time as a result of evaporation, causing variations in the concentration that could have affected the impedance measurements. To address these concerns, the beaker was heated on a hot plate during each impedance measurement, and the temperature of the bath was monitored during the entire EIS experiment using a thermometer placed in the bath.

Additionally, whenever the PBS bath level became too low, new PBS was added although we were careful to add the new PBS only after the impedance measurement was made for that day, so as to keep the concentration at relatively similar levels from measurement to measurement.



Accelerated Aging Results & Experimental Outlook

Figure 5.14 Results from the accelerated aging study. (A–C) Raw impedance data at 1 kHz and 10 Hz for the (A) untreated MXene, (B) rGO/MXene, and (C) Hex-MXene samples over 1 month of accelerated aging in 73 °C PBS. Points represent averages, and errorbars give the standard deviations for N = 3 electrodes for each film type. (D) Bar plot of the 1 kHz impedance of all film types at t_0 vs. t_f . (E–G) Phone camera images of the electrodes at the final timepoint, after removing them from PBS, demonstrating delamination of the MXene films from the glass substrate.

Initially, the Hex-MXene electrodes had the lowest overall impedance of all compositions—at 1 kHz, the impedance was $68 \pm 7 \Omega$ —while the untreated Ti₃C₂T_x electrodes (76 ± 13 Ω) were similar but slightly higher, and the rGO/MXene electrodes had the highest impedance of all compositions (173 ± 30 Ω). After just 24 days in 73 °C PBS, though, it became apparent that the most stable films were the Hex-MXene and rGO/MXene layered films (Figure 5.14). The impedance of the untreated MXene electrodes fluctuated quite significantly, and overall the untreated films showed trends of increasing impedance from Day 12 onward (Figure 5.14, panel A). In fact, the untreated MXene films did not even survive beyond Day 24: the MXene films delaminated from their substrate and dispersed into the PBS solution, and no impedance was measurable from Day 27 onward. The Hex-MXene impedance was highly stable until Day 12, when it, too, started to increase; however, the rate of

increase was slower than for the other films (Figure 5.14, panel B). The rGO/MXene layered films saw an initial rise in their impedance values between the first and third measurements, but after Day 6, they appeared to have stable impedance, as well (Figure 5.14, panel C). The Hex-MXene and rGO/MXene electrodes also survived until the final timepoint at Day 30, when the 1 kHz impedances averaged only \sim 3*x* higher than the value of impedance at the first measurement. Table 5-3 summarizes the results for all film types.

Table 5-3 Summary of the accelerated aging study results for all dried film types. All values of impedance are reported as avg. \pm std. dev. across N = 3 electrodes. Asterisks (*) denote that the final timepoint for the untreated Ti₃C₂T_x dried films were different from the final timepoints of all other films.

Sample	Avg. Electrode GSA [cm²]	Z_{1kHz} at t_0 [Ω]	Z_{1kHz} at t_f [Ω]	1 kHz Z _f / Z ₀ [unitless]
Untreated Ti ₃ C ₂ T _x	0.13 ± 0.06	76.25 ± 12.92	304.00 ± 134.55*	3.99
rGO/MXene	0.15 ± 0.06	173.01 ± 30.05	398.00 ± 61.60	2.30
Hex-MXene	0.11 ± 0.02	68.32 ± 6.65	83.90 ± 7.89	3.05

From this study, it appeared to us that Hex-MXene and rGO/MXene were indeed comparable to one another in terms of their ability to improve the lifetime and stability of $Ti_3C_2T_x$ dried films, especially in a harsh aging environment. The fact that they survived under accelerated aging conditions for longer than $Ti_3C_2T_x$ alone also suggests that the presence of Hex and/or rGO really can benefit the dried film's stability in the long-term. Accordingly, future work may seek to investigate one or both of these proposed MXene composites for inclusion in the next generation of bioelectronic interfaces that could be implanted for chronic *in vivo* studies. Indeed, it remains to be seen how the impedance properties of these electrodes may impact the recording quality of implantable electrodes, as well as how long these films may last when faced with the foreign body response.

Chapter 6. Ti₃C₂T_x for high-fidelity electrocardiography

Having explored the lifetime of $Ti_3C_2T_x$ dried films and developed working theories to explain the material's degradation over time, and having also developed robust working protocols to realize MXene-based bioelectronic interfaces, here in Chapter 6, I will explore the full benefits of $Ti_3C_2T_x$ MXene for high fidelity biosignal recording applications in the field of electrocardiography (ECG). First, I will provide a brief background on the field of ECG, the hallmark of human biosignals recoding. Next, I will explain the development and benchmarking of $Ti_3C_2T_x$ -based dry ECG textile electrodes, and highlight the benefits these electrodes offer compared to clinical standards. Finally, I will demonstrate the use of our MXene-based bioelectronic interfaces for high-fidelity ECG and motion artifact (MA) recording studies. This chapter will underscore the exciting promise of $Ti_3C_2T_x$ MXene compared to traditional materials and device designs, and offer a finalized product suitable for chronic epidermal studies, such as wearable health monitoring, or longterm clinical electrodiagnostics.

Background

The Electrocardiogram (ECG)

The electrocardiogram, or ECG, is a hallmark of human biosignals recording (Mirvis and Goldberger 2012; Al Ghatrif and Lindsay 2012). ECG arises from the depolarization and repolarization of cardiac muscles during each cardiac cycle (*i.e.*, during each heartbeat). This signal propagates through the thorax and abdomen to reach the skin surface, where surface recording electrodes may measure a standard waveform, called the PQRST complex (Dolinsky et al. 2018) (Figure 6.1).

During a single heartbeat, the heart first relaxes and dilates, allowing blood to fill the ventricles; this phase is called diastole. Subsequent to diastole is systole, wherein the cardiac muscles contract, driving blood through the aorta and pulmonary artery, and out to the rest of the body. Immediately following systole, the heart reenters diastole and again relaxes. The electrical activity of the various cardiac muscles during a single heartbeat cycle results in distinct peaks in the ECG signal, corresponding to each of the letters in 'PQRST' (Mirvis and Goldberger 2012). 'P' may be associated with depolarization of the atria. The 'Q' wave relates to depolarization of the interventricular septum (IVS), separating the right and left atria of the heart. 'R' and 'S' together describe ventricular depolarization. Finally, the 'T' wave appears as a result of repolarization of the ventricles. Taken altogether, the PQRST complex allows clinicians to quickly assess the health and condition of the heart as it completes a single cardiac cycle. Thus, monitoring the ECG—which is simply reading PQRST complexes completed across time—enables quick and sure assessment of the heart's rate and rhythm, and may even act as a proxy to allow monitoring of blood and oxygen supply levels throughout the body (Kalra, Lowe, and Al-Jumaily 2019).



Figure 6.1 Two PQRST complexes in a "normal" ECG recording. Individual peaks of the complex, and the associated physiologic processes, are described for the first wave.

Now, the PQRST complex as just described is considering the case of a normal, healthy human subject. There are numerous variations and abnormalities that may arise in the ECG signal, though, and such abnormalities, if they are truly physiologic in nature, are referred to as arrhythmias. The ECG is valuable for monitoring such arrhythmias, and may assist clinicians in identification of events such as myocardial infarction (i.e., heart attack), ischemic heart disease (*a.k.a.* coronary heart disease), atrial fibrillation, or electrolyte abnormalities (*e.g.*, hypo- or hyperkalemia) (Al Ghatrif and Lindsay 2012). The ECG is also an important tool in efforts to combat the large family of cardiovascular diseases (CVDs) (Deng et al. 2018), which are the leading cause of death in the United States (Nabel 2003) and globally (WHO 2013). Even in the veterinary sciences, ECG has been beneficial for tracking cardiomyopathy in dogs (Wess et al. 2010).

Not all variations or abnormalities that arise in the PQRST complex's shape may be related to CVD or other underlying health problems, though (Chenniappan and Sahib 2017). Indeed, the ECG is also useful in a wide variety of other fields for simple monitoring of everyday health. In sports medicine, for example, the ECG is a widely adopted tool to help trainers and clinicians alike observe and advise the cardiovascular health of athletes during typical exercise (Baggish et al. 2010; Lawless and Best 2008; Gajda 2020). Additionally, ECG may be used to corroborate photoplethysmography (PPG) measurements for continuous heart rate monitoring (HRM), as is the case in the Apple Watch (Apple Inc.) (Isakadze and Martin 2020) and other HRM platforms (Weiler et al. 2017). Indeed, the importance and benefits of the ECG for general human health monitoring has been highlighted in numerous reports (Rutten et al. 2000; Gajda 2020; Providencia et al. 2018; Looi et al. 2015). It is worth noting, though, that there are many non-physiologic factors that could also affect the recorded ECG signal, including what type of electrodes are being used, what electrode configuration is adopted, and what signals processing techniques are applied to clean or simplify the signal for interpretation postcollection.

How the ECG is measured: Lead Configurations & Signals Processing

Clinically, there are standard electrode configurations that may be employed to measure the heart's electrical activity. The gold standard is the 12-lead configuration (Al Ghatrif and Lindsay 2012; Kalra, Lowe, and Al-Jumaily 2019) (Figure 6.2, panel A), however this is a highly involved electrode arrangement that requires the placement of multiple monopolar contacts in precise locations across the subject's chest, with the goal of monitoring the minutiae of the PQRST wave. Even the slightest misplacement of the ECG leads can cause significant changes in the recorded signal using this particular configuration, and previous studies have highlighted the importance for precise lead placement in order to achieve diagnostic accuracy (Rajaganeshan et al. 2008; Khunti 2014; Bickerton and Pooler 2019).

Simpler configurations are also possible, such as the 5- and 3-lead configurations, with the 3-lead configuration being the simplest case. Most often, in a 3-lead configuration, the electrodes are placed at either of the subject's wrist and then at the left ankle (Figure 6.2, panel B), following from the theory of the "Einthoven triangle" (Islam et al. 2021; Kalra, Lowe, and Al-Jumaily 2019). Nevertheless, an even simpler 3-lead configuration may involve placement of electrodes only across the subject's chest (Pounds 2018; Kearney, Thomas, and McAdams 2007) (Figure 6.2, panel C), with the key being that the reference electrode must be placed near the right collarbone, so that the recorded ECG at the primary working electrode (located beneath the heart) records a signal that is referenced across the heart itself.



Figure 6.2 Different lead configurations for recording ECG. (A) Standard lead placement for the 12-lead configuration. Figure taken from (Islam et al. 2021). Not shown are the reference (2 leads) and ground (2 leads) electrodes that are placed at the subject's wrists and ankles, respectively. (B) The 3-lead configuration following the standard "Einthoven triangle" methodology. Each sub-panel describes the direction in which the signal is measured, and provides a schematic of the expected PQRST shape. (C) A simplified 3-lead configuration, involving placement of 3 electrodes only across the subject's chest. Reference is beneath the right collarbone (RA), while ground (LA) is directly above the working electrode (LL), which is located under the heart. Schematic taken from (Pounds 2018).

Depending on the lead placement and configuration used, the recorded PQRST complexes within the ECG signal may have different shapes in time, but aside from lead placement, processing of the ECG signal after (or sometimes during) collection may also impact the shape of the PQRST waveform. A very wide variety of techniques exist in the literature exploring a plethora of ways to filter ECG data to aid with faster interpretation and more accurate diagnosis (Martis, Acharya, and Adeli 2014; Acharya et al. 2017; Deng et al. 2018; Rivera-Juárez et al. 2019; Luo and Johnston 2010; Venkatachalam, Herbrandson, and Asirvatham 2011; Sabherwal, Agrawal, and Singh 2017; Kearney, Thomas, and McAdams 2007; C. Zou et al. 2017), but among the various publications, there are a few common themes which highlight the main focuses and motivations for signals processing of recorded ECG. First and foremost, there is the need to define both upper and lower cutoff frequencies, in order to eliminate aliasing and to suppress baseline wandering, respectively (Luo and Johnston 2010; Martis, Acharya, and Adeli 2014). Secondly, there is the need to reduce artifacts due to motion or EMG activation of surrounding muscle groups (Luo and Johnston 2010; Kearney, Thomas, and McAdams 2007; C. Zou et al. 2017). Perhaps the most robust bandpass filtering of ECG signals may be achieved in the frequency band from 0.05–150 Hz (Luo and Johnston 2010). To handle most motion or EMG-induced artifacts, the wavelet transform is a commonly adopted technique (Kalra, Lowe, and Al-Jumaily 2019).

How the ECG is measured: Standard Electrode Technologies

Naturally, which electrodes are used for recording ECG can also have a major impact on the signal quality, as well. In particular, the impedance and adhesion of the

electrode to the skin surface can affect both the SNR and the motion artifact (MA) susceptibility of the recordings. A high impedance electrode will record with lower SNR due to the presence of Johnson noise (Scalia, Sperini, and Guidi 2012), which may cause serious morphological changes in the ECG signature that can complicate interpretation (Taji et al. 2014). The MA susceptibility of skin-based electrodes may also increase with a higher impedance, or poorer adhesion, further degrading the PQRST waveform (Buxi et al. 2012; Wiese et al. 2005).

In the clinical setting to date, the most common type of ECG electrode is any of the variety of commercially available, non-polarizable, pre-gelled Ag/AgCl electrodes (Kalra, Lowe, and Al-Jumaily 2019; Heikenfeld et al. 2017). One such example is the 3M 2360 Red Dot[™] Resting ECG/EKG Electrode (3M Company). The primary electrode is a flat Ag contact, whose surface is coated with a conductive and often adhesive electrolytic gel containing Ag⁺ and Cl⁻ ions. This gelled electrolyte coating not only maintains sufficient electrical contact between the electrode and the skin, but also serves to improve adhesion of the electrode to the skin surface. However, this same gel may dry out over time or irritate the subject's skin (Kang 2007; Kalra, Lowe, and Al-Jumaily 2019; Murphy et al. 2020), and additionally, the true MA susceptibility of gelled electrode technologies has been previously questioned (Huigen, Peper, and Grimbergen 2002; Meziane et al. 2013; Rodeheaver et al. 2021).

The alternative to gelled electrodes are dry contact electrodes, which operate via capacitive coupling with the skin layers, facilitated by moisture and sweat trapped between the electrode contact and skin surface (An and Stylios 2018; Heikenfeld et al. 2017). Dry electrodes tend to take longer to stabilize than gelled electrodes, though, since their performance depends on the skin's hydration levels (Kalra, Lowe, and Al-Jumaily 2019; Matsukawa et al. 2020). Nevertheless, they offer perhaps a better potential for long-term HRM and other health monitoring capabilities, since there is no portion of the electrode that may dry out or physically deteriorate over time. Indeed—and as was discovered and highlighted in Chapter 4—for dry electrode technologies, perhaps the most major concern for chronic applications will be not the electrode behavior itself, but rather the skin's behavior over time (Matsukawa et al. 2020; Heikenfeld et al. 2017; Paolo Cattarello and Merletti 2016; White, Orazem, and Bunge 2013; Baek et al. 2008; Pylatiuk et al. 2009; H. L. Peng et al. 2016; Huigen, Peper, and Grimbergen 2002; David J. Hewson et al. 2003).

Fabrication & Characterization of Ti₃C₂T_x-based ECG Interfaces

Typically, dry ECG technologies have comprised metallic contacts (Meziane et al. 2013; Searle and Kirkup 2000; Rodeheaver et al. 2021), but recently, there have been progressively more reports proposing dry ECG technologies based on textile substrates infused with metal nanoparticles (Arguilla, Webb, and Anderson 2020; Taji et al. 2014; An and Stylios 2018) or conductive carbon-based inks (Driscoll et al. 2021; Tsukada et al. 2019). Other reports have explored purely carbon nanomaterialbased adhesive patches as wearable HRMs, as well (Lou et al. 2016; T. Kim et al. 2016). Of course, many of the reports that have proposed novel EMG interfaces could also be adapted for ECG monitoring, as well, so there is extensive literature supporting the use of ultrathin electrode designs (Nawrocki et al. 2018; 2016), mesh geometries (Tian et al. 2019), as well as paint-on electrodes (Ershad et al. 2020) and electronic tattoos (Guo et al. 2019; Youhua Wang et al. 2018). Still, textile-based electrode interfaces are especially promising, given the prospect of incorporating such fabricbased HRM platforms into everyday clothing designs (Pani, Achilli, and Bonfiglio 2018; X. Wang, Liu, and Zhang 2017; Y. Liu, Pharr, and Salvatore 2017; Meziane et al. 2013).

For our own study, then, we decided to explore textile-based ECG electrodes, inundated with $Ti_3C_2T_x$ MXene solutions. Adapting the work of my colleague and the MXene-infused textiles technology known as "MXtrodes" (Driscoll et al. 2021), we designed an ECG MXtrode interface encapsulated in thin polyimide tape substrates (Kapton). Subsequently, we applied these electrodes for ECG and MA monitoring, and benchmarked their performance against clinical standards, in a similar fashion to our previous HDsEMG study (Murphy et al. 2020).

Development of the ECG MXtrodes

The fabrication protocol to realize textile-based ECG MXtrodes is outlined in Figure 6.3. First, a CO₂ laser (Universal Laser Systems PLS 4.75) was used to pattern a nonwoven, hydroentangled 55% cellulose / 45% polyester blended textile substrate (Texwipe TechniCloth) into the appropriate electrode geometry. While the original MXtrodes publication used polydimethylsiloxane (PDMS) as the primary encapsulation material (Driscoll et al. 2021), here, we patterned Kapton backings in the same geometry as our ECG electrodes, and affixed the textiles to these backings for support during future processing steps. Kapton also provided the overall device encapsulation in a similar fashion to PDMS or other biocompatible polymers, such as parylene-C. $Ti_3C_2T_x$, obtained from Murata Manufacturing Co., Ltd., was then inked into the textile substrates, right from its starting concentration of ~30 mg mL⁻¹. Given that the textiles we used are highly absorbent, the inking process is not only fast and
easily scalable, but also allows for packing of a high amount of MXene material into the electrode. As higher concentration solutions of $Ti_3C_2T_x$ —often referred to as slurries—are known to be more stable than lower concentrations (Igbal et al. 2021; Bhat et al. 2021; Zhao, Vashisth, et al. 2020), this encouraged us about the potential long-term stability of our MXtrode ECG interfaces. Following the inking step, the electrodes were baked overnight (~15 hrs.) in an 80 °C vacuum oven (~500-550 mTorr) to ensure that the $Ti_3C_2T_x$ was sufficiently dried and any moisture from the slurry had been forced out of the substrate. Finally, the electrodes were actually defined, using precut Kapton vias. Notably, by applying a spray-on adhesive (e.g., Adapt[™] 7730 Medical Adhesive Spray from Hollister, Inc.) to these top Kapton encapsulation layers before placing them over the dried ECG MXtrodes, it was possible to improve the final electrode's attachment and conformability to the skin surface. Alternatively, the top masks could be made from adhesive materials themselves, such as EcoFlex or gummy PDMS. All ECG electrodes that we fabricated with this process featured 9-mm-diameter vias (accordingly, the electrode GSA = 0.6362 cm²).



Figure 6.3 Fabrication flow for realizing $Ti_3C_2T_x$ ECG interfaces. (A) The ECG electrodes are cut out of a textile fabric in the correct form factor. (B) The patterned textiles are affixed to Kapton backings for encapsulation and support through future processing steps. (C) $Ti_3C_2T_x$ slurry is injected into the patterned textiles and subsequently baked dry. (D) The top Kapton encapsulation layers are applied, featuring pre-cut masks defining the specific electrode area. (E–H) Phone camera images of samples at various points in the process. Panel (E) corresponds to panel (B) in the process flow, while panels (F,G) and the top panel of (H) show the steps taken during panel (C). The lower panel of (H) corresponds to the encapsulation step illustrated in panel (D).

Following their fabrication, we also characterized the ECG MXtrodes using light microscopy, XRD, and Raman (Figure 6.4). Light microscopy allowed for the clear distinction between the basic textile substrate and the $Ti_3C_2T_x$ -infused MXtrode. We observed that the MXene slurry fully coated the individual fibers of the hydroentangled mesh, which was well within expectations based on previous reports (Driscoll et al. 2021). Furthermore, the results from XRD and Raman spectroscopy were within our expectations for untreated $Ti_3C_2T_x$ MXene. The (002) peak was particularly clear in XRD, and a *d*-spacing value of 11.80 ± 0.38 Å was determined across 3 separate samples, which is within the correct range based on previous reports (Tang et al. 2019). The A_{1g} peaks associated with out-of-plane vibrations of the Ti, O, C, and other functional groups in the $Ti_3C_2T_x$ lattice also occurred in the correct locations in the Raman spectra (Sarycheva and Gogotsi 2020), and were only noticeable for the MXene-infused ECG samples.



Figure 6.4 Preliminary characterization of the $Ti_3C_2T_x$ ECG electrodes. (A,B) Light microscopy imaging of the textile substrate before being infused with MXene, showing the individual fibers of the hydroentangled mesh. (C,D) Light microscopy imaging of the ECG MXtrodes after top layer encapsulation. Panel (C) Shows the 9 mm Kapton mask atop the MXtrode, while panel (D) shows the fibers of the textile, clearly loaded with $Ti_3C_2T_x$. (E) XRD of the blank textile and MXene-loaded textiles, respectively. Peaks associated with $Ti_3C_2T_x$ are denoted by an asterisk (*), and the *d*-spacing value from Bragg's equation is provided for the (002) peak. (F) Raman spectra of the blank textile on a reflective Si substrate, and the MXtrode ECGs, with the main A_{1g} peaks for $Ti_3C_2T_x$ clearly labeled.

Benchmarking the ECG MXtrodes against the ANSI AAMI EC12 Standard

There is a standard against which all skin-based, passive recording electrodes are assessed, called the "ANSI AAMI EC12:2000/(R)2015 Disposable ECG Electrode Standard" (American National Standards Institute 2020). This document is actually used in an official setting, to assess whether electrodes fabricated for ECG recording are suitable for approval by the United States Food and Drug Administration (FDA), and can thus be sold commercially and used in a real clinical setting. Given the availability of this standard, we sought to benchmark our ECG MXtrodes against this document, to assess their potential for FDA approval. In order to compare our fabricated arrays to commercially available electrodes, we also completed ANSI AAMI EC12 testing for pre-gelled Ag/AgCl electrodes, which we purchased from 3M (hereafter referred to as "3M 2360" electrodes).

Table 6-1 Summary of the electrode performance requirements according to the ANSI AAMI EC12 Standard. Table taken from (American National Standards Institute 2020).

Section	Requirement description	Test conditions	Units	Value (Min/Max)
4.2.1	All requirements of this standard shall be met	Up to the "use before" date according to 4.1 a), under the storage conditions according to 4.1 d)		
4.2.2.1	Average value of 10-Hz impedance for 12 electrode pairs	Pairs connected gel-to-gel, impressed current not exceeding 100 μA	kΩ	2 (max)
	Individual pair impedance		kΩ	3 (max)
4.2.2.2	Offset voltage	Pair connected gel-to-gel, after 1-min stabilization	mV	100 (max)
4.2.2.3	Combined offset instability and internal noise	Pair connected gel-to-gel, after one-minute stabilization period, in the passband of 0.15 to 100 Hz, for 5 min	μV	150 (max)
4.2.2.4	Defibrillation overload recovery (polarization potential)	Pair connected gel-to-gel, 5 seconds after each of four discharges of 200 volts	mV	100 (max)
	Rate of change of polarization potential	During 30-sec interval following polarization potential measurement	mV/sec	1 (max)
	After test, 10-Hz electrode impedance		kΩ	3 (max)
4.2.2.5	DC voltage offset	Pair connected gel-to-gel, continuous 200 nA DC current applied over clinical use period (in no case less than 8 hours)	mV	100 (max)

The ANSI AAMI EC12 performance requirements are outlined in Table 6-1, taken directly from the Standard. Briefly, five (5) major requirements are outlined for wearable electrode technologies, wherein all measurements must be conducted by connecting two of the test electrodes together across a gelled medium, and the electrode pairs are connected to a test circuit or other sort of data acquisition system (DAQ), such as a potentiostat. Briefly, the specific device requirements are:

1. a 10 Hz AC impedance < 2 k Ω between 12 connected electrode pairs;

- 2. an offset voltage between connected electrodes pairs < 100 mV after 1 min. of stabilization;
- 3. an internal noise level < 150 μ V for a connected electrode pair over a 5 min. recording period;
- 4. a polarization potential < 100 mV for a connected electrode pair that undergoes four (4) designed capacitor discharges, and a final electrode impedance < 3 k Ω following these discharges, to simulate electrode stability and recovery during defibrillation; and
- 5. a DC voltage offset < 100 mV when 200 nA of DC current is applied across the connected electrode pair over an extended period of time.

Each of these tests which could be completed with a potentiostat were run using a Gamry Reference 600 potentiostat. For these tests, the reference and counter leads were shorted together and attached to one of the electrodes in each pair, while the working and working-sense leads were shorted and attached to the other of the pair. For test 4.2.2.3 (to measure the internal noise of the electrode pairs), an Intan was used as the DAQ instead of the Gamry, while for test 4.2.2.4 (to assess the electrode polarization potential following a simulated defibrillation discharge), an oscilloscope was used as the primary DAQ. Test circuits for these same two tests were also built following the EC12 Standard's guidance, and using breadboards with commercially available resistors, capacitors, and switches (Figure 6.5). A standard laboratory power source with a maximum voltage of 25 V was used to charge the primary capacitor of the defibrillator setup for test 4.2.2.4.



Figure 6.5 Diagrams and constructed circuits for ANSI AAMI EC12 tests 4.2.2.3 and 4.2.2.4. (A,B) Circuit diagrams for the (A) internal stability (Section 4.2.2.3) and (B) defibrillator (Section 4.2.2.4) tests. Taken from (American National Standards Institute 2020). (C,D) Phone camera images of circuits constructed in-house for the same tests as in (A) and (B), respectively.

All fabricated electrodes were evaluated according to the ANSI AAMI EC12 Standard, and thus featured a conductive hydrogel medium between electrode pairs with enhanced ionic content, courtesy of 3M Company. To test for the AC impedance (Section 4.2.2.1), 'Potentiostatic EIS' was completed on the Gamry from 1-10⁵ Hz, although only the 10 Hz value was considered; the impressed current was determined as the *I_{DC}* value in the *EChem Analyst* software after the measurement had been completed. To determine the DC offset voltage (Section 4.2.2.2), a 'Corrosion Potential' test was completed with the Gamry over a 1.5 min. interval, and the maximum observed voltage was considered as the maximum DC offset within this time period. With electrodes connected to our custom-made internal noise circuit (Figure 6.5, panel C), the baseline noise (Section 4.2.2.3) was recorded over a 6-7 min. period using the Intan; subsequently, the signal was bandpass-filtered from 0.15–100 Hz, and the average of the root-mean-square (rms) of the recorded signal after the first minute of stabilization was used to represent the combined offset instability and internal noise of the electrodes. With the electrode pairs connected to the defibrillator circuit (Figure 6.5, panel D), four consecutive discharges of 20 V across the 10 µF capacitor were completed, and the polarization potential was measured on the oscilloscope as the value of the voltage 5 seconds after each of the 4 discharges

(Section 4.2.2.4). In between discharges, the electrode pairs were left to sit for 5 mins. in order to allow for complete discharging of the voltage through all the components of the circuit. Following the final defibrillation discharge and a 5 min. post-discharge stabilization period, 'Potentiostatic EIS' was run on the electrode pairs with the Gamry to determine their final impedance values. Finally, a 'Galvanostatic' experiment was carried out with the Gamry at a fixed DC current of 200 nA for 15 mins. to determine the bias current tolerance of the electrodes (Section 4.2.2.5), and the potential value at end of this measurement was used to determine the maximum DC offset voltage.

The full results of our benchmarking tests are summarized in Table 6-2 and Figure 6.6. To reach the highly encouraging conclusion before offering further explanation: our $Ti_3C_2T_x$ -based ECG electrodes passed *all* of the ANSI AAMI EC12 tests! However, there were some conditional outcomes that are worth explaining in greater detail.

Table 6-2 Summary of the results for 3M 2360 and MXene-based ECG electrode pairs against the ANSI AAMI EC12 Standard. All values of AC impedance for test 4.2.2.1 are reported as avg. \pm std. dev. across N = 12 electrode pairs. For the remaining tests, at least N = 3 pairs were tested, but only the maximum value from out of those pairs is reported. The note ' \checkmark – C*' in the 'Pass?' columns explains that the electrode pairs "conditionally passed" a specific test.

Section	Test Name	3M 2360 Result	Pass?	MXtrodes Result	Pass?
4.2.2.1	AC impedance	Avg. Z_{10Hz} = 0.66 ± 0.05 kΩ Max I_{DC} = 17.72 μA Max Z_{10Hz} = 0.76 kΩ	~	Avg. Z_{10Hz} = 1.16 ± 0.48 kΩ Max I_{DC} = 64.29 μA Max Z_{10Hz} = 1.75 kΩ	~
4.2.2.2	DC offset voltage	$V_{max} = 0.30 \text{ mV}$	~	$V_{max} = 7.40 \text{ mV}$	~
4.2.2.3	Combined offset instability and internal noise	$\Delta V_{max} = 35.78 \ \mu V$	√	$\Delta V_{max} = 44.11 \ \mu V$	~
4.2.2.4	Defibrillation overload recovery	$V_{max} = 24 \text{ mV}$ Final $Z_{10Hz} = 1.01 \text{ k}\Omega$	✓ – C*	$V_{max} = 85 \text{ mV}$ Final $Z_{10Hz} = 2.89 \text{ k}\Omega$	✓ – C*
4.2.2.5	Bias current tolerance	$\Delta V_{max} = 15.37 \text{ mV}$	✓ - C*	$\Delta V_{max} = 67.21 \text{ mV}$	✓ – C*

For test 4.2.2.4, the required voltage for each of the 4 designed defibrillator discharges is 200 V according to the ANSI AAMI EC12 Standard. However, as we did not have access to a power source that could supply >25 V, we had to make do with 4 pulses powered by a 20 V source. Accordingly, although the ECG MXtrodes passed test 4.2.2.4, it was using 1/10th of the required voltage. Along similar lines, test 4.2.2.5 of the ANSI AAMI EC12 Standard requires measuring the voltage response to 200 nA of DC current over a period of time no less than 8 hrs., whereas we only recorded for 15

mins. in our own study. We ended our studies for test 4.2.2.5 after only 15 mins. since we believed that the trends that appeared in the data would only further stabilize given the longer time periods. However, this again meant that, while the ECG MXtrodes passed test 4.2.2.5, it was only a "conditional pass," as we did not assess the electrodes beyond 15 mins.



Figure 6.6 Results for the ANSI AAMI EC12 Standard tests 4.2.2.4 and 4.2.2.5. (A) Plot of the polarization potential *vs.* pulse number for the 3M 2360 and ECG MXtrode electrode pairs. Three (3) separate electrode pairs are shown for each electrode type. The solid red line denotes the 100 mV polarization potential performance requirement set by the ANSI AAMI EC12 Standard. (B) DC voltage *vs.* time for electrode pairs in response to 200 nA of DC current. Once again, 3 separate electrode pairs are shown for each electrode pair, and the solid red line at 100 mV denotes the performance requirement set by the ANSI AAMI EC12 Standard.

The results shown in Figure 6.6 highlight an interesting—but entirely expected—difference between our fabricated $Ti_3C_2T_x$ ECG electrodes and the pregelled Ag/AgCl commercial electrodes. While the Ag/AgCl electrodes appear to be largely stable with successive defibrillator discharges, and also stable over time in response to 200 nA of impressed current, the ECG MXtrodes appear to have continually increasing voltage responses to the same conditions. This result is to be expected, though, given that (i) Ag/AgCl electrodes are non-polarizable and behave in a largely faradaic manner (Roberto Merletti et al. 2009; H. L. Peng et al. 2016; Yamagami et al. 2018), whereas (ii) $Ti_3C_2T_x$ is a highly capacitive material (Ando et al. 2020; Lukatskaya et al. 2017; S. Xu et al. 2017; Murphy et al. 2020) that behaves in many non-faradaic ways.

Altogether, it is finally worth noting that the ECG MXtrodes had surface areas of 0.6362 cm², compared to the 3M 2360 electrodes, which had surface areas of approximately 6.2 cm². Thus, our small, fully dry, $Ti_3C_2T_x$ -based ECG MXtrodes still

passed nearly all of the ANSI AAMI EC12 tests, with only two tests passed "conditionally" due to equipment or time restrictions. This was a highly encouraging result to us, and so we subsequently pursued ECG recording during MA tasks with our fabricated contacts, and compared their performance to the same 3M 2360 commercial electrodes used for the ANSI AAMI EC12 Standard testing.

Validation of Ti₃C₂T_x-based electrodes for ECG recording

Experimental Design

Six (6) healthy human subjects (3 male, 3 female; avg. age = 26 ± 2 years) were recruited and consented to participate in a pilot study at the University of Pennsylvania, in order to acquire preliminary biopotential data using our fabricated electrodes in their envisioned form factor. For these studies, we were most interested in assessing the SNR and MA susceptibility of our dry, MXene-based devices, as they compared to gelled, commercial electrodes. Accordingly, we fabricated one set of devices for ECG recording, and also made an equal number of electrodes to use for recording "pure" MA. After asking the subjects to remove their shirts, we cleaned the skin beneath their pectoral muscles with an alcohol prep pad, and further abraded the skin surface using 3M TracePrep[™] abrasive tape. Thereafter, we placed the $Ti_3C_2T_x$ and 3M 2360 ECG electrodes using an electrode placement scheme as illustrated in Figure 6.7 (Kearney, Thomas, and McAdams 2007; Cömert, Honkala, and Hyttinen 2013). The 3M 2360 clinical electrodes were used as the reference and ground electrodes for all subjects, and the ordering of the ECG MXtrodes and 3M 2360 contacts was randomized from subject to subject to mitigate possible skin-region effects. We purposefully did not remove any natural hair from subjects' chest areas. and in order to ensure the best attachment of the electrodes to the skin, we used 3M Transpore[™] Surgical Tape overtop all electrodes after placement to hold them in place against each subject's body.

The tasks we asked each subject to complete are also illustrated in Figure 6.7. After electrode placement and a 5 min. equilibration period, we recorded the impedance of the electrodes and then measured the baseline, or "resting state" heart rhythm over a 3 min. interval. After this, we asked each subject to stand, and we again measured their impedance and recorded their heartrate for a 2–3 min. period. Next, we asked the subject to slowly raise and lower their right arm, and had them repeat this task continually over a 2 min. period while their ECG was being recorded. As a final MA task, we asked the subjects to walk on a treadmill at a pace of 1–2 miles per hour (\sim 2–3 kilometers per hour). Finally, after a 5 min. resting period following the treadmill walk, we once more recorded each subject's final skin impedance and resting state heart rhythm. While the subjects completed each MA tasks, their ECG

was continuously recorded at the ECG working electrodes (WEs), whereas the MA WEs simultaneously recorded a significantly smaller ECG signal, and mostly suffered from artifacts as a result of the subject's motion. Some EMG from the muscles along the subject's side, front, and back may have also affected the MA WE results. With this relatively simple experimental design, though, we hypothesized that we would be able to accurately quantify each electrode type's MA susceptibility, and at the same see how the MA susceptibility impacted the ECG signal.



Figure 6.7 Experimental design of the $Ti_3C_2T_x$ ECG and motion artifact study. (A) Schematic of the electrode setup for ECG and MA recording. Ref and Gnd refer to 3M 2360 reference and ground electrodes, respectively, while the MA and ECG WEs are the Motion Artifact and ElectroCardioGraphy Working Electrodes, respectively. (B) Phone camera image of the experimental setup for one of the healthy human subjects recruited for the ECG pilot study. NB: 3M TransporeTM Surgical Tape was used to ensure the best attachment of all electrodes to the skin surface. (C–F) The MA tasks proposed in this study. The subject first sat (C), then stood (D), then completed right arm raises (E), before completing a treadmill walk (F) at a prescribed pace. During each task, the subject's ECG was continuously recorded. The schematics include references to the right (R) and left (L) sides of each subject, for each task.

ECG Signals Processing & Metrics Used for Comparison

Following data collection, all ECG data from each subject was bandpass filtered between 0.05–150 Hz (Luo and Johnston 2010), and a 60 Hz notch filter was also applied to negate powerline noise. As we purposefully wanted to explore MA susceptibility in this pilot study, no wavelet transform was applied to the data after bandpass filtering. Instead, in order to quantify the recording quality and MA susceptibility, we defined two specific metrics. The signal quality metric was a simple SNR calculation at the ECG WEs, using the equation

$$SNR [dB] \equiv \frac{1}{N} \sum_{i=1}^{N} 20 \log_{10} \left(\frac{V_{\text{R peak}}}{V_{\text{rms baseline}}} \right)$$
(1),

where $V_{\text{R peak}}$ is the amplitude of the R wave peak in the ECG signal (see Figure 6.1), and $V_{\text{rms baseline}}$ represents the rms voltage amplitude during the baseline period between each PQRST activity pattern (Figure 6.1: the period after the T wave and preceding the P wave). The SNR was averaged over a total of *N* heartbeats for each subject, and the purpose of the logarithm was to allow for comparison across a relatively wide dynamic range.

To quantify the MA susceptibility, we compared the amplitude of the rms voltage at the MA WEs during each of the MA tasks (Figure 6.7, panels D–F), $V_{\rm rms\ baseline}^{\rm MA\ Task}$, to the rms amplitude of the same WEs during the "resting state" task (Figure 6.7, panel C), $V_{\rm rms\ baseline}^{\rm Rest}$, using the equation

$$MA_{sus} [dB] \equiv \frac{1}{N} \sum_{i=1}^{N} 20 \log_{10} \left(\frac{V_{\text{rms baseline}}^{\text{MA Task}}}{V_{\text{rms baseline}}^{\text{Rest}}} \right)$$
(2).

Again, we averaged the MA susceptibility results over *N* total heartbeats for each subject. The benefit of this particular MA metric—again, determined solely from the MA WEs—was that we were able to quantify a relatively "pure" MA susceptibility for each electrode type, at each MA task, free from any significant ECG signal. Necessarily, as follows from Equation(2), the MA susceptibility of all electrodes during the resting state is defined as 0 dB: thus, the MA susceptibility was expected to be a sensitive metric for discovering changes in each subject's motion across tasks.

From both Equations(1,2), then, we believed we would be able to compare and contrast each electrode type, and we expected to demonstrate that the MXene-based electrodes were at least comparable to clinical electrodes in terms of their recording

performance and their susceptibility to MA. We also planned to use the skin-electrode impedance as a third metric for comparison between electrode types.

Pilot Study Results & Statistical Analyses

The starting impedance of the ECG MXtrodes at 20 Hz (the lowest recording frequency possible with the Intan), across all subjects, was $1.56 \pm 1.29 \text{ M}\Omega$, compared to 240.12 ± 16.57 k Ω for the 3M 2360 electrodes. Similarly high skin impedance values (100s of k Ω up to a few M Ω) have been previously reported for textile-based ECG electrodes in the literature, but it is also worth noting that due to the variability of the thickness of human skin over different regions of the body, there is no exact expected impedance value for the chest area (An and Stylios 2018). Still, given the impedance magnitude and the frequency regime of interest, it is possible to compute a theoretical value of the expected Johnson noise, *V*_{thermal}, using the equation (Scalia, Sperini, and Guidi 2012; Mierzejewski et al. 2020)

$$V_{thermal} = \sqrt{4k_BT \times \text{Re}[Z] \times \Delta f} \tag{3}$$

where k_B is the Boltzmann constant and T is the ambient recording temperature. Re[Z] refers to the real part of the impedance, typified by the resistance, and expressed by the impedance magnitude, while $\Delta f \equiv f_2 - f_1$ represents the frequency range of interest, which for ECG monitoring we can define as the same as the bandpass filtering window of 0.05 - 150 Hz. Accordingly, applying Equation(3) to our measured impedance values with the 3M 2360 and $Ti_3C_2T_x$ ECG electrodes at room temperature (300 K), the theoretical thermal noise values obtained are: 0.7694 µV for Ag/AgCl and 1.9610 μ V for Ti₃C₂T_x, respectively. As these values are within similar ranges, and also both significantly lower than even the baseline noise values of the two types of electrodes (see Table 6-2), we believe it is safe to say that the \sim 5x higher impedance observed for the dry MXene-based electrodes did not have a significant impact on the recording quality of these electrodes compared to the 3M 2360 electrodes. It is also worth noting, as well, that when the electrode impedance is normalized by the respective surface areas of the separate contacts, the impedances for the Ag/AgCl and $Ti_3C_2T_x$ electrodes are 1.48 ± 0.10 M Ω cm² and 992.47 ± 820.70 k Ω cm², respectively, meaning that the initial normalized impedance at the skin-electrode interface was roughly equivalent for both electrode types, across all subjects, anyways.

Likely due to the similar interfacial impedance and low Johnson noise values, the recorded ECG signal during each subject's resting state was similar between the ECG MXtrodes and 3M 2360 contacts (Figure 6.8, panel A). As the subjects completed subsequent MA tasks, the artifacts themselves became apparent in the ECG recordings, most noticeably during the baseline period between successive P and T

waves (Figure 6.8, panels B–D). The MA electrodes also picked up clear ECG signals during each MA task, but as Figure 6.8 clearly shows, due to their further distance from the heart compared to the ECG WEs, the ECG signal was much less intense on the MA WEs. Due to the low intensity PQRST signal, the MA susceptibility for these electrodes was much higher amplitude.



Figure 6.8 Results of the $Ti_3C_2T_x$ ECG and MA susceptibility study. (A–D) Filtered ECG signals for one subject during the (A) resting state, (B) standing task, (C) arm raising task, and (D) treadmill walk task of the experiment. In each case, the top two traces shown the recordings from the ECG WEs, while the bottom two traces track the MA WE measurements. The individual components of the PQRST complex are labeled accordingly, where identifiable. Also included are the average heart rates of the subject in each task. (E,F) SNRs for the (E) 3M 2360 and (F) $Ti_3C_2T_x$ ECG electrodes across all subjects tested with either electrode type. (G,H) MA susceptibilities for the (G) 3M 2360 and (H) $Ti_3C_2T_x$ ECG electrodes across all subjects tested with either electrode type. For panels E–H, points represent means, and errorbars show standard deviations across at least *N* = 30 heartbeats for each subject, in each task, after bandpass and 60 Hz notch filtering of the data.

Indeed, both electrode types also had similar baseline noise levels at rest: for the ECG MXtrodes, the baseline noise in the resting state was $39.340 \pm 15.80 \mu V$ across at least 30 heartbeats in all subjects, compared to $30.76 \pm 11.74 \mu V$ for the 3M 2360 electrodes over the same number of trials. Similar values were also observed in the SNR values (Figure 6.8, panels E and F), and MA susceptibility values (Figure 6.8, panels G and H) for the electrodes as calculated from Equations(1,2). This was true not only in the resting state, but also during subsequent MA tasks. Thus, both electrode types exhibited similar trends in their recording performance. As more motion was introduced into each subject's recording session, the SNRs on both

electrode types decreased according to the definition of SNR from Equation(1). Concurrently, we observed a marked rise in the MA susceptibility for each electrode type, as defined by Equation(2).

To further explore the similarities or differences between the gelled 3M 2360 electrodes and our fully dry $Ti_3C_2T_x$ electrodes, we ran statistical analyses on the impedance data, as well as on the SNR and MA metrics data (Table 6-3 and Figures 6.9 and 6.10). To compare impedance values first, we ran simple *t*-tests comparing the MXene and 3M 2360 electrode impedances, both before and after GSA normalization (Figure 6.9, panels A and B), during each task individually. We subsequently ran *t*-tests to explore whether the MA tasks caused significant changes in the independent impedance values for each electrode type, compared to the electrode impedance for the same electrode type during the resting phase (Figure 6.9, panels C and D).



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Figure 6.9 Comparison of the 3M 2360 and $Ti_3C_2T_x$ ECG electrode impedance at 20 Hz. (A, B) Comparative bar plot of the (A) raw impedance magnitude and (B) GSA-normalized impedance magnitude at 20 Hz for the 3M 2360 and $Ti_3C_2T_x$ ECG electrodes. Significance bars in panel (A) denote p < 0.05 statistical significance between the electrode groups. (C,D) Bar plot of the raw impedance magnitudes for the (C) 3M 2360 electrodes and (D) $Ti_3C_2T_x$ ECG electrodes individually, at each MA task. For all panels, bars represent means, and errorbars show standard deviations across all subjects, in each task (N = 6).

As may be expected, we found significant differences between the 3M 2360 and $Ti_3C_2T_x$ ECG electrode impedance values at each of the individual MA tasks when we considered the non-normalized impedance values (Figure 6.9, panel A). In every case, the 3M 2360 electrodes had statistically significantly lower impedance at 20 Hz compared to the MXtrodes (p < 0.05). However, when the electrode impedance was normalized by the GSA of the electrodes, there was no statistically significant difference observed between the two electrode types (Figure 6.9, panel B). This may account for the similar recording performance and similar trends in SNR and MA susceptibility observed in Figure 6.8. Additionally, when we considered the impedance values of the 3M 2360 and $Ti_3C_2T_x$ ECG electrodes separately, and looked at whether the MA tasks themselves resulted in significant changes in the impedance values, we observed no statistically significant result (Figure 6.9, panels C and D). Thus, we could conclude that the skin-electrode interfacial impedance was not only comparable between our electrode types, but was also consistent over the length of the entire MA study, and was unaffected by the MA tasks themselves. This is an interesting outcome to highlight, though, since it has long been shown that impedance plays a significant role in signal quality (Cogan 2008; Huigen, Peper, and Grimbergen 2002; Mierzejewski et al. 2020), and since it is also thought that the impedance is impacted by MA, as well (Taji et al. 2014; Rodeheaver et al. 2021). Perhaps the consistency of impedance values across the length of the study was a direct results of the electrodes being well-attached to the skin, so that electrode adhesion was not a major cause of concern, nor a cause for MA, in these recordings. The use of the Transpore[™] Surgical Tape certainly improved adhesion, although further studies may seek to explore adhesive coatings to the surface of the ECG MXtrodes as a more fair comparison to the already adhesive, gelled 3M 2360 contacts.

After impedance analysis, we also ran two separate *t*-tests between the 3M and MXene SNR values, and the 3M and MXene MA susceptibility values, across at least 30 heartbeats for each subject, for each MA task. The significance levels were set to p = 0.025 after a Holm correction, to adjust for multiple comparisons between heartbeats within each subject's dataset under each task. Table 6-3 and Figure 6.10 summarize the statistical results of this analysis. What we found was no statistically significant difference between the SNR values of the 3M 2360 electrodes and the MXene-based ECG electrodes. This implied that both electrode types are capable of recording ECG with equal quality. Furthermore, no significant difference was found for the baseline rms amplitudes, nor for the MA susceptibilities of the two electrode types. This is particularly noteworthy, as it suggests that our smaller area, totally dry, Ti₃C₂T_x-based ECG interfaces are perfectly equivalent to the larger-area, pre-gelled 3M 2360 electrodes currently in use in many clinical settings, in terms of their sensitivity to MA.

Table 6-3 Summary of the metrics and statistics results for 3M 2360 and $Ti_3C_2T_x$ ECG electrodes during each MA task. Impedance values are reported both in raw form and after GSA normalization. The value of MA susceptibility was 0 dB for all electrode types according to Equation(2); therefore, the baseline noise values have been supplemented into the table instead for the MA metric at 'Rest.' SNR and MA *p*-values are the result of *t*-tests between the 3M 2360 dataset for all subjects, and the MXtrodes ECG dataset for all subjects, with a 5% significance level (*p* < 0.025 after a Holm correction). Note that only signals from the ECG WEs were considered for baseline noise and SNR analysis, while data from only the MA WEs were considered for the MA susceptibility analysis.

		Rest	Standing	Arm Raise	Treadmill
	$Z_{20 Hz}$	240.12 ± 16.57 kΩ [1.49 ± 0.10 MΩ cm ²]	$169.05 \pm 19.05 \text{ k}\Omega$ [1.05 ± 0.12 MΩ cm ²]	151.04 ± 18.09 kΩ [0.94 ± 0.11 MΩ cm ²]	$\frac{188.78 \pm 136.92 \text{ k}\Omega}{[1.17 \pm 0.84 \text{ M}\Omega \text{ cm}^2]}$
3M 2360	SNR	31.59 ± 2.04 dB	29.40 ± 2.00 dB	27.40 ± 3.22 dB	23.93 ± 3.85 dB
	MA	Baseline Noise: 30.76 ± 11.74 μV	3.09 ± 2.43 dB	11.34 ± 6.08 dB	16.36 ± 4.84 dB
MXtrodes	Z _{20 Hz}	1.56 ± 1.29 MΩ [0.99 ± 0.82 MΩ cm ²]	2.53 ± 0.79 MΩ [1.61 ± 0.50 MΩ cm ²]	1.61 ± 0.88 MΩ [1.03 ± 0.56 MΩ cm ²]	$1.78 \pm 0.16 \text{ M}\Omega$ [1.13 ± 0.10 M Ω cm ²]
	SNR	33.17 ± 0.97 dB	31.39 ± 2.09 dB	29.35 ± 1.77 dB	26.30 ± 2.88 dB
	MA	Baseline Noise: 39.340 ± 15.80 μV	4.32 ± 2.81 dB	12.33 ± 2.03 dB	18.12 ± 1.78 dB
SNR <i>p</i> -values		0.1568	0.1623	0.2706	0.3020
MA <i>p</i> -values		0.2771	0.4808	0.7376	0.4670



Figure 6.10 Comparison of the 3M 2360 and $Ti_3C_2T_x$ ECG electrode signal quality. (A) Baseline noise of each electrode type during Rest. (B) SNR values for each electrode type during each MA task. (C) MA susceptibility for each electrode type during each MA task. For all plots, bars represent means, and errorbars show standard deviations, across at least *N* = 30 heartbeats for all subjects, for each task. Again, only the ECG WEs were considered for the baseline noise and SNR analysis, and only the MA WEs were considered for the MA susceptibility analysis. No significant differences were found between the $Ti_3C_2T_x$ ECG electrodes and the 3M 2360 electrodes for any of these metrics.

Beyond comparing the recording performance and MA susceptibility of the dry $Ti_3C_2T_x$ and gelled Ag/AgCl electrodes to one another, we were also curious to know whether our proposed metrics in Equations(1,2) were actually capturing significant changes in the collected data. After all, since the impedance did not change significantly for either electrode type during any of the MA tasks compared to the resting state, it was still a significant result alone that the SNR and MA susceptibility metrics *did* change. Therefore, we sought to validate that either the SNR metric or the MA metric-if not both metrics together-were sufficiently defined so as to distinguish between the basic resting state and a given MA task in our experimental setup. Since the MXtrodes and the 3M 2360 had similar trends, and as the electrodes were not significantly different in any of the individual MA tasks, we accordingly pooled together the SNR values of the 3M 2360 and MXene electrodes, and ran a simple *t*-test comparing the SNR of all electrodes in each of the MA tasks to the SNR of all electrodes during Rest. We also conducted a similar *t*-test for the MA metrics of all electrodes. The results are summarized in Table 6-4 and Figure 6.11. Once again, in order to correct for multiple comparisons across subjects, a Holm correct was applied, thus adjusting the *p*-value for a 5% significance level to p = 0.025.

Table 6-4 Statistical analysis of the SNR and MA metrics during ECG recoding for all electrode types. Again, the value of MA susceptibility is 0 dB for all electrodes according to Equation(2). The significance level was set to p = 0.025 after a Holm correction, to account for multiple comparisons among heartbeats across all subjects. Values of Avg. SNR and Avg. MA are reported as mean ± std. dev. across all electrode types (2), all subjects (6), and for at least N = 30 heartbeats per subject.

	Rest	Standing	Arm Raise	Treadmill
Avg. SNR [dB]	32.40 ± 1.72	30.39 ± 2.20	28.38 ± 2.66	25.11 ± 3.44
SNR p-value	—	0.0826	0.0030	3.45×10^{-3}
Avg. MA [dB]	0	3.71 ± 2.56	11.83 ± 4.30	17.24 ± 3.56
MA p-value	—	7.06×10^{-3}	8.60×10^{-2}	2.97×10^{-4}

In total, we found that there was a statistically significant difference in the SNR values of the ECG signal collected during the Arm Raising and Treadmill Walk MA tasks, when compared to the resting state ECG recordings (Figure 6.11, panel A). However, we did not observe a significant difference between the SNR values of the ECG signal collected during the Standing phase compared to the resting state. Accordingly, we concluded that the SNR metric as defined by Equation(1) may only be a sufficiently sensitive metric for distinguishing between resting state and MA tasks that involve more complex or full-body motions, such as motion of the arms or ambulation. This would also imply that the recording quality of our two electrodes was largely unaffected by the Standing task. In essence, as the primary ECG signal does not change significantly between a subject sitting and standing, the SNR metric is insufficient to distinguish between the two states.



Figure 6.11 Sensitivity of the SNR and MA metrics to "true" motion artifact. (A) Comparison of the SNR values across all electrodes and all subjects, at each of the MA tasks, to the SNR values at Rest. (B) Comparison of the MA susceptibility values across all electrodes and all subjects, at each MA task, to the MA metric value at Rest. Only the ECG WE data were considered for the SNR metric analysis, however all electrode types were considered; thus, bars represent averages and errorbars provide the standard deviations across both the Ti₃C₂T_x ECG and 3M 2360 electrodes, for all subjects, for all MA tasks. Similarly, only the MA WE data were considered for the MA susceptibility analysis, but all electrode types were considered. At least 30 heartbeats were considered for each subject, during each task. Triple-asterisks (***) denote a significance level of *p* < 0.025. Refer to Table 6-4 for actual values.

Similar to the SNR results, we found that the MA metric as defined by Equation(2) was sufficiently sensitive to distinguish between the Arm Raising and Treadmill Walk MA tasks, compared to the resting state. However, we also found that the MA values computed during the Standing task were also statistically significantly different from the values during Rest (Figure 6.11, panel B). Accordingly, we concluded that our proposed MA metric is a truly robust metric that is particularly sensitive for determining the MA susceptibility of the ECG electrodes as explored in the experimental design of this pilot study. Again, we may ascribe the robustness of our proposed MA metric to the fact that it defines the MA during Rest as strictly 0 dB for *all* electrode types, thus allowing for a finer sensitivity to even slight increases in the MA for various electrodes. Comparison of the SNR and baselines noise values of individual electrodes during Rest is certainly a more accurate way to distinguish between various electrode types at baseline.

Conclusion & Outlook

In the pilot study described above, we first fabricated fully dry electrodes for ECG recording based entirely on $Ti_3C_2T_x$ slurries injected into highly absorbent textile substrates. Following fabrication, we benchmarked our fabricated electrodes against an established Standard for ECG electrodes, and discovered that our electrodes

passed many of the performance requirements put forth in this Standard. Accordingly, the $Ti_3C_2T_x$ -based dry ECG electrodes may hold the potential for true commercialization and use beyond a simple research setting.

Following benchmarking, we recruited healthy human subjects and recorded ECG during simple tasks involving increasingly greater motion, in order to assess the recording performance of our electrodes, as well as outline their motion artifact susceptibility. To demonstrate the feasibility of our devices for high-fidelity ECG recording, we compared their performance to a state-of-the-art electrode design—the 3M 2360 Red DotTM Resting ECG/EKG Electrode. Student's *t*-tests revealed no significant differences between the recording performance or motion artifact susceptibility of the Ti₃C₂T_x-based ECG electrodes, compared to the much larger and gelled Ag/AgCl electrodes from 3M. Furthermore, both electrode types had similar baseline noise levels, and similar impedance properties when normalized by the electrode surface area. Finally, we demonstrated that our proposed methodology for examining recording performance and motion artifact susceptibility is, indeed, a sound methodology, given the sensitivity of our computed metrics at distinguishing between a resting state and a state of motion.

Altogether, this pilot study has demonstrated that our fabricated textile electrodes likely perform at the same level as current clinical standards. Future work will have to compare the MXene-based electrodes to clinical standards strictly according to the ANSI AAMI EC12 Standard. Additional work will also need to explore the long-term stability of these interfaces, for truly chronic HRM applications. Nevertheless, given the ease of processability of $Ti_3C_2T_x$ MXene, we expect the relative costs to manufacture dry, MXene-based devices to be much less compared to gelled, metallic devices, which are the current state-of-the-art. Accordingly, we still propose our $Ti_3C_2T_x$ ECG MXtrodes, as described in this section, as a promising new technology for basic electrodiagnostics, with the potential to greatly cut costs and increase accessibility, without significant losses in device performance or diagnostic accuracy.

Future Work

In further work already being conducted at this time, we are exploring alternative $Ti_3C_2T_x$ MXene solutions to help boost the shelf-life and overall lifetime of this particular class of surface recording electrode technology. Synthesizing the work done in our studies in Chapters 3 & 5 with the work outlined here and in previous reports (Driscoll et al. 2021), we have already explored high-density MXtrode arrays featuring hexametaphosphate- (Hex-MXene) and *l*-ascorbic acid-treated MXene solutions (VC-MXene), and compared these directly with untreated $Ti_3C_2T_x$. Figure 6.12 illustrates the three alternative MXene-based MXtrode arrays.



Figure 6.12 High-density MXtrode arrays featuring additive $Ti_3C_2T_x$ solutions. (A) An untreated MXene MXtrode, made using ~30 mg mL⁻¹ $Ti_3C_2T_x$ slurry. (B) A Hex-MXene MXtrode, made using a ~10 mg mL⁻¹ hexametaphosphate-treated $Ti_3C_2T_x$ solution. (C) A VC-MXene MXtrode, made using a ~10 mg mL⁻¹ VC-treated $Ti_3C_2T_x$ solution. All electrodes were 3 mm in (exposed) diameter, and each array featured 10 individual channels. Devices were developed according the original MXtrodes protocol, outlined in (Driscoll et al. 2021).

As a simple preliminary study of the effects that the additives may have on the electrode impedance—and, by extension, the recording performance—we have completed a short stability study of the MXtrode array impedance over 72 hrs. of continuous wear on a single subject (Figure 6.13 and Table 6-5). Informed by our equivalent circuit modelling investigation in Chapter 4, we also took special care to explore the impact of skin treatments on the long-term impedance characteristics. Accordingly, electrodes applied to the subject's forearm experienced both No Treatment and the PBS skin treatment that was determined to be sufficient at reducing the impedance for $Ti_3C_2T_x$ -based electrode interfaces. We were also careful to monitor environmental conditions, such as the temperature and relative humidity of the recording environment, in order to explore the potential impact of these factors on the total impedance over time.



Figure 6.13 Preliminary assessment of the long-term impedance stability of $Ti_3C_2T_x$ highdensity surface recording electrodes. (A–C) The 10 Hz skin impedance values for MXtrodes devices featuring different MXene composites, and exploring No Treatment and PBS skin conditions, over 72 hrs. of continuous wear. The different compositions of MXene are (A) untreated MXene (~30 mg mL⁻¹ concentrated slurry), (B) Hex-MXene (~10 mg mL⁻¹ solution), and (C) VC-MXene (~10 mg mL⁻¹ solution). For all plots, points are averages and errorbars are standard deviations across N = 10 channels in each of the MXtrode arrays. (D) Plot of the room temperature (in blue) and the relative humidity (in red) *vs.* time for the entirety of the recording study. Notice that the trends in temperature in particularly appear to be correlated with the impedance changes observed in panels (A–C).

Results from our preliminary assessment showed interesting trends in the impedance data over time (Figure 6.13 and Table 6.-5). All electrodes had similar 10 Hz impedance values at the initial timepoint, t_0 , within the range of 30–200 k Ω , which is, again, within the expected range for most textile-based epidermal electrode technologies (An and Stylios 2018). As expected, the untreated MXene device had the lowest interfacial impedances, likely due to the higher MXene loading afforded by use of the 30 mg mL⁻¹. Nevertheless, the VC-MXene and Hex-MXene devices still showed promising skin impedance values. Also as expected from our previous work, the PBS skin treatment reduced the impedance for all electrode types compared to the impedance without any skin treatment. Over time, the impedance for all electrodes fluctuated, but generally increased; by the final timepoint, t_{fi} all electrodes had approximately 1–2 M Ω impedance. The VC-MXene and Hex-MXene devices had slightly lower impedances than the untreated MXene device.

Solution	Skin Prep	Z_{10Hz} at t_0 (k Ω)	$Z_{10 Hz}$ at t_f (M Ω)
Untroated Ti C T	No Treatment	143.00 ± 7.85	1.70 ± 0.14
Untreated H ₃ C ₂ T _x	PBS Treatment	39.59 ± 0.75	1.34 ± 0.08
VC MVono	No Treatment	67.49 ± 1.44	1.94 ± 0.14
v C-Mixelle	PBS Treatment	44.65 ± 1.41	0.91 ± 0.09
How MYono	No Treatment	206.27 ± 4.62	1.17 ± 0.03
пех-млене	PBS Treatment	153.93 ± 3.72	0.95 ± 0.06

Table 6-5 Skin-electrode impedance of the modified MXtrode arrays at 10 Hz, over the course of 72 hrs. of continuous wear. Values are reported for the t_0 and t_f timepoints, and are reported as avg. \pm std. dev. across all 10 channels in each array.

Perhaps of more interest, though, was the relationship we observed between the impedance fluctuations in our MXtrode devices over time, and the fluctuations observed in the environmental conditions (Figure 6.13, panel D). Particularly looking at the temperature, we observed that increases in the ambient temperature seemed to cause similar increases in the impedance, and *vice versa* for decreases in the ambient temperature. Increases in temperature were typically correlated with decreases in the relative humidity of the recording environment, though, so perhaps the trends observed in the impedance data may track more closely with the relative humidity of the environment than with the ambient temperature of it. Such would certainly make sense based on previous reports, as the skin-electrode impedance is known to have an inverse relationship with the level of skin hydration, which is itself related to environmental conditions (Matsukawa et al. 2020; Heikenfeld et al. 2017; Yamamoto and Yamamoto 1976). As we know from Chapter 5, as well, $Ti_3C_2T_x$ is particularly susceptible to moisture ingress, so perhaps the humidity plays a larger role for this reason, as well.

Future work will certainly require a deeper investigation not only into the benefits of the modified MXene solutions for device stability, but also the impact of environmental conditions of the impedance and recording signal quality. Indeed, the MA artifact study proposed and explored in this same Chapter may be a good way to delineate between various MXene-based electrode types, but a novel study may need to be designed and implemented to more accurately correlate or define the role that environmental temperature and relative humidity play on the impedance and signal quality. Indeed, only a very few number of reports have previously explored or even discussed the role that the environment can play on impedance characteristics and recording performance (Matsukawa et al. 2020; Pani, Achilli, and Bonfiglio 2018; Heikenfeld et al. 2017; Ortiz-Catalan et al. 2012).

Chapter 7. Conclusions & Future Directions

Contributions

Electrode technologies will always be valuable tools for probing the human condition, whether that entails observation and monitoring through recording, or modulation and tempering of function and behavior through stimulation. However, while state-of-the-art electrode technologies function well enough to meet their prescribed purposes at present, they are largely incompatible with the human body due to mechanical mismatch. Furthermore, they do not survive long enough for truly chronic applications. To address and possibly mitigate these concerns, there has been an incessant push in the fields of materials science and biomedical device design, towards realizing highly functioning, fully biocompatible platforms based in part or entirely on two-dimensional nanocarbons. This shift in focus comes as a result of the many enhanced mechanical, electrical, and electrochemical properties that most carbon-based nanomaterials exhibit.

Chief among the most promising carbon-based nanomaterials for biomedical applications is $Ti_3C_2T_x$ MXene, which has a higher conductivity, better electrochemical capacity, and more impressive mechanical and optical tunability in comparison to many of the other nanocarbon alternatives. A significant amount of work has already been done to date to demonstrate the feasibility of $Ti_3C_2T_x$ for use in human-oriented electrode technologies, but there is still work to be done to fully realize a MXene-based bioelectronic interface that is robust enough to be useful in acute or chronic studies, be it *in vivo* as an implantable design, or epidermally as a long-term wearable device. To help reach this goal, this thesis outlined various process flows for realizing $Ti_3C_2T_x$ MXene bioelectronic interfaces, and also demonstrated their use for a variety of biosensing applications. It also compared the performance of $Ti_3C_2T_x$ -based electrode technologies in every instance to state-of-the-art technologies, so as to highlight the exciting benefits, as well as possible areas of improvement, to be explored in the future.

Chapter 3 outlined the optimal solutions-processing methods necessary to reproducibly create $Ti_3C_2T_x$ dried films with suitable electronic and electrochemical capabilities for their incorporation in high-fidelity, skin-based recoding electrode arrays. Tests using arrays featuring spray-cast $Ti_3C_2T_x$ thin films revealed significantly improved recording capabilities for the MXene-based interfaces compared to size-matched electrodes made from stiffer metals. Furthermore, by including larger, pre-gelled electrodes in our study, we highlighted the superior impedance and recording characteristics of our dry, $Ti_3C_2T_x$ -based interfaces compared to state-of-the-art Ag/AgCl electrodes that are used in the clinical setting. Using our fabricated arrays on healthy human subjects allowed for high-density

tracking of muscle activation patterns in small muscle groups in the human hand, with millimeter-scale spatial and millisecond-scale temporal resolution, something which is impossible to achieve with the large monopolar contacts that are in standard use today.

Expanding on the work of Chapter 3 in **Chapter 4**, I further elaborated on the importance of the electrode-medium interface, and highlighted how the impedance properties of fabricated $Ti_3C_2T_x$ electrodes change depending on the medium type and the environment. I explored and proposed three separate equivalent circuit models to describe the complete family of interactions that occurs for both clinical and $Ti_3C_2T_x$ -based electrodes interfacing with the two most standard media of biopotential sensing, namely physiologic saline, and human skin. Trends in the data directly derived from these equivalent circuit models supported previous observations made in Chapter 3, and in previously published literature, highlighting the ability of our proposed models to truly capture the behavior of the electrode-medium interface. A methodological approach was also proposed to investigate the particularly complex interactions that occur at the interface between human skin and gelled and dry electrode technologies. With this methodology, future studies may more accurately investigate changes in surface electrode behavior over time, which has serious implications for chronic wearable applications.

In **Chapter 5**, I explored the long-term stability of $Ti_3C_2T_x$ dried films, and investigated strategies for improving their lifetime. As MXene is known to degrade over time, it was necessary to first understand how this degradation occurs, and then to develop ways to combat these processes. Accordingly, we aged $Ti_3C_2T_x$ solutions featuring a variety of stabilizing additive agents, based on previous suggestions in literature, and thereby determined a few "modified MXene" solutions worth testing alongside untreated MXene in our dried films study. The subsequent dried films study firmly established that the major mechanism driving deterioration of the excellent electronic and electrochemical properties of $Ti_3C_2T_x$ dried films is the ingress of moisture between the individual flakes of the MXene layers. Moisture ingress results in flake separation, driving degradation of the overall film properties, and although the moisture that has infiltrated the films may be baked out, the aging process itself may not be so reversible for fully realized devices implanted in the brain or interfacing with the human skin on a regular basis. Accordingly, we sought to develop specific strategies to combat the ingress of moisture into the $Ti_3C_2T_x$ dried films, and found two promising approaches. The first strategy showed that either vitamin C or hexametaphosphate salt may be added to the precursor MXene solution, as these additives resulted in improved stability against moisture ingress and film degradation. In the second strategy, we explored coating the $Ti_3C_2T_x$ films with a reduced graphene oxide (rGO) coating, and found that the $rGO/Ti_3C_2T_x$ combined

structure also had improved resistance to moisture ingress and oxidation over time. As a final point, we also discovered that the synthesis and processing conditions can have significant effects on the stability of $Ti_3C_2T_x$ dried films, which again highlights an area of investigation worth exploring in future studies.

Finally, in **Chapter 6**, I integrated the knowledge of each of the proceeding chapters into realizing a mature bioelectronic interface based on a textile substrate inundated with $Ti_3C_2T_x$ MXene, which I then explored for electrocardiography and motion artifact testing in a small cohort of healthy human subjects. The fabrication flow developed to realize these electrode technologies was designed to be high-throughput and low-cost, and resulted in $Ti_3C_2T_x$ bioelectronic interfaces which were capable of passing the performance requirements set by the ANSI AAMI EC12 Standard, which defines the bar against which all FDA-approved medical devices are compared. In subsequent recording experiments, we discovered no statistically significant differences between the impedance behavior, recording quality, or motion artifact susceptibility of our dry $Ti_3C_2T_x$ -based ECG electrodes compared to pre-gelled, clinically-relevant 3M 2360 ECG electrodes. Preliminary investigations into the stability of our technologies for longer-terms studies also showed promise, and again highlighted the importance of environmental factors and the electrode-medium interface on long-term electrode performance.

In total, we conclude that we have developed many promising new bioelectronic interfaces for a variety of electrodiagnostic applications, with the real potential to cut costs, while increasing accessibility of the technology, and without any significant losses in the overall device performance.

Future Directions

In the end, this work sought to outline and establish the entire process flow necessary for realizing mature $Ti_3C_2T_x$ -based electrode technologies. I first approached this goal by starting at the basic materials science level, optimizing the solutions-processing methods needed to reliably create $Ti_3C_2T_x$ dried films. Nevertheless, much work can still be done in this realm, particularly to optimize the MXene synthesis process, as well as to define the optimal spray-coating concentration and baking conditions. Alternative film casting methods may also be worthwhile to explore, including screen printing, dip-coating, or blade-coating of MXene solutions onto the target substrate. These alternative film casting techniques, while still fully compatible with the unique rheological properties of $Ti_3C_2T_x$ MXene, would yet require optimization and characterization all on their own, as separate from the work of this thesis.

Another future direction of this work—and an area which is noticeably absent from the current thesis—is the nature of $Ti_3C_2T_x$ MXene for *stimulating* electrode technologies. While MXene has been well-established as a highly capacitive material, and this work and others have demonstrated $Ti_3C_2T_x$ MXene for a wide variety of passive recording applications, there has yet to be extensive characterization of the potential and capacity of MXene dried films for incorporation into bioelectronic interfaces geared towards modulating neural, neuromuscular, or similar dynamics via direct electrical stimulation. Given the many advancements and explorations already made into $Ti_3C_2T_x$ MXene's promise for energy storage applications, however, there could be much promise and benefit in developing a stimulating electrode technology based on this unique 2D carbon-based nanomaterial.

Finally, the final products of this thesis have been real-world electrode technologies suitable for actual use in at least acute physiology and health monitoring studies. We have successfully demonstrated recording of the electromyogram and the electrocardiogram with MXene electrodes, which are two of the most commonly collected and analyzed biopotential signals from humans in both the research and clinical fields. And while we have highlighted certain aspects of both the electrode technology and the environment and media which will require serious consideration when conducting design of experiments, we are nevertheless optimistic that future work should be able to clearly demonstrate the use of Ti₃C₂T_x-based bioelectronic interfaces for longer-term studies. This is perhaps the "final piece of the puzzle" that is absolutely necessary in order to realize MXene-based electrodes for commercial applications in the clinical and research spheres, on par with state-of-the-art metallic and silicon-based technologies, such as many of those described throughout this thesis. Indeed, the overarching goal of this dissertation has been to lay the groundwork for future such chronic studies, with the ultimate hope that one day, $Ti_3C_2T_x$ electrode technologies may see adoption into the next generation state-ofthe-art bioelectronic interfaces for high-fidelity recording or stimulating applications in the long-term.

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