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MICROELECTRODE ARRAYS ON POLYDIMETHYLSILOXANE SUBSTRATE

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ABSTRACT

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Microelectrode arrays (MEAs) have emerged as innovative electronic tools that can be used in laboratory environments to study electrically active cells. MEAs fabricated on compliant substrates, such as polydimethylsiloxane (PDMS), offer improved biocompatibility compared to MEAs constructed on rigid substrates, since PDMS as an elastic material can conform to tissue surfaces. The enhanced ability of MEAs on PDMS to acquire electrical signals from biological specimen, attributed to the formation of a tight tissue-electrode interface due to the elasticity of PDMS, offers an approach to develop more precise diagnostics and treatment methods for cardiovascular diseases and neural disorders compared to MEAs fabricated on rigid substrates.

The aim of this bachelor's thesis is to present fabrication processes by which MEAs exhibiting both biocompatible and optimal electrical characteristics on PDMS can be fabricated, since MEAs constructed on PDMS are seen to be at the forefront of developing innovative treatment approaches and advancing biomedical research. Due to its optimal mechanical properties, PDMS is also utilized as a substrate material in systems integrating electronics and microfluidic channels. These organ-on-chips mimic human physiology with utmost accuracy *in vitro,* which emphasizes the significance of PDMS as a substrate material within the biomedical field.

In this literature review, the material properties of PDMS and their importance in biomedical applications are first introduced. Subsequently, both rigid and flexible MEAs and the standard microfabrication processes applied to fabricate planar MEAs are presented. Thereafter, the focus of the thesis is on diverse fabrication processes by which MEAs on PDMS are fabricated.

The fabrication processes employed to construct MEAs on PDMS can be roughly divided into three categories, determined by whether the fabrication of microelectrodes is based on photolithography, ink-jet printing, or principles of stretchable electronics. Principles of stretchable electronics are typically applied in MEA fabrication via manufacturing polymeric conductive microelectrodes, the wavy electrode configuration of which enables the stretching of the MEA. However, the material properties of the PDMS substrate pose challenges that complicate the fabrication process. As a result, standard microfabrication techniques may not be applicable in fabrication processes where PDMS is utilized as the substrate material. The elasticity of PDMS may cause the properties of the PDMS substrate to change during processing and hydrophobicity of the substrate complicates the introduction of hydrophilic materials to PDMS.

Thus, to minimize the drawbacks occurring in PDMS during processing and enable the construction of MEAs with optimal electrical characteristics, novel fabrication processes are required. Since the elasticity of PDMS poses challenges in MEA fabrication especially in traditional photolithographical fabrication processes, ink-jet printing of microelectrodes, where the elasticity of the PDMS substrate is not problematic, is being developed. To address the challenges caused by the hydrophobic nature of PDMS, resources are directed towards researching oxidizing agents to modify the material properties and surface energy of PDMS. However, regardless of the differences between the manufacturing techniques, all fabrication processes aim to produce a biocompatible MEA with a tight electrode-tissue interface in a time- and cost-efficient manner.

Keywords: microelectrode array, PDMS substrate, microfabrication, photolithography, ink-jet printing

TIIVISTELMÄ

Sonja Kuusinen: Mikroelektrodimatriisit polydimetyylisiloksaanisubstraatilla
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Mikroelektrodimatriisit (MEA:t) ovat biolääketieteen alalla alati yleistyviä sähköisiä työkaluja, joita käytetään laboratorioympäristöissä sähköisesti aktiivisten solujen tutkimiseen. Joustaville substraattimateriaaleille, kuten polydimetyylisiloksaanille (PDMS), valmistettujen MEA:iden bioyhteensopivuus on jäykille substraattimateriaaleille valmistettuja MEA:ita parempi, sillä PDMS elastisena materiaalina mukautuu kudospintoihin. PDMS-substraatin elastisuus mahdollistaa tiiviin elektrodi-kudosrajapinnan muodostumisen, mikä edesauttaa sähköisten signaalien välittämistä biologisesta näytteestä mikroelektrodeille. PDMS-substraatille valmistetuilla MEA:illa nähdäänkin täten jäykkiä MEA:ita merkittävämpi potentiaali kehittää uusia keinoja sydän- ja verisuonitautien sekä hermosairauksien diagnosointiin ja hoitoon.

Tämän kandidaatintutkielman tavoitteena on esitellä valmistusprosesseja, joilla bioyhteensopivuudeltaan ja sähköiseltä toiminnaltaan optimaalisia MEA:ita voidaan valmistaa PDMS-substraatille, sillä PDMS:lle valmistetut MEA:t nähdään innovatiivisten hoitomuotojen kehittämisen lisäksi biolääketieteellisen tutkimuksen edistämisen keskiössä. Optimaalisten mekaanisten ominaisuuksiensa vuoksi PDMS:ää käytetään substraattimateriaalina ihmisen fysiologiaa tarkasti jäljittelevissä elektroniikkaa ja mikrofluidistisia kanavia yhdistävissä järjestelmissä, mikä korostaa PDMS:n roolia substraattimateriaalina biolääketieteellisissä sovelluksissa.

Tässä kirjallisuuskatsauksessa esitellään ensin tärkeimpiä PDMS:n ominaisuuksia ja pohditaan niiden merkitystä biolääketieteellisissä sovelluksissa. Seuraavana käsitellään jäykkiä ja joustavia MEA:ita sekä niiden valmistuksessa käytettäviä yleisimpiä mikrovalmistustekniikoita. Tämän jälkeen tutkielmassa keskitytään PDMS-substraatilla toimivien MEA:iden valmistusprosessien käsittelyyn.

MEA-valmistus PDMS-substraatille voidaan jaotella karkeasti kolmeen luokkaan sen mukaan, perustuuko mikroelektrodien valmistus fotolitografiaan, mustesuihkutulostukseen vai joustavan elektroniikan periaatteiden hyödyntämiseen. Joustavan elektroniikan periaatteita sovelletaan valmistuksessa tyypillisesti polymeeristen johtavien mikroelektrodien avulla, joiden aaltoileva elektrodikonfiguraatio mahdollistaa MEA:n venymisen. PDMS-substraatin ominaisuudet aiheuttavat kuitenkin valmistusprosesseissa haasteita, joiden vuoksi yleisimpiä mikrovalmistustekniikoita ei välttämättä voida käyttää PDMS:n toimiessa substraattimateriaalina. PDMS:n elastisuuden vuoksi sen ominaisuudet saattavat muuttua prosessoinnissa, ja PDMS:n hydrofobisuus vaikeuttaa hydrofiilisten näytteiden adheesiota substraattiin.

Prosessoinnin aiheuttamien substraattimateriaalin haittavaikutusten minimoimiseksi ja sähköisesti optimaalisten MEA:iden valmistamiseksi tarvitaankin täten innovatiivisia valmistusprosesseja. Koska PDMS:n elastisuus vaikeuttaa MEA-valmistusta erityisesti perinteisissä fotolitografisissa valmistusprosesseissa, mikroelektrodien mustesuihkutulostusta, jossa PDMS-substraatin elastisuus ei aiheuta ongelmia, kehitetään jatkuvasti. PDMS:n hydrofobisuuden aiheuttamien haasteiden ratkaisemiseksi resursseja on suunnattu PDMS:n ominaisuuksia hydrofiilisemmiksi muuttavien hapettavien tekijöiden tutkimukseen. Valmistusmekanismien välisistä eroista huolimatta kaikilla valmistusprosesseilla pyritään tuottamaan aika- ja kustannustehokkaasti bioyhteensopiva MEA, joka mahdollistaa tiiviin elektrodi-kudosrajapinnan muodostumisen.

Avainsanat: mikroelektrodimatriisi, PDMS-substraatti, mikrovalmistus, fotolitografia, mustesuihkutulostus

CONTENTS

LIST OF ABBREVIATIONS

CMOS	complementary metal-oxide-semiconductor
CRU	constitutional repeating unit
CTE	coefficient of thermal expansion
MEA	microelectrode array
MMD	multilevel matrix deposition
MPTMS	3-mercaptopropyl trimethoxysilane
OoC	organ-on-chip
PDMS	polydimethylsiloxane
PEDOT	poly(3,4-ethylenedioxythiophene)
PEN	polyethylene naphthalene
PI	polyimide
polyMEA	all-polymer microelectrode array
PSS	poly(4-styrenesulfonate)
RF	radio frequency
RIE	reactive ion etching
SiN	silicon nitride
SNR	signal-to-noise ratio

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1. INTRODUCTION

As cardiovascular diseases and neural disorders continue to rise in our society demanding increasingly substantial healthcare resources, there is a growing need to study the cellular functions associated with them. To offer a mechanism for more accurate diagnostics and enhanced treatment of these conditions, microelectrode arrays (MEAs) are being developed. MEAs are electrical tools intended to interface with living tissues and to transduce electrochemical signals expressed by cells into currents in external electrical circuits. MEAs can be fabricated on diverse substrate materials providing an *in vitro* mechanism to measure the electrical activity of cells generating electrical impulses, such as cardiomyocytes and neural cells. (S. Xu et al., 2023)

In vitro MEAs are used in multiple areas of biomedical research due to their advantageous properties such as noninvasiveness, biocompatibility, and capability of bidirectional transduction of signals. Because of these characteristics, MEAs are utilized in drug screening, pathological mechanisms and biosensors research. (S. Xu et al., 2023) Depending on the application, the electrodes in MEAs can be either planar or non-planar. MEAs with planar electrodes are typically utilized with 2D cell cultures. The planar electrode structure minimizes the invasiveness of the MEA and the mismatch of mechanical impedance between the electrodes and the cell culture. However, planar electrodes have limited capability to study 3D cell models or tissue samples due to the inaccessibility of planar electrodes into 3D structures. Therefore, MEAs with non-planar electrodes allowing detection of electrical signals throughout three-dimensional samples are also fabricated. (Guo, Meacham, Hocham & DeWeerth, 2010)

MEAs fabricated on rigid substrates express limitations in accurate transmission of signals from biological specimen to the microelectrodes due to the incapacity of the rigid substrate to conform with the biological specimen. Thus, to improve the conformability of MEAs and provide a tighter electrode-tissue contact, the usage of compliant substrate materials has increased. (Chircov & Grumezescu, 2022) Polydimethylsiloxane (PDMS) is a widely employed flexible MEA substrate materials as it is elastic, biocompatible, and chemically inert (Ariati, Sales, Souza, Lima & Ribeiro, 2021). These properties render PDMS to be an optimal substrate material in biological applications. Given the enhanced biocompatibility and signal acquisition accuracy of MEAs fabricated on PDMS in contrast

to rigid MEAs due to the elasticity of PDMS, MEAs on PDMS can be seen to hold more potential in developing novel diagnostics and treatment methods for neural and cardiovascular diseases compared to rigid MEAs. (Chircov & Grumezescu, 2022)

In addition to the advantages associated with the adaptability of PDMS substrate to tissue surfaces, PDMS exhibits considerable promise as a substrate material in organ-on-chip (OoC) research. OoCs are systems containing microfluidic chips in which natural or engineered tissue samples can be grown. They are developed to model complex *in vivo* biological systems *in vitro* by replicating cell microenvironments. To better mimic human physiology, flow is induced to the microfluidic channels of OoCs to represent the fluid flow occurring naturally in biological systems. As PDMS is permeable to gases and microfluidic channels can be easily incorporated into PDMS, it is an optimal substrate material utilized increasingly in the fabrication of OoCs. (Cameron et al., 2022)

Due to the favorable technical characteristics of PDMS as a substrate material, efforts are directed towards developing fabrication methods for constructing MEAs on PDMS. The incorporation of microelectrodes on PDMS has imposed challenges since traditional microfabrication techniques may not be suitable to be utilized with PDMS due to its elasticity and hydrophobicity. Construction of MEAs on PDMS has historically relied on photolithographical fabrication, but recent development of microsystem technology has introduced new methods to fabricate MEAs on PDMS. (Guo et al., 2010) In addition to altering the photolithographic fabrication processes via innovative micromanufacturing approaches, ink-jet printing has emerged as a novel fabrication approach by which versatile microelectrode structures on PDMS can be constructed (Adly et al., 2018). Moreover, principles of stretchable electronics have been applied into the fabrication of MEAs on PDMS, typically done via incorporating stretchable polymer microelectrodes with wavy electrode configuration onto PDMS (L. Xu et al., 2021).

The aim of this literature review is to answer the question of how biocompatible MEAs with optimal electrical characteristics can be fabricated on a PDMS substrate. First, background on both PDMS as a material and MEAs are presented, after which the most widely employed microfabrication techniques in constructing MEAs are introduced. Thereafter, the thesis focuses on presenting various fabrication processes by which MEAs on PDMS can be constructed. The advantages and challenges of each fabrication process and how they aim to optimize the properties of MEAs are discussed.

2. POLYDIMETHYLSILOXANE AS A MATERIAL

PDMS is a polymeric material that can be classified into the subgroup of elastomers, which are cross-linked polymers capable of returning to their initial shape after a deformation caused by tension. Properties of elastomers and therefore the characteristics of PDMS include high-yield strain, low Young's modulus, and easy stretchability. Elastomers can be further divided into subclasses, the most common of which are synthetic rubbers, nitrile rubbers and silicone elastomers. (Ariati et al., 2021) PDMS is an inorganic silicone elastomer that is widely applied as a substrate material in microfluidic systems, biomedical devices and electric components due to its beneficial properties in such applications (Wythers, 2012, p. 443).

Synthesis of PDMS is achieved by polymerization reactions of monomers, such as dichlorosilanes, or by mixing two pre-polymers with the assistance of catalysts. Straight-forward polymerization reaction of dichlorosilanes, which includes hydrolysis and condensation reaction of the monomers, results in cyclic and linear chains of polymers, the molecular weight of which may vary greatly. Since controllability of molecular weight is a desired property of polymers, PDMS is more commonly synthesized by ring-opening polymerization reactions of cyclic siloxanes. (Ariati et al., 2021) As straight-forward polymerization reactions produce long chains of PDMS, the usage of which in biomedical applications is not as common as PDMS rubber, PDMS utilized as a substrate material is often synthesized by mixing two polymer precursors together. The mixing reaction of the precursor polymers results in a cross-linked, rubbery structure used in medical applications, microfluidics and as a substrate material for MEAs. (Wythers, 2012, p. 446)

2.1 Material properties

PDMS is a widely used silicon-based elastomer composed of constitutional repeating units (CRUs) which contain oxygen and silicon in the primary backbone and two methyl groups that are covalently bonded to the silicon atom. The chemical structure of the CRU illustrated in figure 1 influences the ease at which the chains can rearrange the substituents. The location of the substituents and chain conformational isomerism largely determine the material properties. Thus, the CRU is important to consider when examining characteristics typical to the material. (Wythers, 2012, p. 446)

Because of the two methyl groups bonded to silicon in the CRU, PDMS is a hydrophobic material. The symmetric arrangement and chemical properties of the methyl groups

make the polymer non-polar and thus hydrophobic. However, by oxidizing the methyl groups into polar hydroxyl groups, the surface properties of PDMS can be altered from hydrophobic to hydrophilic. (Wythers, 2012, p. 446) The hydrophobicity of PDMS results in the material having a low surface free energy, which increases the contact angle of PDMS with hydrophilic materials, such as water. Water as a hydrophilic molecule is repellant to hydrophobic surfaces, and therefore the introduction of aqueous samples to PDMS is difficult. (Wolf, Salieb-Beugelaar & Hunziker, 2018) In addition, the number of CRUs in a polymer chain needs to be considered as it determines the molecular weight of the material. The molecular weight, in turn, affects the viscoelastic properties of PDMS which define the behavior of PDMS when a force is applied to the material. (Ariati et al., 2021)

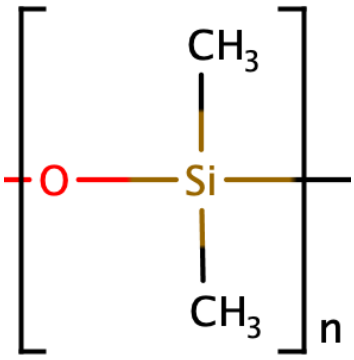

Figure 1. *Constitutional repeating unit of PDMS.*

PDMS presents unique physicochemical characteristics, which are significant in terms of biological purposes. PDMS is a biocompatible material, which ensures that the material is compatible with human tissues and causes only a brief inflammatory reaction when introduced into a biological system. Additionally, PDMS is optically transparent, physiologically indifferent and resistant to biodegradation, and can thus be used in contact with biological samples. (Miranda et al., 2021)

Since PDMS is an elastomer, it possesses elastic properties and can restore to its original form after a force deforming the structure is removed. PDMS has a low Young's modulus, which is in the range from 1 MPa to 3 MPa and tensile strength, which varies between 3.5 MPa and 5.1 MPa. The variation in the values that depict the mechanical properties of PDMS results from the curing agent ratio and the used temperature in material manufacturing, which both may vary in different processes. (Ariati et al., 2021) PDMS is a soft and flexible material due to its elastomeric properties and utilization above the glass transition temperature. Even though the material is highly permeable to gases, it is thermally stable and chemically inert. Thus, PDMS does not react readily with

samples introduced to it, and the properties of the material are stable under various processing conditions. (Miranda et al., 2021)

Thermal and electrical insulation properties of PDMS allow the material to be utilized in contact with electronic components. As the material does not conduct heat nor current, it may be used as an insulative material or as a protective material to improve the resistance of components against mechanical shocks and environmental features. (Miranda et al., 2021) Finally, PDMS is widely used because the handling and processing of PDMS is straight-forward, and the material costs are low (Ariati et al., 2021).

2.2 Advantages of polydimethylsiloxane in biomedical applications

The significance of PDMS in the biomedical field stems from its utilization as a substrate material for biomedical applications and as the material on which OoCs are built (Cameron et al., 2022). In addition to utilizing PDMS as the material in which microfluidic channels in OoCs are created and onto which MEAs are constructed, PDMS can also be used as a medical implant material and as an enabling material to fabricate biomodels for *in vitro* hemodynamic study of diseases (Chircov & Grumezescu, 2022). The variety of these applications emphasizes the importance and usefulness of PDMS in the biomedical field, but moving forward, the focus will be on the applications where electrodes are fabricated on a PDMS substrate.

A comprehensive understanding of the benefits PDMS offers in biomedical applications can be gained by observing the substrate material requirements in applications where PDMS is utilized. While considering the substrate material of MEAs, a flexible substrate material ensures the best possible conformity and contact between microelectrodes and tissue surfaces. Therefore, one of the major benefits of PDMS is its characteristic compliance, which provides a consistent and firm contact between the MEA and the tissue surface. Furthermore, the substrate material must be chemically inactive and biologically compatible in biomedical applications where the material comes in contact with biological specimen. PDMS fulfills both demands as it is biocompatible and chemically inert and therefore does not react to chemical agents or to biological substances introduced to the substrate. (Wythers, 2012, p. 445)

Along with PDMS being unreactive towards substances, it is equally as important that the material is non-toxic and does not impose any destructive reactions in biological agents by releasing toxic substances. The substrate material of OoCs is required to be mechanically stable to maintain fixed dimensions, which enables sealing of microfluidic

channels. (Wythers, 2012, p. 445) Sealing is one of the primary necessities of microfluidic devices, such as OoCs, as it is the process in which microfluidic channels are closed to prevent leakage. PDMS is a favorable material in this relation as well, because sealing is easy to implement with PDMS without the addition of adhesives. (Carrell, McCord, Wydallis & Henry, 2020) Moreover, in OoCs where rapid gas flow is relevant for modeling human physiology, high gas permeability of PDMS is a favorable feature (Schneider, Gruner, Richter & Loskill, 2021).

The processing capability of PDMS is a major advantage when it comes to biomedical applications. The dimensions of OoCs may vary greatly based on the application and thus, it is favorable that PDMS can be processed without the risk of material breakage. In terms of processability, an advantageous property of PDMS is its fast prototyping by micromolding. (Carrell et al., 2020) In biological applications where cells or tissue samples are researched optically, optical transparency of the substrate is important. Since PDMS is transparent, it is possible to conduct visual examinations of the quality and condition of biological specimens and utilize fluorescence detection in applications where luminescence phenomenon is used. Electrical insulation is also an advantageous property of PDMS, because it allows the usage of PDMS as a compliant insulative layer in MEA fabrication and the implementation of electrophoresis in applications utilizing PDMS as the substrate. (Park, Kim & Han, 2009; Wythers, 2012, p. 445)

2.3 Disadvantages of polydimethylsiloxane in biomedical applications

Although PDMS presents many favorable properties making the material suitable for a wide array of biomedical applications, it has also disadvantageous characteristics in terms of biomedical purposes. Since the CRU of PDMS contains two methyl groups making the polymer hydrophobic, introduction of hydrophilic samples to PDMS is difficult. While it is possible to treat the surface with an oxidizing agent, such as oxygen plasma, and make the surface hydrophilic, the required surface treatment increases the amount of work in the manufacturing process. (Victor, Ribeiro & Araújo, 2019) For example, in photolithography-based MEA fabrication processes the PDMS substrate must be either oxidized prior to depositing a hydrophilic photoresist onto it or coated with an adhesion layer (Chen, Lam & Fu, 2012). In addition, in MEA fabrication processes where hydrophilic nanoparticles are ink-jet printed onto PDMS, chemical agents are needed to alter the wettability of PDMS to allow the printing of continuous electrodes (Adly et al., 2018).

Furthermore, oxidative treatment of a PDMS surface with chemical agents makes the surface hydrophilic only temporarily. Therefore, the treatment does not ensure durability and stability of the coating. (Victor et al., 2019) When treated with an oxidizing agent, cracking of PDMS surface may also occur, which in turn restricts the applicability of the material (Adly et al., 2018). Therefore, a substrate material with an inherently hydrophilic surface would provide better affinity between the substrate and the hydrophilic materials it is brough into contact with, without the need of oxidation. (Wythers, 2012, p. 445).

Permeability of PDMS to gases is considered as an advantage of PDMS in OoCs where fast gas flow is essential, but on the other hand, high gas permeability causes water evaporation. Significant evaporation may in turn affect the osmolality of cell cultures and lead to unideal cell culture conditions. (Schneider et al., 2021) In addition to gas permeability, PDMS permeates small molecules such as proteins and lipophilic molecules, which may impose an issue as the absorption influences solute concentrations and device throughput. Protein absorption makes quantifying low concentrations of analyte difficult and lipophilic molecules may pose challenges when serving as intercellular signals in microscale cell cultures. (Carrell et al., 2020)

Although being impermeable to liquids, PDMS swells when exposed to solvents. This is disadvantageous in many applications as it may cause feature distortion and interference in cell cultures due to occurring changes in the electrolyte concentration in cell culture media. (Ariati et al., 2021) The resistance of PDMS to many organic solvents is also low, which may cause inaccurate results in biological assays (Neckel et al., 2021). In addition, the coefficient of thermal expansion (CTE) of PDMS is high, and the material may therefore shrink during curing, especially in high temperatures. This slows down the fabrication of OoCs as faster chip manufacturing is achieved via rapid curing in hot temperatures. (Schneider et al., 2021)

Another drawback of PDMS is the challenging electrode fabrication on PDMS substrate because of the elasticity and hydrophobicity of PDMS. Due to these properties, standard microfabrication techniques used to construct MEAs on rigid substrates may not be applicable to be utilized with PDMS substrates. While the elasticity of PDMS provides numerous advantages, the low Young’s modulus limits the number of suitable processing techniques and thus complicates the MEA fabrication process. (Hill, Qian, Chen & Fu, 2016) Furthermore, the elasticity of PDMS limits the structural support the material is able to provide which can impose disadvantages in applications where high support from the substrate material is required (Schneider et al., 2021).

3. MICROELECTRODE ARRAYS

MEAs are biopotential devices which facilitate the translation of electrochemical signals generated within biological samples into external electrical circuits. The microelectrodes can both collect signals from biological specimen and stimulate the target tissue the electrodes are in contact with. (Qi et al., 2017) MEAs can be classified into *in vivo* and *in vitro* MEAs, of which *in vivo* MEAs are invasive since they are implanted directly into an organ in a biological system. Despite the capability of precise recording and stimulation of organisms with *in vivo* MEAs, the destructiveness of *in vivo* MEAs limits their usage. As opposed to *in vivo* MEAS, *in vitro* MEAs are typically used in cell culture laboratories. The key benefit of *in vitro* MEAs is their non-invasiveness, which allows the study subject to remain undisturbed as the electrodes are not inserted into it. (L. Xu et al., 2021)

Since the aim of this thesis is to present fabrication processes for the construction of MEAs on PDMS utilized in laboratory environments, the thesis will focus on *in vitro* MEAs. The characteristic dimensions of MEAs are in microscale, and they are produced via microfabrication processes commonly based on semiconductor chip manufacturing techniques. (L. Xu et al., 2021) Due to the development of microfabrication techniques, the fabrication of more advanced microfluidic structures and the spread of novel cell culture approaches, *in vitro* MEAs have become a promising tool not only to understand the underlying physiological mechanisms of cells transmitting electrical signals, but also a method to improve diagnostics and develop new treatment approaches for neurological disorders and cardiovascular diseases. (Qi et al., 2017)

3.1 *In vitro* microelectrode arrays

In vitro MEAs are increasingly recognized as an advanced technique within the realm of bio-sensing to detect electrical signals generated in cells. The operating principle of MEAs relies on establishing a direct interface between the MEA and the biological specimen to be analyzed, followed by subsequent recording and interpretation of electrical signals. The non-destructiveness of *in vitro* MEAs is due to the utilization of planar microelectrodes with 2D cell cultures. MEAs with planar electrodes interact with 2D cell cultures without puncturing the electrodes into the cells that are measured. However, planar MEAs are restricted in terms of electrode accessibility. (L. Xu et al., 2021) To overcome this limitation, 3D MEAs with non-planar electrodes have been

fabricated for targeting sites inside 3D cell models and tissue samples. (Choi, Lee, Rajaraman & Kim, 2021)

Alongside the improved accessibility of 3D MEAs, non-planar electrodes have a greater electrode surface area compared to planar structures, which affects the electrode impedance and the signal-to-noise ratio (SNR) favorably. The aim is to minimize the electrode impedance and maximize the SNR describing the ratio between the power of the signal and the background noise. (Choi et al., 2021) Higher SNR and reduced impedance are also pursued in planar MEAs by coating the electrodes with a rough-surfaced or porous material, typically nanoscale metal particles, to increase the active area of the electrodes (Zhao, Gong, Zheng & Wang, 2016).

Despite the advancements in 3D MEA fabrication, MEAs with planar electrodes remain widely utilized because of their non-invasive characteristics. Planar MEAs can be manufactured using simple microfabrication techniques which can be combined with other technologies to increase the functionality of the device. (S. Xu et al., 2023) For example, functionality can be enhanced by combining planar electrodes and microfluidic channels. Via this incorporation, OoCs capable of mimicking human physiology accurately can be created. (Cameron et al., 2022) Traditionally, MEAs include three layers, each of which have different requirements. The conductive layer is typically metallic, and it includes both the electrode and the contact sites connected by wires. The insulative layer acts as a protective sheet separating the conductive layer and the tissue surface. Both insulating and conductive layers are held in the substrate, and the structure of such typical MEA is depicted in figure 2. (S. Xu et al., 2023)

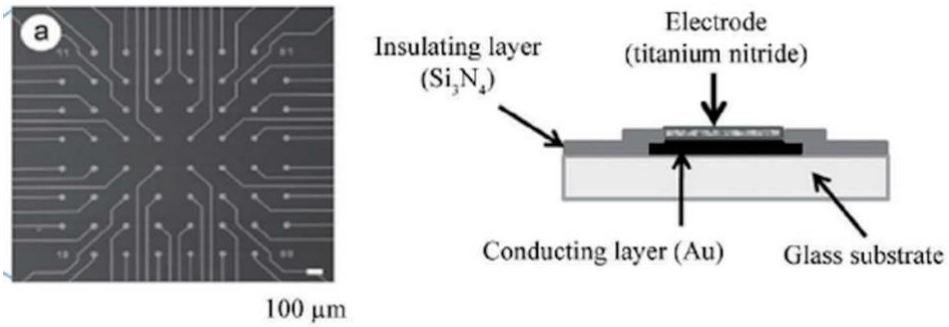

Figure 2. *Planar MEA electrode design on the left and cross-sectional image of the structure on the right. (Liu, Chen, He, Li & Chen, 2012)*

In vitro MEAs can be divided into high density and traditional MEAs, the latter of which can be further classified into flexible and rigid MEAs based on the compliance and Young's modulus of the substrate material. High density MEAs are produced by using a similar manufacturing process as with complementary metal-oxide-semiconductor

(CMOS) microelectronic chips. CMOS microelectronic chips consist of pixel units sensitive to light, which gather optical signals and transduce them into electrical signals. This phenomenon can be utilized in detection of bioelectric signals on cellular and biomolecular level. In addition, CMOS-based MEAs are used in intracellular electrophysiological imaging. Although high density MEAs have many potential applications due to their numerous signal processing and amplification possibilities, the difficult fabrication processes, high fabrication costs and limited scalability of high density MEAs have restricted the number of institutes that focus on the research and development of CMOS MEAs. (L. Xu et al., 2021)

Traditional *in vitro* MEAs are more widespread than CMOS MEAs and they are constantly developed further to improve time-space resolution detection, decrease phase delay, and increase SNR. Moreover, effort is dedicated into ensuring the precision and safety of these MEAs by applying a high charge injection density and limit to enhance the electrical stimulation capability of MEAs. Research is conducted to fulfill these desired properties even better, and both rigid and flexible MEAs are developed to study the electrical signals generated in cells with utmost accuracy. (S. Xu et al., 2023)

3.1.1 Rigid microelectrode arrays

Rigid planar MEAs are used as platforms to conduct experiments for *in vitro* cell cultures, and their structure corresponds to the cross-sectional image depicted in figure 2. Rigid planar MEAs are fabricated on a rigid substrate material, typically glass or ceramics. The rigid substrate material must be biocompatible, and transparency of the substrate is desired in applications where the biological sample under observation is studied optically. Typical electrode materials utilized in rigid planar MEAs are gold and platinum, as they are biocompatible and allow the usage of the MEA in biological applications. (L. Xu et al., 2021) To increase the SNR and reduce the electrode impedance, titanium nitride and platinum black are commonly deposited onto the metal electrodes. The porosity of these materials increases the active area of the electrodes and enables better electrical performance of the electrodes. (S. Xu et al., 2023)

In addition to nanoscale metal particles, the metallic microelectrodes can also be coated with carbon nanomaterials or conductive polymers. New coating options for rigid MEAs are under research as it has been demonstrated that the porosity of platinum black may render the electrodes to be weaker and more readily damaged than metal electrodes without coating. However, in biomedical applications, the external stimuli that could

possibly damage the electrodes are often very small and thus, the nanoscale metal film coatings are widely employed. (S. Xu et al., 2023)

Rigid planar MEAs are manufactured via thin-film processes which are easy to conduct on rigid substrates. Both the conductive and insulative layer can be implemented onto a rigid substrate material by standardized semiconductor processes, which lowers the fabrication costs and speeds up the manufacturing process of rigid MEAs. Photolithography, dry etching and both chemical and physical material deposition methods can be successfully conducted when fabricating rigid MEAs. A functional rigid MEA contains the three layers presented earlier and a cell culture chamber made of glass or PDMS in which biological samples are grown. (L. Xu et al., 2021)

3.1.2 Flexible microelectrode arrays

Multiple compliant substrate materials are accessible for the fabrication of flexible MEAs. The fundamental requirements the substrate material must fulfill are a low Young's modulus and an application specific ratio of bending stiffness to material thickness. Low Young's modulus ensures that the material is bendable and is in terms of mechanical properties as close to the stiffness of biological samples as possible. Generally, materials with low bending stiffness are utilized as substrates in flexible MEAs, but the desired value of bending stiffness depends on the structure of the material. Bending stiffness is proportional to the material thickness, and conformability of the material is inversely proportional to the bending stiffness, which makes these variables significant in material selection. The relation between thickness and conformability of the material offers a means to achieve enhanced tissue contact and thus biocompatibility by decreasing the thickness of the substrate material. (L. Xu et al., 2021)

Polymer materials are the most widely used substrate materials in flexible MEAs, since they possess biocompatible and insulative characteristics which allow their usage in contact with biological specimen. The processability of polymer materials is simple, and many microfabrication techniques, such as etching, electrochemical deposition and photolithography are possible to apply to polymeric materials. Typical substrate materials in flexible MEAs are polyimide (PI), polyethylene naphthalene (PEN) and PDMS, each of which is used in slightly different applications based on their individual material properties. (L. Xu et al., 2021)

The most significant advantage associated with the utilization of flexible MEAs as opposed to rigid MEAs results from enhanced signal acquisition. This augmentation is

due to the capacity of flexible MEAs to interface with a soft-tissue substrate by establishing direct contact between the electrodes and the target tissue. The elasticity of the substrate material is the enabling factor in the formation of a consistent contact between the MEA and the tissue. Thus, electrical signals are gathered most accurately when using a flexible substrate material, such as PDMS silicone. (L. Xu et al., 2021) Another reason to utilize flexible substrate materials, mainly PDMS, is the fabrication of OoCs. When serving as a substrate material in OoCs, PDMS allows easy formation of multi-layered microfluidic channels and introduction of a flow-based environment, which are required to precisely mimic *in vivo* environments. (Cameron et al., 2022)

Compared with rigid MEAs, the biocompatibility of flexible MEAs is superior. Flexible MEAs can be categorized as biocompatible because they do not cause significant adverse responses in the host. The non-destructiveness of flexible MEAs stems from the capability of the devices to generate little stress and strain at the interface between the MEA and tissue. As opposed to rigid substrates, the compliant substrates used in the fabrication of flexible MEAs can conform to a specified form and follow the movement of the object to be measured. Thus, the mechanical properties of flexible MEAs make them suitable to be used in contact with biological specimens. (L. Xu et al., 2021)

3.2 Microfabrication techniques used in fabricating microelectrode arrays

In vitro MEAs are typically fabricated in cleanrooms on diverse substrate materials depending on the application and the desired functionality of the MEA. While constructing MEAs, the fabrication processes may differ in terms of stepwise manufacturing mechanisms, but the most typical microfabrication techniques, photolithography and surface micromachining, are used in most MEA manufacturing processes, regardless of the substrate material. (Guo et al., 2010)

The operating mechanism of photolithography is the same for all materials, but there are differences in the process depending on which materials are chosen. As the interest of this thesis is focused on the fabrication of MEAs on PDMS, the behavior of PDMS in photolithographic manufacturing needs to be considered. When used as a substrate material, the hydrophobicity of PDMS introduces additional manufacturing steps in photolithographic pattern transfer. Surface micromachining, on the other hand, is a broad concept containing several material deposition, material removal and surface treatment processes, which can be conducted by various techniques. However, surface patterning

of PDMS is challenging and limits the number of applicable surface micromachining techniques. (Hill et al., 2016)

3.2.1 Photolithography

Photolithography, also referred to as optical lithography, is one of the most widely used microfabrication techniques because it enables the fabrication of microscale structures with high resolution. Photolithography is a two-step pattern transfer process where the desired pattern is initially transferred optically from a mechanical plate to a photosensitive film on a substrate. Secondly, the pattern created on the photosensitive film is transferred to the substrate via occurring chemical or physical processes. The photolithographically constructed patterns may be modified further in subsequent manufacturing processes such as electroplating, material deposition or etching, depending on the application. (Mack, 2007) The significance of photolithography in fabricating electrical components in microscale is vast as traditional fabrication processes of MEAs have relied on photolithographical patterning of the electrodes in cleanroom environments (Meacham, Giuly, Guo, Hochman & DeWeerth, 2008).

Single layer photolithography is a relatively straight-forward process in which the essential components are a photoresist, an aligner, a mask, and energy delivered in the form of photons. The photoresist is an optically sensitive polymer which coats the substrate and enables the transfer of the mask's pattern onto the photoresist when the resist is exposed to light. The energy provided by photons causes photochemical reactions in the photoresist and thus, the mask's image is transferred. The purpose of the aligner is to ensure that the mechanical mask is correctly aligned with the wafer and pattern transfer occurs as desired. (Mack, 2007)

The patterning process begins with deposition of the photoresist onto the substrate. In the deposition process, the aim is to achieve a uniform photoresist thickness across the entire substrate, and this is best accomplished via spin-coating. To conduct the first pattern transfer, the photoresist is exposed to light to create either the positive or negative image of the mask's pattern on the photoresist. The photochemical changes in the photoresist occur during exposure, and the constructed pattern is determined by whether the photoresist is positive or negative. The development of positive photoresists creates the mask's image on the wafer as the solubility of the exposed areas increases due to chain scission reactions. Similarly, negative photoresists form the negative image of the mask on the substrate since the resist becomes insoluble in exposed areas during

exposure because of occurring cross-linking reactions. Hence, the solubility of non-exposed areas is greater, and those areas are diluted. (Mack, 2007)

In the next process step, the pattern is transferred onto the wafer via subtractive transfer or additive transfer approaches (Mack, 2007). Although photolithography is a suitable microfabrication technique for many materials, its utilization is challenging when PDMS is used as the substrate material. The deposition of hydrophilic photoresists onto an untreated PDMS surface is difficult as PDMS has a low surface energy because of its hydrophobic nature. (Chen et al., 2012) To conduct photolithography and enable the adhesion of hydrophilic photoresists to PDMS, an intermediary adhesion layer may be introduced. This makes the pattern transfer process more complex due to difficult removal of the added layer. (Hill et al., 2016) Moreover, the adhesive layer, typically composed of metal or parylene, may distort the properties of the underlying PDMS substrate. When a metal film is employed as an adhesive layer, it may buckle spontaneously causing undesirable changes in the PDMS surface. While a parylene film serves as an adhesion layer, the etching process required to transfer the layer onto the substrate may damage the PDMS surface. (Diebold & Clarke, 2011)

Hence, to avoid the usage of an adhesion layer, one option is to use direct photolithography to pattern chemically modified PDMS which possesses photosensitive characteristics. However, there are concerns regarding the biocompatibility and thus potential cytotoxicity of photosensitive PDMS. (Hill et al., 2016) Another, more widely used technique to avoid the need for employing an adhesion layer is to use oxygen plasma to chemically modify the PDMS surface from hydrophobic to hydrophilic state. Hydrophilicity of PDMS increases photoresist adhesion on the substrate and allows the usage of conventional photolithography without problems associated with photosensitive PDMS biocompatibility. (Chen et al., 2012)

3.2.2 Surface micromachining

In surface micromachining, microstructures are constructed on substrates through various thin-film processes. Sacrificial and structural layers are both deposited and etched in a certain order to achieve desired structures on substrates via surface micromachining. There are numerous material deposition methods such as chemical and physical vapor deposition, from which the most suitable technique for a specific application can be chosen. Similarly, there are various etching techniques that can be applied to remove material. These can be categorized into chemical wet etching and

vapor or plasma-assisted dry etching. With PDMS, however, only dry etching can be used. (Hill et al., 2016)

High-resolution features that enable the enhancement of structure complexity can be constructed on PDMS by implementing reactive ion etching (RIE) after photolithography (Chen et al., 2012). RIE is an application of plasma-assisted dry etching in which reactive ionized species are produced in a low-pressure glow-discharge plasma. The ions are directed into the PDMS surface by a DC bias voltage, and they remove material via direct ion bombardment. In RIE, the configuration of the plasma etched feature depends on the pressure of the plasma: a greater base pressure results in a more isotropic etch profile. (Hill et al., 2016) In addition, radio frequency (RF) power and chamber gas composition affect the obtained final structure (Chen et al., 2012). Effective RIE is achieved when the base pressure is lowered to a threshold value causing the ions to move perpendicularly in relation to the PDMS surface. The ionized species that collide with the PDMS substrate diffuse into the surface and etch the material by breaking the bond between silicon and oxygen in PDMS backbone causing anisotropic surface profiles. (Hill et al., 2016)

During fabrication of both rigid and flexible MEAs, a metallization process is usually required to produce the conductive layer from which the electrodes are formed (S. Xu et al., 2023). Typical options to conduct metallization are electron-beam evaporation and sputter deposition, which both are applications of physical vapor deposition. In electron-beam evaporation, the material to be deposited is first evaporated by targeting a beam of high-energy electrons into it. The kinetic energy of the electrons causes the material to evaporate, and the resulting vapor is used to create the conductive layer. The electron-beam evaporation system, illustrated in figure 3, must allow the resulting vapor to reach the substrate to be coated while the evaporation process is controlled with a magnetic field. (Sree Harsha, 2006, p. 400)

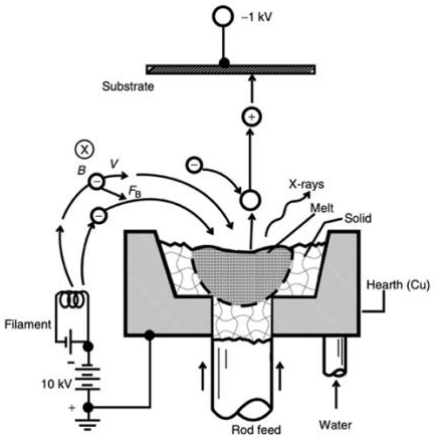

Figure 3. *Electron-beam evaporation system. (Sree Harsha, 2006, p. 401)*

In sputter deposition, a thin film of a target material is deposited onto the substrate. First, the target material is bombarded with ions causing atoms to be sputtered away from the target. These sputtered atoms are subsequently deposited onto the substrate. The deposition of the atoms is commonly controlled using either a DC bias voltage, RF power, or a combination of both, as is in the case of magnetron sputtering. In magneton sputtering, both electric and magnetic fields control the movement of ions and sputtered atoms. It is a widely used coating technique as it enables higher sputter deposition rates due to longer electron path lengths. (Murty, Yeh & Ranganathan, 2014, p. 84)

Finally, to ensure the reliability of MEAs and the accuracy of transmitted signals, the feedlines that are not to come in contact with biological samples need to be coated with an insulation layer. This layer must maintain a high impedance and appropriate insulation characteristics throughout the usage of the MEA. Typically, rigid MEAs have been insulated with a silicon nitride (SiN) layer, which is applied by plasma-enhanced chemical vapor deposition. Even though SiN is a common insulation material, the humidity, immersion of ions, and enzyme activity occurring in cell culture media causes damages to SiN and may destruct the insulation characteristics of the layer. In addition, small pinholes that enhance corrosion, are often present in SiN insulative films. (Karttu et al., 2022) To address the problems with SiN insulation, spin-coating of an epoxy-based photoresist, SU-8 or alternatively PI, is a widely applied method to produce a uniform and pinhole-free insulation layer for rigid MEAs (Zhang et al., 2010).

While constructing flexible MEAs, the usage of SiN, SU-8 or PI as insulating layer material poses problems. The mechanical properties of these traditional insulation layer materials render them to be stiff and rigid, which makes them susceptible to cracking when used with compliant substrates. To provide adequate insulation and to prevent cracking of the passivation layer in flexible MEAs, the material used in the insulation layer must also be compliant. Therefore, deposition of an insulative PDMS layer via spin-coating is conducted to insulate MEAs on PDMS. Although the motivation to use PDMS as an insulating layer has arisen from the fabrication of flexible MEAs, the usage of PDMS has also been suggested to insulate rigid MEAs. However, PDMS insulation of rigid MEAs has not become widespread since patterning of PDMS is more difficult than that of the conventional insulation layer materials, and PDMS may shrink during curing due to its high CTE. (Park et al., 2009)

4. FABRICATION OF MICROELECTRODE ARRAYS ON POLYDIMETHYLSILOXANE

Along with developing microfabrication processes, numerous solutions to fabricate MEAs on PDMS have been presented. These solutions can be grouped into categories based on the employed manufacturing process, although distinct approaches within each category may vary significantly in relation to the application and the goals they aim to achieve. (Adly et al., 2018) Figure 4 shows a stretchable MEA on PDMS, the fabrication of which is strived to be accomplished efficiently in terms of both time and cost. The MEA shown in figure 4 is a commercial product manufactured by BMSEED, a company specializing in the manufacture of flexible MEAs. (BMSEED, 2021) Despite various alternatives to construct MEAs on PDMS, challenges inevitably arise during fabrication due to the incompatibility of standard microfabrication processes with flexible materials. The material properties of PDMS cause difficulties in several process steps, such as fabrication of functional electronics on the surface, surface patterning of PDMS and implementation of the insulating layer. To overcome these challenges, expensive instrumentation, extensive research, and vast resources have been required throughout the years. (Adly et al., 2018)

Figure 4. *A commercial stretchable MEA on a PDMS substrate manufactured by BMSEED. The PDMS substrate is the transparent rubbery area visible within the cell culture ring in the figure. (BMSEED, 2021)*

Fabrication techniques that have produced the most promising results in fabrication of MEAs on PDMS can be divided into lithography-based methods and techniques based on ink-jet printing. Additionally, the implementation of flexible conductive polymers as microelectrodes has risen as one alternative to construct MEAs on PDMS. Within each of these categories, the objective is to manufacture a bioelectronic interface that enables dependable and accurate signal transmission between the target tissue and the electrodes. Despite the common aim, each of the fabrication subgroups exhibits diverse

advantages and disadvantages in terms of the fabrication processes applied to construct MEAs. Their popularity among the scientific community has fluctuated over time, and as microsystem technology has advanced, there has been a recurring return to previously discovered fabrication processes to optimize MEA functionality by adding new features into previously invented manufacturing processes. (Meacham et al., 2008; Blau et al., 2011; Adly et al., 2018;)

4.1 Lithography-based microelectrode array fabrication

A typical fabrication mechanism of planar MEAs on PDMS relies on photolithographical patterning of a conductive material between two thin films of PDMS. There is a wide array of possible alternatives for producing MEAs lithographically and the materials used in different applications may vary. The initially developed lithography-based manufacturing method for MEAs on elastomeric substrates establishes the foundation for other, more recent lithographic manufacturing mechanisms. (Meacham et al., 2008) Thus, the fundamental lithography-based fabrication process is first described, after which novel photolithographic MEA fabrication processes, the fabrication of bilayer-nanomesh MEAs and conical-well MEAs are presented (Guo et al., 2010; Ryu, Qiang, Jang, Suh & Fang, 2022).

4.1.1 Lithography-based fabrication process

The crucial fabrication steps that are applied in a standard lithography-based manufacturing process are depicted in figure 5. Regardless of the fabrication mechanism, gold is the most commonly used electrode material in lithography-based MEAs, and the fabrication process takes usually place on a rigid substrate, such as glass. As the first process step, the rigid substrate is coated with a thin non-stick layer which prevents adhesion between the rigid wafer and PDMS. (Guo et al., 2010) The purpose of the non-stick layer, which is commonly made of gold, is to enable the removal of the fabricated structure from the rigid substrate. To prevent detaching of the PDMS substrate from the glass substrate during manufacturing, an adhesive tape is applied around the glass perimeter prior to evaporating the gold non-stick layer onto the glass substrate. After applying the anti-adhesion layer onto the glass and subsequently removing the adhesive tape, the glass forms stronger adhesion forces with the PDMS substrate compared to the non-stick gold layer. This ensures that the PDMS substrate stays in place during processing. (Meacham et al., 2008)

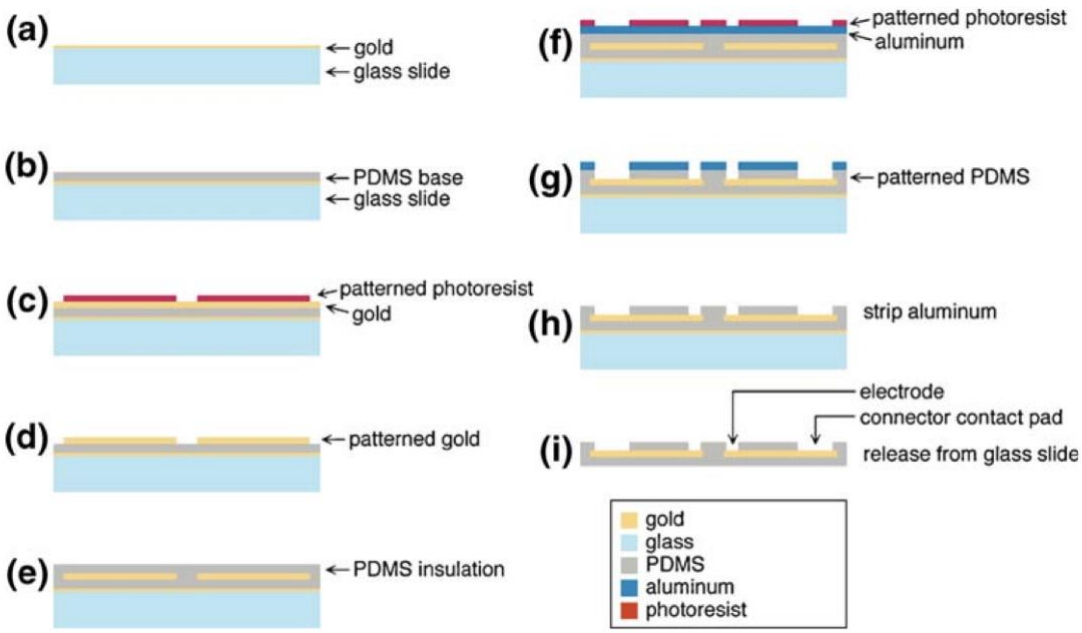

Figure 5. *Stepwise photolithographic fabrication process. (Meacham et al., 2008)*

The fabrication process illustrated in figure 5 continues with spin-coating the first PDMS layer onto the gold non-stick layer on the glass substrate to a uniform thickness. The deposition is followed by baking the sample on a hot plate to cure the PDMS base layer on the glass slide. The microelectrodes and wire traces are created photolithographically from a gold film deposited on the PDMS base layer. This process involves a mask with the desired electrode pattern along with a positive photoresist. The photoresist patterning follows a standard lithography process, after which the desired electrode structure is formed by etching the gold layer in specific etchants and rinsing it in de-ionized water. (Meacham et al., 2008)

The robustness of the MEA can be altered by changing the thickness of the gold film: the thicker the film, the more mechanical stress and electrolytic corrosion the MEA withstands. However, the film quality decreases when film thickness of 500 nm is exceeded. After patterning the electrodes, the photoresist is removed by flood-exposing the sample and soaking it in a developer. (Meacham et al., 2008) In flood-exposing the entire photoresist-coated substrate is exposed to light, after which it is rinsed in a developer. This treatment eliminates the need to use solvent strippers, such as acetone, which cause adverse effects in the PDMS substrate. (Guo et al., 2010)

The next process step in figure 5 is the production of a PDMS insulating layer. Following the curing of the insulative PDMS layer, a protective photolithographically patterned metallic mask is deposited onto the surface. The mask, typically made from aluminum,

ensures that material removal occurs only in desired areas when the material is etched to create electrode openings. Thereafter, contact pad and electrode openings are constructed by RIE that removes PDMS from the areas that are not covered with the aluminum mask. During RIE, the gold electrodes between the deposited PDMS layers act as an etch-stop material and ensure that only the insulating PDMS layer is etched. However, the electrodes may be damaged during etching, which must be considered when choosing the RIE process parameters. The final process step in fabricating a functional MEA on PDMS is the removal of the protective aluminum film which covers the insulative PDMS layer. Lastly, the fabricated MEA on the PDMS substrate is detached from the glass substrate. (Meacham et al., 2008)

Even though the traditional photolithography-based fabrication mechanism described above is still widely applied while constructing MEAs on PDMS, enhanced MEA characteristics have been achieved via altering the standard lithography-based fabrication process. The conformability of MEAs to biological samples has been successfully improved by fabricating ultrathin bilayer-nanomesh MEAs and a tighter electrode-tissue contact has been implemented by constructing conical-well MEAs. The stepwise fabrication processes of these bilayer-nanomesh and conical-well MEAs are lithography-based due to the photolithographically patterned microelectrodes. However, in these novel applications, fabrication of the electrodes and the electrode openings deviate from the stepwise fabrication process presented in figure 5. (Guo et al., 2010; Ryu et al., 2022)

The aim in constructing a transparent bilayer-nanomesh MEA on PDMS is to fabricate an ultrathin MEA with enhanced conformability. Figure 6 presents the structure of a bilayer-nanomesh MEA, which in turn is fabricated by the stepwise process shown in figure 7. This fabrication process differs from the previously described standard photolithographic manufacturing process in terms of electrode patterning. In construction of bilayer-nanomesh MEAs, the PDMS substrate is first coated with an etch stop layer, onto which a parylene C -film acting as a stress-balancing layer between the PDMS substrate and the microelectrodes is deposited. Subsequently, polystyrene nanospheres are used to generate a gold nanomesh. Thereafter, the gold nanomesh is deposited with PEDOT:PSS (poly(3,4-ethylenedioxythiophene) and poly(4-styrenesulfonate)) to create the bilayer structure. Finally, the gold electrodes and the interconnects are patterned via photolithography followed by etching. (Ryu et al., 2022)

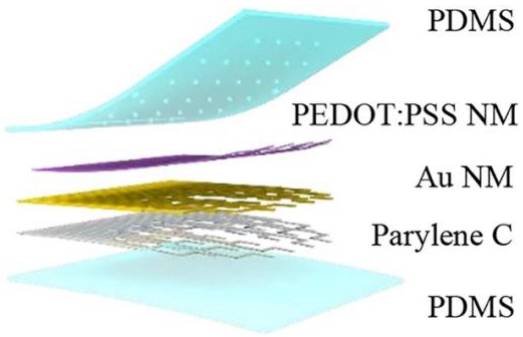

Figure 6. *An illustrative image of a bilayer-nanomesh MEA. (Ryu et al., 2022)*

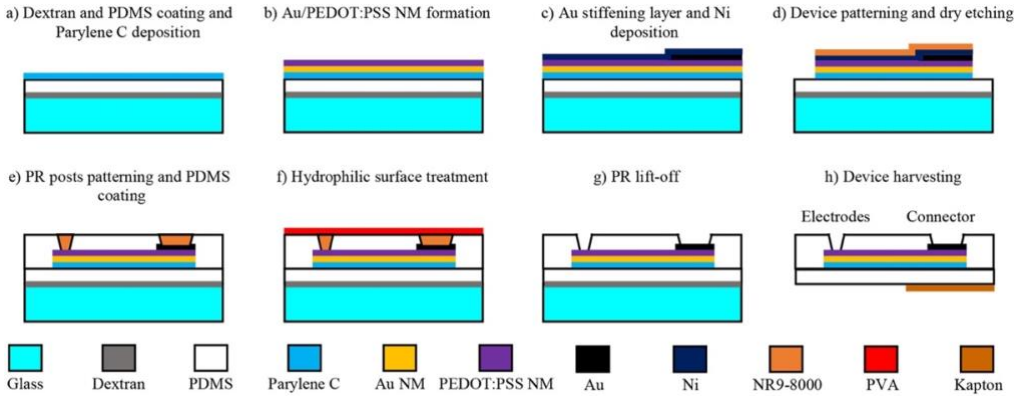

Figure 7. *Stepwise fabrication mechanism of bilayer-nanomesh MEAs. (Ryu et al., 2022)*

The electrode openings in bilayer-nanomesh MEAs are constructed via a lift-off process rather than implementing RIE on the PDMS insulating layer, as is done in the standard lithography-based fabrication process. As shown in figure 7, negative photoresist posts marking electrode opening sites are photolithographically patterned onto the surface prior to insulative layer deposition. After determining the electrode opening sites, an insulative PDMS layer is spin-coated onto the surface and treated with oxygen plasma and polyvinyl alcohol solution to present hydrophilic characteristics. The surface hydrophilization is conducted for subsequent cell measurements, rather than being an essential step in the technical device fabrication process. Finally, after curing the hydrophilic PDMS insulation layer, the lift-off of the posts marked by the negative photoresist creates the electrode openings and the last process steps are the same as described earlier. (Ryu et al., 2022)

In addition to improving MEA characteristics with the construction of bilayer-nanomesh MEAs, conical-well MEAs increasing microelectrode conformability can be fabricated. The stepwise manufacturing process associated with conical-well MEA fabrication is illustrated in figure 8. The major differences between the fabrication of conical-well MEAs and the standard lithography-based MEAs results from how the electrodes and the electrode openings are constructed. Instead of depositing a uniform gold layer onto the

PDMS base layer and constructing the electrodes via photolithography and etching as is presented in figure 5, the PDMS base layer is directly patterned photolithographically and the electrodes are constructed by utilizing a lift-off process. (Guo et al., 2010)

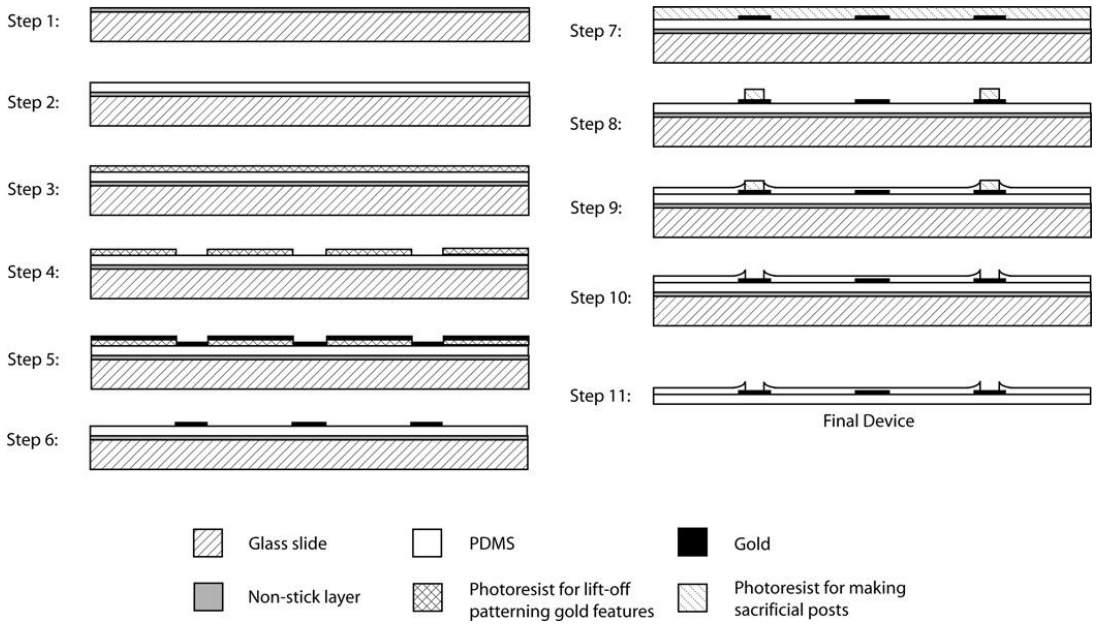

Figure 8. *Stepwise fabrication mechanism to construct conical-well MEAs on PDMS. (Guo et al., 2010)*

Since direct photolithographic patterning of the PDMS substrate is conducted to create the microelectrodes, the PDMS substrate is first activated with oxygen plasma to improve photoresist adhesion. Thereafter, a positive photoresist is deposited onto the PDMS substrate and baked to remove excess solvent and increase photoresist adhesion. A standard lithography process is employed to pattern the photoresist, after which the electrode material, gold with a titanium priming layer, is deposited onto the photolithographically patterned surface. The microelectrodes are created from the parts of the gold film that directly touch the PDMS surface. Therefore, the remaining resist is dissolved and the gold film coating the photoresist is lifted off. (Guo et al., 2010)

Although the lift-off process in theory is an operation that does not cause any negative impacts on the microelectrode pattern, the elastomeric characteristics of PDMS induces fabrication drawbacks and limits the utilization of traditional lift-off metallization. Lift-off on PDMS is challenging due to its high CTE. Materials with high CTE shrink in elevated temperatures during baking in photolithography process, which results in cracking of the deposited photoresist. In addition, PDMS absorbs chemicals and swells when brought into contact with solvents, such as stripping liquids. (Guo & DeWeerth, 2010)

Therefore, most stripping liquids that are utilized in rigid-substrate lift-off processes cannot be applied with PDMS because the PDMS substrate deforms and stripping of the

photoresist mask is difficult. Furthermore, construction of high-resolution microstructures on PDMS is demanding, because the usage of conventional photoresists is limited and desired features may be lifted off when PDMS swells. (Guo & DeWeerth, 2010) These challenges have been solved by using a positive photoresist and stripping it off with its developer, but the process step increases the fabrication workload (Guo et al., 2010).

To construct the conical-well electrode openings, sacrificial posts are created photolithographically. The hydrophilized PDMS surface is coated with a negative photoresist and exposed to light, and as a result, the exposed areas remain coating the MEA structure. The thickness of the photoresist layer determines the elevation of the sacrificial posts, thereby influencing the depth of the conical-wells. To encapsulate the MEA, an insulative PDMS layer is deposited and cured. The deposited PDMS layer must be thinner than the height of the sacrificial posts. This is critical for achieving a functional conical-well system because the desired structure is formed when the insulative PDMS layer sticks to the sacrificial posts and rises along their sides because of the capillary effect. Without the capillary effect, the depth of the conical-wells would not be the same as the thickness of the photoresist, which is the factor determining the structure of the wells. (Guo et al., 2010)

To define the conical-well electrodes, the sacrificial posts are removed with acetone. While acetone treatment imposes issues such as deformation of untreated PDMS and stripping off gold features when creating the microelectrodes, such problems do not occur during removal of sacrificial posts with acetone. The difference in how acetone affects the PDMS in separate fabrication process steps can be attributed to the alterations occurring in the insulative PDMS layer during processing. The changes in the properties of PDMS make penetration of acetone to the polymer more difficult and thus, the encapsulated device can endure the effects of acetone. The sacrificial posts could be removed with a positive photoresist and its developer as is done in the earlier lift-off process, but acetone is utilized because it provides a more time- and cost-efficient method to create the conical-well structure. (Guo et al., 2010) Regardless of the PDMS layers resisting the acetone treatment after processing, this process step may impose harmful effects to the PDMS layers if immersed in acetone for too long. (Meacham et al., 2008)

Finally, as described earlier, the fabricated structure is peeled off the rigid glass substrate, and the MEA on PDMS is ready to be used in *in vitro* applications. Even though the fabrication process associated with constructing conical-well MEAs is favorable as it takes place in a clean environment and no etchant residue remains, the process is not time-efficient. (Guo et al., 2010) More generally, each of the discussed photolithography-

based fabrication processes requires deposition of multiple materials which are subsequently cured, patterned, and etched. Therefore, these stepwise manufacturing processes utilizing photolithography contain numerous stages and can be seen as laborious and time-consuming.

4.1.2 Methods to improve microelectrode array characteristics

In novel manufacturing processes utilizing photolithography-based pattern transfer as a foundation to construct MEAs, efforts are made to optimize the characteristics of the manufactured MEA by modifying the standard photolithographic fabrication process. Through these changes, the goal is to produce MEAs which best enable the bidirectional transmission of electric signals. This is achieved via increasing the signal detection capability and conformability of the MEA. (Guo et al., 2010; Ryu et al., 2022)

Enhancement of the MEA electrical properties can be achieved by modifying the standard lithography-based manufacturing process depicted in figure 5. Adjustments to the RIE process parameters, which define the electrode openings, enhance material removal precision. By changing the RIE process parameters, bulk PDMS can initially be removed, followed by removal of the remaining PDMS residues from the insulative layer with modified etching process parameters. This enables the fabrication of electrode openings while leaving the gold film containing the electrodes intact. In a customized RIE process the RF power is lowered, and chamber pressure is increased after bulk PDMS has been removed to ensure that the ions removing PDMS residues do not impose any damages to the electrodes. This improves electrode functionality since their structure is not disrupted during RIE. (Meacham et al., 2008)

A typical starting point for improving the performance of MEAs on PDMS is to consider methods how the contact between the MEA and the tissue can be enhanced. Even though PDMS is a flexible material capable of elastic deformation, a MEA containing straight rigid electrodes is uncapable to stretch, which inevitably reduces the conformability of the MEA to tissues. This limitation is tried to overcome with various methods, the most successful of which contain utilization of serpentine electrode traces as well as fabrication of bilayer-nanomesh MEAs and conical-well MEAs, the fabrication processes of which were described earlier. (Meacham et al., 2008; Guo et al., 2010)

One option to enhance the conformability and stretchability of MEAs is to alter the placement of planar electrode traces or the microelectrodes themselves. Thus, intersecting electrode traces creating a more elastic serpentine pattern are constructed.

Generally, MEAs with straight electrode traces can stretch only 3% of their initial length, whereas the serpentine pattern allows up to 8% deformation. (Meacham et al., 2008) In addition, the diameter of the electrodes can be altered to improve the compliance of the MEA. The smaller the diameter of the electrodes, the less rigid they are and thus, the more accurately they comply to tissue surfaces. The improvement of photolithography has been a key factor in the reduction of electrode sizes since the electrode pattern is conducted photolithographically. (Guo et al., 2010)

Another approach to increase MEA conformability is to fabricate transparent bilayer-nanomesh MEAs on PDMS. The gold electrodes in bilayer-nanomesh MEAs are patterned with polystyrene nanospheres, enhancing electrode adaptability to tissue surfaces. Electrochemical deposition of PEDOT:PSS onto the gold nanomesh completes the formation of the bilayer structure. (Ryu et al., 2022) Utilizing PEDOT doped with PSS as the electrode material increases the accuracy of the MEA, since PEDOT:PSS may interact better with cellular interfaces than noble metal alone (Adly et al., 2018). Another advantageous property of bilayer-nanomesh MEAs is their thinness. As the thickness of these MEAs can be restricted to 10 μm, they are highly flexible ensuring optimal contact between the electrodes and biological samples. Additionally, the transparency of the bilayer-nanomesh MEA across a wide wavelength spectrum enables simultaneous electrophysiological recording and optical imaging of electrically active cells. By combining the measurements, spatial and temporal resolution advantages of both methods can be obtained. (Ryu et al., 2022)

MEA characteristics can also be enhanced by fabricating conical-well microelectrodes. The conical-well structure essentially improves the compliance of the MEA because the conical-well structure is elevated from the PDMS substrate enabling the formation of a better contact between the MEA and the tissue. Thus, the non-planar conical-well MEA allows the formation of an isolated electronic microenvironment due to the height of the wells. This, in turn, results in better electrical interfacing and increased uniformity of current density. In addition, the raised wells protect the microelectrodes from material breakdown caused by mechanical irritants. (Guo et al., 2010)

4.2 Ink-jet printed microelectrode arrays

Another widely used approach to fabricate MEAs on PDMS is ink-jet printing. Ink-jet printing is a fabrication technique among the diverse array of 3D printing methods, the rapid development of which has facilitated easier manufacturing of more versatile MEA structures. Ink-jet printing utilizes a conductive ink material directly deposited onto the PDMS substrate, which forms the desired electrode pattern. Ink-jet printing initially aimed

to align the mechanical properties of MEAs and tissues, but throughout its implementation, it was noted that the fabrication mechanism possesses also various other advantages. (Adly et al., 2018)

Along with rapid advancements in 3D printing, the popularity of ink-jet printing high-resolution microelectrode structures on diverse substrate materials has risen. The widespread adoption of ink-jet printing results from the versatility of the additive manufacturing method. With ink-jet printing, it is possible to change the structure of the MEA during manufacturing. Hence, the technique offers greater flexibility in construction of different microelectrode geometries compared to fabrication processes based on photolithography. Since ink-jet printing relies on extrusion of a conductive ink material through a nozzle to create the microelectrode pattern, mechanical lithographic masks are not needed. Therefore, the challenges associated with photolithographic pattern transfer can be avoided. (Adly et al., 2018) Moreover, ink-jet printing offers a more time-efficient manufacturing process by eliminating the numerous deposition and etching steps required in photolithography-based fabrication (Peng et al., 2023).

Although the equipment required for ink-jet printing is complex, the manufacturing process itself is simple, and the same equipment can be used for the extrusion of various ink materials. This, in turn, lowers the costs of the fabrication mechanism. Additionally, a favorable feature of ink-jet printing is the application of new electrode materials, the most often used of which are carbon and PEDOT:PSS. The noble metals typically applied to construct microelectrodes due to their conductive nature have high impedances and thus reduce the conformability of the MEA. Therefore, especially carbon, which has a low impedance, is a popular ink material from which microelectrodes can be ink-jet printed. Furthermore, carbon has excellent electrochemical stability and a wide electrochemical water window, which are both factors facilitating the receiving and transmitting of electrical signals. (Adly et al., 2018)

4.2.1 Fabrication process based on ink-jet printing

To construct a functional MEA on a PDMS substrate via ink-jet printing, microelectrodes, feedlines, and a passivation layer must be fabricated. The printing material utilized to construct the required structures varies since each of them fulfills different purposes. A schematic illustration of the fabrication process is presented in figure 9. First, outer feedlines are constructed from silver nanoparticle ink to create the framework for the microelectrodes. Subsequently, carbon nanoparticle ink is applied to manufacture the inner feedlines and the microelectrodes. The final process step in manufacturing is the printing of a PI passivation layer. An insulative PI layer is required in applications where

the MEA is brought into contact with samples containing water, as is the case with most biological specimen. PI is chosen as the insulative layer material because it is dielectric, biocompatible, and chemically inert in a cell culture medium and provides therefore sufficient insulation. (Adly et al., 2018)

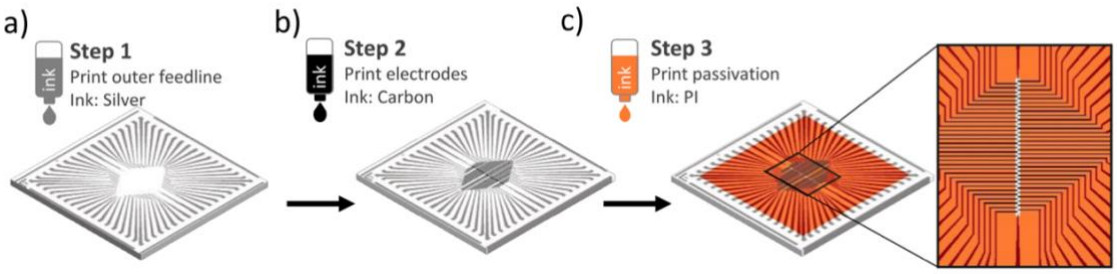

Figure 9. *Ink-jet printing procedure to fabricate MEAs on PDMS. (Adly et al., 2018)*

Even though the ink-jet printing procedure and thus the MEA fabrication process is relatively straight-forward, dewetting of the PDMS substrate presents challenges in manufacturing. To reliably collect signals from biological samples using the constructed microelectrodes, the circuits must be closed, which is achieved by ink-jet printing electrically continuous lines. Due to the hydrophobicity of PDMS, the creation of functional entities from hydrophilic, water-based ink droplets is difficult. Hydrophobic characteristics of the PDMS substrate lead to a high contact angle between the substrate and the ink droplets, which causes dewetting and thus disruption of the deposited liquid structure. (Adly et al., 2018) To facilitate the formation of closed circuits and functional MEAs on PDMS via ink-jet printing, the properties of PDMS must be altered during the fabrication process. The challenges posed by the hydrophobicity of PDMS can be addressed through various methods, the most commonly used of which are multilevel matrix deposition (MMD) and oxidative treatment. (Wu et al., 2015)

4.2.2 Optimization of fabrication process

To enable the usage of ink-jet printing in MEA fabrication, several methods to tackle the dewetting of PDMS substrate have been developed. MMD method relies on depositing droplets with a constant distance in both x- and y-directions. During MMD, the printed images are overlapped, and deposition continues until the whole surface is coated. This method is suitable for avoiding coalescence of adjacent droplets not only on PDMS but also on other hydrophobic substrate materials since the technique is not based on altering the properties of PDMS. MMD can also be utilized even after modifying the surface properties of PDMS with chemical agents to further improve the wettability characteristics of the substrate. (Wu et al., 2015)

A common method to increase the surface energy of PDMS is to oxidize the material with oxygen plasma. Despite the improvements in wettability due to oxidation, this method may cause the PDMS surface to crack during drying. (Adly et al., 2018) Therefore, the utilization of 3-mercaptopropyl trimethoxysilane (MPTMS) to increase the wettability of PDMS is more widely applied. MPTMS is a coupling agent that not only can change the methyl groups in the CRUs of PDMS into hydroxyl groups and make the material more hydrophilic, but also alter the surface characteristics of PDMS. Via modifying the surface properties, crack formation can be prevented and adhesion between the substrate and the deposited ink increased. (Wu et al., 2015)

A major advantage associated with MPTMS is the possibility to control the surface energy of PDMS and thus the dispersion of the printed ink droplets by altering the incubation time. The optimal wetting degree of PDMS depends on the utilized ink material and the intricacy of the structure. During printing of fine structures, the wettability of PDMS must be different from the process conditions applied in large-area film construction. Therefore, the surface energy of PDMS must be modified with MPTMS between printing the high-resolution microelectrodes and the PI passivation layer. After MPTMS treatment, oxygen plasma may also be used to alter the surface energy as MPTMS treatment prevents crack formation. (Adly et al., 2018)

Ink-jet printing can also be utilized to construct 3D MEAs that allow the detection of electrical signals from 3D specimens. Compared to lithography-based fabrication of 3D MEAs, additive manufacturing provides a more efficient method to produce 3D MEAs in terms of cost and time. (Peng et al., 2023) While 3D MEAs on PDMS have not yet been ink-jet printed, piezoelectric drop-on-demand ink-jet printing has been applied to create 3D MEAs on other compliant substrates, such as PEN. In this process, a conductive metal-based ink is printed to create the feedlines and microelectrodes, followed by electroplating the potentially cytotoxic electrodes with a biocompatible material, usually gold. The printed feedlines are passivated with an acrylate ink and cured with UV light. (Grob et al., 2021) As the interest in PDMS as a substrate material for MEAs is growing, ink-jet printing of 3D MEAs on PDMS may become feasible in the future.

4.3 Stretchable microelectrode arrays

One approach to fabricate MEAs on PDMS with enhanced electrode-tissue interaction is the construction of stretchable MEAs. In stretchable MEAs, the substrate and the microelectrodes are composed of flexible polymers and therefore, they are called all-polymer MEAs (polyMEAs). (Qi et al., 2017) Traditionally, metals or semiconductor

materials are used as electrodes in MEAs due to their high conductivity and simple processability. Even though it has been demonstrated that an integrated stretchable MEA with noble metal electrodes can exhibit uniaxial stretching up to 5%, the rigidity of metals limits the stretchability of the MEA and causes a risk of repositioning which may impose damage to biological specimen. (Guo, Guvanasen, Liu, Tuthill, Nichols & DeWeerth, 2013) Therefore, fabrication of stretchable MEAs relies on the application of polyMEAs, where the flexible electroconductive polymeric microelectrodes are capable of stretching while the tissue in contact with the polyMEA stretches (Qi et al., 2017).

PolyMEAs are thus electrical *in vitro* tools working on the same principle as conventional MEAs, with the exception that the conductive material in a polyMEA is a polymer instead of a metal. The electrode materials applied widely in polyMEAs are polypyrrole and PEDOT:PSS. The primary advantages associated with polyMEAs are their adaptability to tissues and the low Young's modulus of the microelectrodes which allows them to stretch when a tissue experiences strain. Additionally, polyMEAs possess better electrical characteristics in contact with tissues compared to conventional MEAs due to their low impedance, which results from the capability of polymers to transfer charge by both electronic and ionic mechanisms. (Qi et al., 2017)

The fabrication mechanisms used to construct stretchable polyMEAs deviate from each other in terms of electrode patterning techniques and material selection. A low-cost fabrication mechanism that is suitable for various conductive polymer materials begins with photolithographical patterning of a replica master which defines the structure of the polyMEA, the interconnects and the contact pads. Photolithography is followed by stamping the created master structure into a PDMS substrate, and cavities for the electrodes are created where the master's protrusions pierce through the entire PDMS layer. To construct the electrodes and the interconnects, the cavities in the PDMS sheet are filled with a conductive polymer, typically PEDOT:PSS. Lastly, the fabricated MEA structure is encapsulated with an insulating polymer film. (Blau et al., 2011)

However, with this fabrication mechanism, the electrodes made of PEDOT:PSS are prone to breaking upon stretching. To increase their stretchability and resistance to mechanical stress, PEDOT:PSS may be replaced with PDMS to which graphite flakes have been introduced. Despite its high stretchability and bendability, the graphite-flaked PDMS does not allow the fabrication of an optimal MEA for cell recordings as it is non-transparent. (Blau et al., 2011) Hence, a more widely applied method to fabricate transparent stretchable MEAs is the construction of polymeric microelectrodes with wavy electrode configuration (Qi et al., 2017).

Wavy electrode patterning presents an effective method to fabricate stretchable microelectrodes capable of reversible stretching without strains, but the weak electrode-substrate adhesion of wavy electrodes must be considered. The adhesion is facilitated by Van der Waals forces, and since the contact area between the substrate and the wavy electrodes is smaller compared to that of straight planar electrodes, the wavy structure may detach from the substrate. A solution to this problem is to bury nanowires in the PDMS substrate that act as a transition layer enhancing the adhesion between the MEA and the substrate, and this is illustrated in figure 10. In addition to assisting in electrode-substrate adhesion, the nanowires better the mechanical stability of the MEA and ease the electrode transfer onto the PDMS substrate. (Qi et al., 2017)

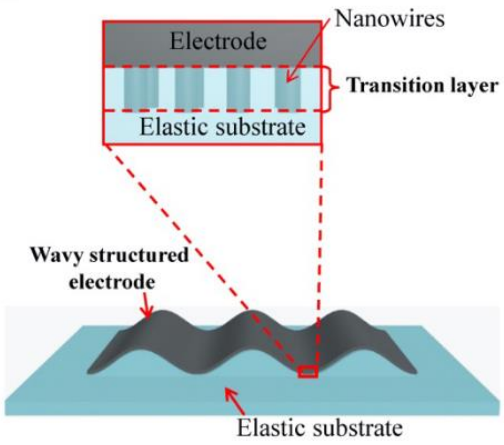

Figure 10. *An illustrative figure of a wavy polyMEA. (Qi et al., 2017)*

An applicable fabrication mechanism to produce polyMEAs with wavy electrode configuration exhibiting reliable electrical characteristics is based on photolithographical patterning of polyporryle nanowires and electrodes on a rigid glass substrate. Thereafter, the fabricated electrode structure is transferred to PDMS. To transfer the MEA from the glass substrate to PDMS, the microelectrode structure is covered with a prestretched PDMS layer which is subsequently cured. During curing, the adhesion forces between the polypyrrole electrodes and PDMS become stronger than the forces between the MEA and the glass substrate, and the MEA is transferred to the PDMS substrate. The wavy pattern is formed when the prestretched PDMS substrate is allowed to relax, upon which compressive forces induced to the MEA cause the conductive polymer electrodes to adopt a wavy configuration. (Qi et al., 2017)

The various MEA fabrication processes discussed are summarized for comparison in table 1. The table concludes how the elasticity and hydrophobicity of the PDMS substrate are taken into account in each manufacturing process, how the MEA structure is fabricated and how each process aims to optimize the electrical characteristics of the MEA.

Table 1. *A concluding table comparing the presented fabrication processes.*

MEA fabrication process	**Elasticity and high CTE of PDMS**	**Hydrophobicity of PDMS**	**Construction of electrodes and electrode openings**	**Principle employed to improve the characteristics of the MEA**
Standard photolithographical fabrication **Meacham et al., 2008**	Baking at a suitable temperature only for the required time	PDMS is not directly patterned photolithographically	Photolithographical electrode patterning, RIE process etches electrode openings in the PDMS insulative layer	Serpentine electrode traces: increased stretchability Customized RIE process: intactness of the electrodes
Fabrication of transparent bilayer-nanomesh MEAs **Ryu et al., 2022**	Stress-balancing parylene C-film, usage of a positive photoresist and its developer in the lift-off process	PDMS is not directly patterned photolithographically Hydrophilization of PDMS with oxygen plasma and polyvinyl alcohol for subsequent cell measurements	Electrode patterning with photolithography and polystyrene nanospheres, lift-off process to construct electrode openings	Ultrathin device: increased flexibility Electrode patterning with polystyrene nanospheres: increased adaptability
Fabrication of conical-well MEAs **Guo et al., 2010**	Usage of a positive photoresist and its developer in the lift-off process	Hydrophilization of PDMS with oxygen plasma prior to coating the PDMS substrate with a photoresist	Photolithographical electrode patterning, lift-off process to construct electrode openings	Conical-well electrodes: increased conformability and reduced electrode impedance
Ink-jet printing **Adly et al., 2018**	Elasticity of PDMS does not complicate the ink-jet printing process and elevated temperatures are not required	MMD, hydrophilization of PDMS with MPTMS and/or oxygen plasma between separate fabrication steps	Ink-jet printing of the desired microelectrode structure	Several possible electrode materials: increased conformability Fabrication of versatile MEA geometries
Fabrication of all-polymer MEAs with a wavy electrode configuration **Qi et al., 2017**	Fabrication of the MEA structure on a glass substrate, curing of the pre-stretched PDMS layer at a suitable temperature	PDMS is not directly patterned	Photolithographical electrode patterning on a glass substrate, transferring of the MEA to a pre-stretched PDMS substrate	Polymeric wavy compliant microelectrodes: increased stretchability and biocompatibility

As can be seen in table 1, the challenges posed by the properties of PDMS are considered in different ways in each fabrication process and as a result, each process has its own advantages and challenges. Furthermore, the principles used to optimize the properties of MEAs on PDMS in different manufacturing processes vary. This can be seen as an advantage, as the desired properties of MEAs required in different biomedical applications may differ, and thus alternative fabrication processes for the construction of MEAs on PDMS are needed.

5. CONCLUSIONS

MEAs can be seen as one of the most potential approaches to develop both diagnostics and treatment methods in future healthcare. MEAs are increasingly utilized in biomedical research as they allow signals from electrically active cells to be studied, which can be employed in the diagnosis and treatment of neurological disorders and cardiovascular diseases. (Ryu et al., 2022) The usage of PDMS as a MEA substrate material has increased since the elasticity of the PDMS substrate enables the formation of a close interface between the microelectrodes and the biological sample by conforming to tissue surfaces. This, in turn, improves the ability of MEAs fabricated on PDMS to acquire electrical signals from biological specimen, enabling a more precise method for studying the electrical activity of biological samples compared to rigid MEAs. (L. Xu et al., 2021)

Since MEAs fabricated on PDMS and PDMS-based OoCs play a significant role in advancing biomedical research, several alternatives for fabricating MEAs on PDMS have been developed. The common aim in fabrication is to construct a MEA exhibiting optimal electrode-tissue interaction, and in construction of such a MEA, the elasticity, hydrophobicity, and high CTE of PDMS substrate must be considered. (Adly et al., 2018) Although these properties of PDMS pose challenges in fabrication and limit the usage of conventional microfabrication techniques, traditional photolithography-based fabrication process remains widely employed and is likely to retain its significance due to its simplicity and relatively low manufacturing costs. However, the standard photolithographic fabrication process is increasingly altered via novel microfabrication approaches, resulting in the construction of MEAs with improved biocompatibility and enhanced electrical properties. (Guo et al., 2010)

The most successful methods by which photolithographically fabricated MEA properties have been optimized include modifying the microelectrode arrangement and fabricating bilayer-nanomesh MEAs and conical-well electrodes. (Guo et al., 2010; Ryu et al., 2022) However, the optimized photolithographic stepwise fabrication processes are laborious and time-consuming. Moreover, in these fabrication processes, the structure of the photolithographically patterned MEA is a replica of the mechanical mask used in the photolithographic pattern transfer, which limits the possible MEA geometries and makes modifying of the MEA structure challenging. One potential solution to address this limitation could be the use of direct writing technologies, where the photoresist is patterned by a moving laser beam instead of using mechanical masks. Another option to increase the versatility of MEA structures is provided by ink-jet printing. Fabrication

processes based on ink-jet printing utilize a moving print head to construct the microelectrodes from conductive ink droplets, facilitating the fabrication of versatile MEA structures more efficiently compared to photolithographic fabrication (Adly et al., 2018).

Although the hydrophobicity of PDMS causes challenges during ink-jet printing, the advantages of ink-jet printing outweigh the difficulties occurring in fabrication. In addition to allowing the construction of a customized MEA pattern, an ink-jet printer can be used to print different inks on various substrates, making the fabrication process both time- and cost-effective. (Adly et al., 2018) Additionally, principles of stretchable electronics can be applied into fabrication of MEAs on PDMS, resulting in the construction of polyMEAs. In polyMEAs, both the MEA and the substrate are polymeric materials capable of elongation, thereby enhancing the biocompatibility and signal acquisition capability of the MEA. (Qi et al., 2017) Typically, the stretchability of a polyMEA results from the implementation of a wavy electrode configuration. Despite the challenges in electrode-substrate adhesion with wavy electrodes, the fabrication of wavy electrode configurations via ink-jet printing could be explored in the future. As wavy electrodes have been successfully fabricated from silicon nanoparticles, their construction from other non-polymeric electrode materials, such as carbon nanoparticle ink utilized in ink-jet printing, could be researched.

When comparing all the presented fabrication processes, ink-jet printing demonstrates the most promise in fabricating MEAs on a PDMS substrate with the lowest efforts, the highest accuracy, and the most versatile characteristics. Since ink-jet printing is part of a wide range of rapidly growing 3D printing techniques, significant resources are being devoted to the development of this fabrication process. Thus, in the future, ink-jet printing could provide an efficient method for fabricating non-planar MEAs which could be used to accurately study the electrical activity of 3D biological specimen. The emphasis on fabricating 3D MEAs will continue to rise in the future, for which ink-jet printing offers an inexpensive, easily implementable, and adaptable fabrication method.

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