3D Printing of Flexible Electrodes for Clinical Applications

Laura Blanco Peña

University of Wollongong

UNIVERSITY OF WOLLONGONG

COPYRIGHT WARNING

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author.

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

Recommended Citation


Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au
3D Printing of Flexible Electrodes for Clinical Applications

Laura Blanco Peña

Supervisors:
Dr. Sepidar Sayyar, A/Prof. Michael J. Higgins, Prof. Gordon G. Wallace

This thesis is presented as part of the requirement for the conferral of the degree:
Master of Philosophy (Biofabrication)

The University of Wollongong
School of Chemistry

July 2018
Abstract

The cochlear implant (CI) is the only successful tool for management of sensorineural hearing loss. Implanted within the patient’s inner ear, it adopts the cochlea’s seashell shape and electrically stimulates the auditory nerve at different points, allowing auditory perception to occur. 3D printing the CI electrode array would allow to customize it to the patient inner ear anatomy and increase the complexity of the stimulation provided due to the high spatial control offered by the 3D printing techniques, which would solve some of the issues associated with the electrode such as a poor nerve-electrode interface or reduced number of stimulation channels. In order to develop 3D printed flexible electrodes that could be used for the CI, two approaches were explored: inkjet printing a Pt-precursor ink (10% H2PtCl6) on a polydopamine-coated PDMS substrate followed by reduction to Pt; and 3D printing of conductive rLCGO/PDMS coaxial fibres. The resulting printed Pt-patterns did not showed the expected conductivity and more characterization and optimization is required to address the issue. On the other hand, coaxial printing rLCGO/PDMS fibres allowed creating a prototype construct that was both flexible and electrically conductive. More optimization of the printing process must be done before these techniques can be implemented for the CI electrode fabrication.
Laymen’s Summary

Hearing loss is a health problem that has serious consequences at both personal and societal level and it must be properly handled to ensure the wellbeing of the individual and the progress of the society. While patients suffering from mild hearing loss can benefit from hearing aids that amplify the sounds in the ear, people with severe or profound hearing loss – usually due to disfunction of the inner ear (cochlea) – need a different device: the cochlear implant (CI). The CI is implanted within the patients head and has an electrode array that contacts the auditory nerve and sends electrical signals to it, making hearing possible. This electrode could be improved by customization of its shape to the patient’s cochlear anatomy, so the contact between the electrode and the auditory nerve would be better; or by increasing the complexity of the electrical signals that can be sent to the nerve. 3D printing the electrode would allow to achieve those goals due to the high spatial control that those techniques offer. Two different techniques were explored. The first one consisted in dispensing an ink on a biocompatible flexible substrate that would become Pt after exposing it to high temperatures and thus the printed patterns would become conductive. Since the substrate was flexible, it could adapt to the cochlea’s seashell shape. Nevertheless, we did not obtain the desired conductivity of the patterns. On the other hand, we developed a technique that allowed us to extrude a fibre made of a flexible conductive fibre surrounded by a flexible insulating material. Using a 3D printer, we could arrange several of these fibres together to create a construct that was both flexible and conductive. This technique is promising and more research must be carried out to optimize it and be able to create constructs adequate for the CI.
Acknowledgments

I wish to acknowledge and thank my supervisors, Dr Sepidar Sayyar and A/Prof Michael Higgins for their guidance, help and support to develop this project. I would like to thank Prof Gordon Wallace for taking the time to seat with every student to discuss the progress of their projects and always suggest creative ideas.

To Adam Taylor, thank you for your invaluable assistance with the inkjet printer and the design of the coaxial nozzles; thank you for your patience and your will to make things work. Many thanks to Ali Jeiranikhameneh for your support with the extrusion printer. I appreciated the expertise of Kezhong Wang for the rLCGO fibres provided to use in this study and the help of Tony Romeo for obtaining SEM images of my samples. I wish to express a sincere thank you to Binbin Zhang, for creating the foundation for this study. Finally, thank you to all the fantastic colleagues I have shared this months with and asked so many questions to.

I want to thank Prof Jos Malda, A/Prof Jacqueline Alblas, and Roos Nieuwenhuis for facilitating the international double master’s degree in Biofabrication and for all the support before and during my stay at the University of Wollongong.

Finally, I wanted to thank my family, my friends, and H.N. for all your love, encouragement, and support through all these years and, specially, this last one. Being the furthest possible from you, your backing has been essential in this journey.
Certification

I, Laura Blanco Peña, declare that this thesis submitted in fulfilment of the requirements for the conferral of the degree Master in Philosophy (Biofabrication), from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Laura Blanco Peña
3rd July 2018
List of Names or Abbreviations

CAD – Computer-aided design
CI – Cochlear implant
DPN – Dip-pen nanolithography
EDS – Energy-dispersive X-ray spectroscopy
EG – Ethylene glycol
H₂PtCl₆ – Chloroplatinic acid
PDMS - Polydimethylsiloxane
Pt – Platinum
rLCGO – Reduced liquid crystalline graphene oxide
SEM – Scanning electron microscopy
Si – Silicon
# Table of Contents

Abstract .......................................................................................................................... 1

Laymen’s Summary ....................................................................................................... 2

Acknowledgments .......................................................................................................... 3

Certification .................................................................................................................... 4

List of Names or Abbreviations ................................................................................... 5

Table of Contents .......................................................................................................... 6

List of Figures ................................................................................................................ 7

Chapter 1 ....................................................................................................................... 10

Introduction: background and research plan .............................................................. 10
  Epidemiology and socioeconomic impact of hearing loss impairment ..................... 10
  Prevention and Management of hearing loss ............................................................... 11
  Cochlear Implant: present and future ....................................................................... 11
  Research approach: purposes and significance ........................................................ 13

Chapter 2 ....................................................................................................................... 14

Inkjet Printing of Platinum Nanoparticles on PDMS substrates .................................. 14
  Introduction ............................................................................................................... 14
  Materials & methods ................................................................................................. 17
  Results and Discussion .............................................................................................. 19

Chapter 3 ....................................................................................................................... 29

3D Printing of rLCGO/PDMS Coaxial Fibres ............................................................... 29
  Introduction ............................................................................................................... 29
  Materials & methods ................................................................................................. 32
  Results and Discussion .............................................................................................. 36

Chapter 4 ....................................................................................................................... 47

Conclusions .................................................................................................................... 47

Bibliography or List of References ............................................................................... 48

Appendices ..................................................................................................................... 54

Appendix 1: Sketches of the nozzles ........................................................................... 54
List of Figures

**Figure 1.** Elements of the cochlear implant. Illustration of the components of the CI and relevant anatomy (A): sound processor (a), coil and electromagnetic transducer (b), electrode array (c), cochlea (d), auditory nerve (d). Internal parts and materials in the CI (B): electromagnetic transducer with a titanium or ceramic case (1), magnetic coil that receives the signals from the external sound processor (2), extracochlear electrodes (3), electrode array made of platinum/iridium (90/10) wires within a polydimethylsiloxane (PDMS) carrier and 22 platinum contacts (4), removable magnet (from the transducer) (5), PDMS reinforcement (6) (Wallace, Higgins, Moulton, & Wang, 2012).

**Figure 2.** Inkjet-printed nano-silver circuit (Nehlsen, 2016).

**Figure 3.** Components in thermal and piezoelectric (acoustic) inkjet printers (Murphy & Atala, 2014).

**Figure 4.** PDMS surface hydrophobicity prevent single drops to fuse and create patterns (Kim et al., 2014).

**Figure 5.** Effect of polydopamine coating and air plasma treatment on PDMS wettability for water and Pt-precursor ink. A reduction in contact angle (mean ± SD) translates to a higher wettability for the two different liquids. The decrease in contact angle right after treatment (t = 0h) is shown as the relative percentage change with respect to the control (A). The effect of different treatments on PDMS wettability for water (B) and Pt-precursor ink (C) immediately after treatment (t = 0h) was compared (n=6, mean ± SD; *** p<0.001, one-way ANOVA).

**Figure 6.** Comparing the effect of polydopamine and air plasma on PDMS wettability for water and Pt-precursor ink over time. Higher wettability translates to lower contact angle and may change over time (A). The wettability for water (B) and Pt-precursor ink (C) was evaluated over time and the effect of each treatment at 0h, 1h, 2h, 3h, 4h, 5h, 24h, 48h, and 72h was compared to the effect at 0h (n=6, mean ± SD; * p<0.05, ** p<0.01, *** p<0.001, two-way ANOVA).

**Figure 7.** Behaviour of PDMS wettability for water (A, B, C) and Pt-Precursor ink (E, F, G) over time after three different surface treatments. Contact angle was measured at 0h, 1h, 2h, 3h, 4h, 5h, 24h, 48h and 72h (n=6, mean ± SD; * p<0.05, ** p<0.01, *** p<0.001, two-way ANOVA). Linear regression of time-dependent data was performed (D, H).

**Figure 8.** Pt-precursor ink reduced by either air plasma (A) and heat (B). The metallic grey areas are deposited Pt-particles while the yellow areas correspond to unreduced Pt-precursor.

**Figure 9.** Square Pt patterns with lines in multiple directions. The CAD file (A) was printed onto untreated (B) and polydopamine-coated (C) PDMS substrates and reduced. The Pt-precursor ink droplets stay individually when no surface modification is applied to enhance PDMS surface wettability for the ink resulting in a dotted Pt pattern (optical microscopy image) (B), while they extend and fuse creating a continuous pattern when the PDMS substrate has being coated with polydopamine (SEM image) (C). The bright circles appreciated on the optical microscopy image (B) correspond to air bubbles present within the transparent PDMS substrate and do not affect the homogeneity of the PDMS surface, as observed on the SEM image (C). Wrong “Z” values lead to faulty printed patterns (D, E).
Figure 10. SEM images of Pt printed lines on polydopamine-coated PDMS. 1-, 3-, and 5-layers lines (from bottom to the top) (A) show different opacity due to differences in the amount of Pt deposited. A magnified detail of a 3-layers line (B) shows cracking on the printed pattern. And EDS image (C) of the same magnified area maps the presence of Pt (Pt M series). Actual width of printed lines and width increase with respect to the expected 0.5 mm width (on polydopamine-coated PDMS) (D). The number of layers printed has an effect on the final width, the 5-layers lines being wider than its counterparts (n=12, mean ± SD; *** p<0.001, one-way ANOVA).

Figure 11. SEM image of a printed “50 µm width” line on polydopamine-coated PDMS. Instead of this, two parallel sequences of dots were obtained. The dots have a diameter of 67.5 ± 5.2 µm and both sequences are 215.71 ± 9.15 µm apart (mean ± SD).

Figure 12. Molecular structure of graphene exhibiting a honeycomb-like structure (Papageorgiou, 2017) (A). Oriented graphene oxide sheets result in nematic liquid crystalline graphene oxide dispersions (Jalili et al., 2014) (B). SEM image of a wet-spun rLCGO fibre (C) and its cross-section showing different carbon sheets composing it (D).

Figure 13. Extrusion-based 3D printing uses pneumatic and mechanical (piston, screw) forces to dispense continuous threads of material (Murphy & Atala, 2014) (A). Coaxial nozzle showing two different channels filled in with two different materials that concentrically converge resulting in the extrusion of coaxial structures (Cornock et al., 2014) (B).

Figure 14. Potential 3D printed coaxial construct for the CI. Coaxial fibres have a rLCGO fibre as conductive core and PDMS as insulating, outer layer. 3D printing these fibres would allow fabricating a flexible, solid construct with multiple parallel rLCGO fibres acting as an electrode array surrounded by PDMS.

Figure 15. Sketch of the longitudinal section of the nozzle showing the internal architecture (A) and 3D model of the nozzle (B).

Figure 16. Optical microscopy image showing one fibre segment delimited by silver paint acting as electrical contacts.

Figure 17. Characterization of rLCGO and Pt-rLCGO fibres. While platinizing the fibre did not affect fibre diameter (A), it did lead to a dramatic increase in electrical conductivity (B).

Figure 18. Temperature dependence of PDMS rheological behaviour. The dynamic test comprised a temperature ramp step from 25 ºC to 150 ºC (5 ºC/min) (0 – 25 min) followed by a 10-minutes time sweep step (25 – 35 min). G’ and G” were measured over time. G”>G’ at t = 0 min meaning that PDMS shows a viscous behaviour. G’ becomes larger than G” at t = 16.5 ºC, when the temperature is 108 ºC, therefore PDMS shows an elastic behaviour at temperatures above that.

Figure 19. Image of one printed nozzle (A) and a partially sectioned nozzle with an inserted 30G needle tip showing the inner architecture (B). Optical microscopy image of the sectioned nozzle’s lower section showing the fusion of the vertical channel – for the rLCGO fibre – and the inclined channel – for the PDMS (C). Bottom view of the nozzle showing the vertical channel open (optical microscopy) (D). Bottom view of a nozzle with an inserted 30G needle tip; the needle tip can be observed through the nozzle’s channel when focusing a deeper plane (E), what ratifies the perfect alignment of the lower vertical channel with the needle tip.
Figure 20. Manually placed rLCGO/PDMS coaxial fibres using a extrusion pump. ...............................39

Figure 21. Printing setup. The rLCGO fibre (1) is passed through the needle tip (2) and collected in a syringe tube (3) attached to the tip (A). The loose end of the fibre is passed through the nozzle (4) vertical channel by inserting the needle tip into it. The cartridge attaching system is positioned in a 45° angle thanks to the printed adaptor piece (5) (B). The nozzle (4) is attached to the metal barrel (6) that contains the PDMS (C). This barrel is connected to the printer pneumatic system (7) and placed within a thermal jacket (8) in case warming up the material is necessary. The substrate (9) is placed on a hot plate (10). ..................................................................................................................40

Figure 22. Printed rLCGO/PDMS coaxial fibres through the 700 µm (A) and 400 µm (B) diameter nozzles. The end of the fibres was cut clean (A) to eliminate any defect due to dragging of the rLCGO fibre (B) and to expose the rLCGO fibre on the section. 3D printed continuous rLCGO/PDMS fibre showing dragging of the rLCGO fibre in the corners (C). 3D printed continuous rLCGO/PDMS fibres with a circle of radius 3 mm at the corners. Dragging of the rLCGO fibre stopped after the first loop (D). ..................................................................................................................42

Figure 23. Top view of a 3D printed two-layer rLCGO/PDMS construct with a stepped alignment of the fibres (A). Portions of the fibres stand out of the construct. Given the transparency of the PDMS, the perimeter of the construct has been marked in red to help visualizing. Top (B) and section (C) view of the 3D printed two-layer rLCGO/PDMS construct after cutting out the ends. Due to differences in light reflection across the curvy PDMS surface, some of the rLCGO fibres cannot be properly visualized. 2 layers with 4 coaxial fibres each were printed. The morphology of the second layer was very poor ..................................................................................................................44

Figure 24. Bending test. The construct underwent 100 cycles of bending. .........................................45
Chapter 1

Introduction: background and research plan

Epidemiology and socioeconomic impact of hearing loss impairment

Hearing impairment is the partial or total inability to perceive sounds. The normal-hearing threshold for humans range from 0 to 20 dB in the best ear; disabling hearing impairment occurs when the hearing-threshold is 35 dB or higher (Olusanya, Neumann, & Saunders, 2014; Stevens et al., 2013). It is estimated that 538 million people above the age of 5 have a disabling hearing impairment, the prevalence being high in low- to middle-income countries (Olusanya et al., 2014).

Hearing loss can be congenital or acquired (Olusanya et al., 2014; WHO Media centre, 2017). Hereditary factors and complications during pregnancy and labour are the causes leading to congenital hearing loss – present at birth or acquired soon after it. Birth asphyxia, neonatal jaundice, some maternal infections – e.g., syphilis, cytomegalovirus, and rubella – and incorrect drug use during pregnancy are examples of these complications. In addition to this, individuals can experience hear loss at any age due to chronic ear infections and liquid accumulation in the ear, the use of ototoxic drugs – e.g., some drugs prescribed against malaria, cancer, and drug-resistant tuberculosis (Arslan, Orzan, & Santarelli, 1999) –, ear trauma, some infections – e.g., meningitis, mumps, and measles –, and exposition to loud sounds and noise – occupational, recreational, or accidental (Basner et al., 2014).

Hearing loss is an important global health issue that is intimately related to the development of a region. The prevalence of congenital hear impairment is three times higher in developing countries and the number of cases in individuals under the age of 15 that could have been prevented through effective public health policies is higher in those countries (75%) than in high income countries (49%) (WHO Media centre, 2017). Deaf people often perform badly academically (in poor countries deaf children are rarely schooled), are unemployed, or hold low responsibility positions; besides, non-treated deafness has a strong social and emotional impact: affected people need to join special programs to learn sign language and other communication tools to integrate in society, and they usually feel excluded and isolated, causing loneliness and frustration (Olusanya et al., 2014; WHO Media centre, 2017). Taking into account the cost of educational support, loss of productivity, and medical assistance (hearing-aids excluded), the global economic burden associated to hearing impairment is estimated to be 750 billion international dollars (WHO Media centre, 2017). Consequently, hearing loss prevention, diagnosis, and management, especially in undeveloped areas where the incidence of hearing impairment is higher, would help to attain important Millennium Development Goals, such as
eradication of global poverty and full access to primary education for all children (Olusanya, Ruben, & Parving, 2006).

**Prevention and Management of hearing loss**

Overall, 50% of the global cases are thought to be preventable (WHO Media centre, 2017). Immunization of children and women, good hygiene, and screening against infections in pregnant women, improved labour and neonatal care, avoidance of ototoxic drugs unless prescribed by a qualified physician, screening and treatment of other health conditions related to hearing loss and mentioned above, and avoidance of loud sounds and noise (Olusanya, 2012; Olusanya et al., 2006; WHO Media centre, 2017).

In addition to prevention, early diagnosis and management are key for the correct development and education of deaf children as well as for the societal integration of deaf children and adults (Olusanya et al., 2006). Lip-reading, sign language, and written text are important skills deaf people need to develop to communicate with others (WHO Media centre, 2017). Besides, different devices have been developed to allow or improve hearing: hearing aids and cochlear implants (CI). Hearing aids consist of electronic devices that work either amplifying the sound in the patient’s external ear or emitting sound waves that are transmitted through the skull to the healthy inner ear when hearing loss is due to problems affecting the external ear, such as ear canal malformations thereby benefitting people with mild to moderate hearing loss (Janssen, Hong, & Chadha, 2012; WHO Media centre, 2017). Nevertheless, hearing aids are not useful for people with severe to profound deafness, such is the case of sensorineural hearing loss – where hair cells and spiral ganglion neurons are damaged – and they benefit from CI instead (Irving et al., 2014).

**Cochlear Implant: present and future**

The multi-channel CI replaces the cochlea’s lost function by detecting external sounds, processing them into electric signals, and directly stimulating the auditory nerve. Therefore, the CI is beneficial to manage sensorineural hearing loss (Clark, 2014; Clark, Pyman, & Bailey, 1978). The device consists of both external and internal elements and works as follows (Figure 1). A sound processor that can be worn either behind the ear or on the body will detect the sounds in the environment and transform them in digital codes. The sound processor is connected to a coil magnet placed on the skull surface which will transmit the digital code to the electromagnetic transducer in the implanted part of the device in the head. The implant receives the digital code and transforms it into electrical signals that will be conducted through an electrode array to the cochlea, where the electrode contacts and stimulates the auditory nerve at different locations. Therefore, the damaged inner ear is bypassed and the auditory stimuli can arrive to the brain, where auditory perception occurs (Clark et al., 1978; Cochlear Ltd, 2018; Tisch, 2017).
Figure 1. Elements of the cochlear implant. Illustration of the components of the CI and relevant anatomy (A): sound processor (a), coil and electromagnetic transducer (b), electrode array (c), cochlea (d), auditory nerve (d). Internal parts and materials in the CI (B): electromagnetic transducer with a titanium or ceramic case (1), magnetic coil that receives the signals from the external sound processor (2), extracochlear electrodes (3), electrode array made of platinum/iridium (90/10) wires within a polydimethylsiloxane (PDMS) carrier and 22 platinum contacts (4), removable magnet (from the transducer) (5), PDMS reinforcement (6) (Wallace, Higgins, Moulton, & Wang, 2012)

The electrode array is made of platinum/iridium (90/10) wires embedded in an insulating flexible silicon carrier and has 22 platinum (Pt) electrode contacts on the distal end. Each of the wires represent a single channel of stimulation. Due to the flexibility, the electrode adapts to the cochlea’s seashell shape and provides stimulation to the cochlea through the 22 Pt contacts. On the other hand, the transductor case is made of either titanium or ceramics (Cochlear Ltd, 2018; Wallace et al., 2012). Since the device is implanted within the head of the patient, the materials used for its fabrication must ensure its safety and long-term functionality, and therefore, be biocompatible, resistant to mechanical forces, and stable over time. The materials used for the implant fabrication that are in contact with the patient’s tissues—silicon, Pt, titanium, and ceramics—show the required biocompatibility, corrosion-resistance, low reactivity, and mechanical resistance while ensuring the conductivity and flexibility of the electrode (Stöver & Lenarz, 2009; Wallace et al., 2012).

Since the first implanted device in 1978 (Clark et al., 1978), researchers and manufacturers continue working to improve the current technology. Some of the goals include reducing the size of the features on the electrode array, preventing biofilm formation on the electrode surface that may hamper the electrical stimulation of the nerve, improving the nerve-electrode interface,
increasing the number of channels that can be stimulated in an independent manner, reducing the risk of infection post-implantation, and preventing residual-hearing loss due to post-implantation inflammation and fibrosis (Choi & Oghalai, 2005; Dhanasingh & Jolly, 2017; Im et al., 2015)

**Research approach: purposes and significance**

Applying additive manufacturing techniques – 3D printing constructs in a layer-by-layer fashion from a 3D computer-aided design (CAD) model – to the fabrication of the electrode array is likely to help achieving some of these goals. 3D printing offers superior material deposition control, which allows producing models with high spatial resolution. In this way, smaller and more complex structures with a higher number of stimulation channels and customized to the particular patient’s cochlea anatomy could be produced. Additionally, anti-inflammatory and antibacterial molecules could be incorporated on the implant surface using the same 3D printing techniques.

Although the number of printing techniques is large, we explored two different approaches that seemed suitable to produce 3D-printed flexible electrodes for the CI: inkjet printing a Pt-precursor on PDMS to create a thin, high-resolution conductive pattern on a flexible, insulating substrate; and extrusion printing of coaxial fibres consisting of a conductive core made of a reduced liquid crystalline graphene oxide (rLCGO) fibre and an insulating outer layer made of PDMS. These methods could allow creating flexible, conductive, and biocompatible electrode arrays.
Chapter 2

Inkjet Printing of Platinum Nanoparticles on PDMS substrates

Introduction
Contrary to microwelding highly conductive metals to create different electrode arrays, new techniques aim to create more complex conducting patterns with higher spatial resolution in an easy, straight-forward fashion. Inkjet printing of silver-based inks is the most explored prototyping technique for this aim (Figure 2) (Kim, Ren, Kim, & Noh, 2014; Ko et al., 2007; Murata, Matsumoto, Tezuka, Matsuba, & Yokoyama, 2005).

![Figure 2. Inkjet-printed nano-silver circuit (Nehlsen, 2016).](image)

Inkjet printing consists in dispensing microdroplets (1-100 pl) of material (“ink”) through small gauge nozzles and directing them towards a substrate with high spatial control recreating a digital image. Two different mechanisms are used to propel the droplets out of the nozzle (Figure 3): thermal inkjet printers heat the printhead to evaporate components of the ink and produce air-pressure that forces the ink out, whereas acoustic printers use piezo-electric activators to mechanically eject the droplets (Malda et al., 2013; Murphy & Atala, 2014).

Despite silver showing the highest electrical conductivity of all metals (6.30 x 10^7 S/m at 20 ºC) (Serway & Jewett, 2015), its use for long-term clinical implantation has been discarded due to its high reactivity and cytotoxicity (Hansen, 2008; Park et al., 2011). On the other hand, Pt appears as the preferred metal for electrodes with medical applications, due to its low corrosiveness, good conductivity, and high biocompatibility, among other beneficial characteristics (Cowley & Woodward, 2011; Geddes & Roeder, 2003).
Previous work on developing a Pt precursor ink that could be printed on different substrates, such as glass and non-conductive, flexible and soft silicon (Si) substrates have been done in our research group (O’Connell et al., 2013). A solution of 10% chloroplatinic acid (H₂PtCl₆·xH₂O) in ethylene glycol (EG) was used as a Pt-precursor ink in order to obtain Pt-nanoparticles in situ. H₂PtCl₆ reduces to solid Pt nanoparticles at 160º C in the presence of reducing agent EG (Cho & Ouyang, 2011; O’Connell et al., 2013), creating a thin conductive film with the desired pattern. Treating the Pt-precursor ink with reactive plasma species for at least 10 minutes also leads to the reduction of H₂PtCl₆ to Pt (O’Connell et al., 2013). However, preliminary work done in our group showed that temperature produced a more efficient reduction and therefore is the preferred method, air plasma being a great alternative when working with thermal-sensitive substrates, since it takes place at room temperature. PDMS starts degrading at 350 ºC (Camino, Lomakin, & Lazzari, 2001), therefore it is compatible with the thermal reduction of the Pt-precursor ink at 160ºC.

A key factor to take into account when using inkjet printing is the wettability of the substrate. Wettability is the ability of a solid surface to maintain contact with a liquid and directly affects the size and stability of the printed patterns. Wettability can be easily quantified through the measurement of the contact angle between a sessile drop and a solid surface – when the contact angle is higher than 90º, the surface is said not to provide good wettability for that liquid; on the contrary, the wettability is said to be good when the angle is lower than 90º (Mittal, 2015; Shafrin & Zisman, 1960). PDMS surface is hydrophobic, showing low wettability for polar solvents such as water and ethylene glycol (Kim et al., 2014). Indeed, it has been described how inks containing polar solvents do not spread on the PDMS and produce a line when patterned on PDMS. Instead, they create a sequence of isolated drops regardless of the drop spacing (Figure 3).

Figure 3. Components in thermal and piezoelectric (acoustic) inkjet printers (Murphy & Atala, 2014).
4) or fuse together forming a bigger drop due to the existent cohesive forces within the liquid when the drops get to contact each other.

Figure 4. PDMS surface hydrophobicity prevent single drops to fuse and create patterns (Kim et al., 2014).

Since the Pt-precursor ink is an EG-based solution, improving PDMS surface wettability is necessary in order to allow an optimal patterning. Several methods to increase PDMS wettability for polar solvents – that is, decrease its hydrophobicity – have been described. One of the most extensively applied methods is the exposure of the material to reactive plasma species, since it highly efficiently introduces polar functional groups on the PDMS surface without affecting the bulk material properties. Nevertheless, hydrophobic recovery of the surface over time has been reported (Alves et al., 2008; Chu, Chen, Wang, & Huang, 2002; Grace & Gerenser, 2003; Kim et al., 2014). Another method consisting on the chemical modification of PDMS surface with polydopamine has been suggested (Chuah et al., 2015; Lee, Dellatore, Miller, & Messersmith, 2007). Dopamine hydrochloride undergoes oxidative polymerization in alkaline conditions and shows a strong adsorption to the PDMS surface through covalent bonds and strong molecular interactions, improving PDMS wettability for water. Additionally, polydopamine coating resulted in a long-term improved cell attachment to PDMS, which suggest that the increased wettability is stable over time (Chuah et al., 2015). Besides, Hwa Tan and co-workers (2010) described a method – plasma power of 70W, treatment over 5 minutes – that would allow maintaining plasma-driven wettability of PDMS surfaces for 6h (or a week when the samples were maintained in deionized water in a vacuumed environment).

Dip-pen nanolithography (DNP) printing has been used to dispense H₂PtCl₆ into air plasma-treated silicon substrates followed by reduction of the Pt-precursor ink to Pt-nanoparticles. The resulting films were electrically conductive, although their conductivity was not quantified. Nevertheless, printing straight lines proved challenging, and they could only obtain single-dot successions that never contacted (O’Connell et al., 2013). More previous work done by our research group showed 1-layer solid patterns (squares) printed on polydopamine-coated PDMS using a Dimatix printer and reduced to Pt-nanoparticles. The electrical conductivity of the
resulting Pt films was 800 S/m (the conductivity of Pt metal is $9.43 \times 10^6$ S/m at 20 °C (Lazarev & Shaplygin, 1978; Serway & Jewett, 2015)). Printing other structures that could relate to the CI, such as parallel lines, was not attempted.

Taking all this into account, inkjet printing the Pt-precursor ink on PDMS substrates seems a potentially feasible method to create Pt conductive patterns that may substitute the currently used platinum/iridium (90/10) wires (Cochlear Ltd, 2018; Wallace et al., 2012) on the next generation CI. Despite the pattern conductivity being lower than the actual Pt metal, inkjet printing the conductive patterns would allow to customize the electrode to the patient’s specific cochlear anatomy and create smaller conductive features which would allow the inclusion of a higher number of these increasing the number of independent conductive channels. The high spatial control of the technique would allow creating more complex patterns than single parallel lines, resulting in active microchip-like circuits that would exponentially increase the complexity of the stimulus provided to the cochlea improving the auditory perception (Axisa et al., 2006).

Since very little material is deposited per layer, the thickness of the resulting conductive patterns is very low (0.03 µm, 0.044 µm, and 0.110 µm for 2, 3, and 7-layers patterns, approximately 0.015 µm per printed layer) as shown in previous work done by our research group. This may potentially decrease the overall production cost of the implant, since Pt is one of the most expensive metals available – the price of Pt is $906.50 per Troy ounce, more than 50-fold more expensive than silver, rated at $16.78 per Troy ounce as of June 8th 2018 (‘Bullion by Post UK’, n.d.).

To advance on the inkjet printing of Pt-precursor ink, we aimed to optimise the printing parameters in order to be able to print continuous, straight lines in different directions. Before this, an evaluation of the effect of air plasma and polydopamine coating on PDMS wettability over time was also performed in order to understand the most suitable surface treatment method for printing.

**Materials & methods**

**PDMS substrate fabrication**

Medical grade PDMS silicon elastomer and curing agent (provided by Choclea Ltd, AUS) were mixed in 10:1 (w/w) using a planetary centrifugal mixer (Thinky Mixer ARE-250, THINKY, Japan) via the following program: mixing, 30 seconds, 800 rpm; mixing, 2 minutes, 2000 rpm; defoaming, 1 minute, 2200 rpm. The liquid PDMS was poured into a glass petri dish and placed in the oven at 140 °C for 4h as indicated by the provider. The cured PDMS was then cut into pieces of required size.
*Pt-precursor ink preparation*

A 10% (w/w) \( \text{H}_2\text{PtCl}_6 \cdot \text{xH}_2\text{O} \) (254029, Sigma-Aldrich, US) solution in ethylene glycol was prepared and kept protected from the light.

*PDMS surface modification*

Two different substrate treatments were performed on PDMS films: air plasma exposure and polydopamine coating. PDMS films were immersed in polydopamine coating solution (0.5 mg/ml dopamine hydrochloride (H8502, Sigma-Aldrich, US) in 10mM Tris buffer, pH 8.5) for 12h and then rinsed with distilled water. A brown coating on the PDMS surface will be noticeable. Alternatively, PDMS samples were exposed to reactive air plasma species in plasma cleaner (Harrick Cleaner, PDC-32G-2, New York, USA). Air was pumped into the chamber to a stable pressure between 1000 and 1200 mTorr and the plasma switch was turned to “high” (18W) for 10 minutes. Plasma-treated samples were either kept in contact with air or submerged in distilled water until the sessile drop measurement took place (see next section).

*Wettability analysis of treated PDMS over time*

The wettability of PDMS substrates treated with polydopamine coating, air plasma, or air plasma followed by submersion in water was quantified by the sessile drop method at 0h, 1h, 2h, 3h, 4h, 5h, 24h, 48h, and 72h after treatment. Wettability for both water and Pt-precursor ink was evaluated. A conventional goniometer (Contact Angle System OCA, Dataphysics, Germany) equipped with a 20G blunt needle (inner diameter of 0.61mm) (Precision Tips, 7018169, Nordson EDF, US) was used to dispense 3µl single water drops on the treated PDMS substrate. The contact angle was calculated using the software associated with the instrument (SCA 20, Dataphysics, Germany). 6 drops were dispensed and analysed per time point and treatment group (n=6). This was repeated using the Pt-precursor ink instead of water. GraphPad Prism 7 (GraphPad Software, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) or two-way ANOVAs with post-hoc Tukey’s tests were performed for multiple comparisons. Data were expressed as mean ± SD, and \( p < 0.05 \) was considered statistically significant.

*Inkjet printing Pt-precursor patterns*

Inkjet printing was performed using a PiXDRO printer (PiXDRO LP50, Meyer Burger Technology Ltd, the Netherlands) equipped with a piezoelectric printing head (Spectra SL-128, Fujifilm, Japan). Spherical, Pt-precursor ink droplets were dispensed using the following printing parameters: 80V, -19.9 mbar, 1.5 µs/8 µs/1.5 µs nozzle-opening waveform. PDMS was used as substrate material. The printing height (“Z”) was set to 2.5 mm higher than the actual substrate thickness (measured prior to printing using a digital calliper). A single open nozzle was used to print the patterns.
Pt-precursor reduction to Pt nanoparticles

20 µl of Pt-precursor ink was drop-casted and reduced by either exposition to reactive air plasma species in a plasma cleaner – same procedure as stated in the “PDMS surface modification” section – or by heat, placing the samples on a covered hot plate at 160 ºC for 15 min. All the printed Pt-patterns were reduced by heat.

Imaging

An upright optical microscope (Leica M205 A, Leica Microsystems, Germany) equipped with a camera (Leica IC90 E, Leica Microsystems, Germany) and an scanning electron microscope (SEM) (JSM-6490LV, JEOL, Japan) were used to get morphological information of the printed patterns before and/or after reduction. The SEM operated in low vacuum mode at a pressure of 90 Pa. Back scattered electron imaging was done at 15 kV accelerating voltage with a probe current setting of 60 and the specimen placed at 10 mm working distance. Additionally, energy-dispersive X-ray spectroscopy (EDS) for elemental mapping of the samples was performed with the EDS detector integrated in the system (X-MAX 80 SDD, Oxford Instruments, UK). EDS mapping and image mounting was performed with the proprietary software associated to the machines (AZTEC, Oxford Instruments, UK). Measurements of different printed features (drop diameter, drop distance, line width…) have been done using ImageJ software (n=10) (Rasband, 2016; Schneider, Rasband, & Eliceiri, 2012).

Characterization of electrical properties

4-probe conductivity (RM3000 test unit & multi-height conductivity probe, Jandel, UK) and 2-point electrical-resistance measurements (287 True RMS Multimeter, FLUKE, US) were used to measure electrical conductivity of the samples.

Results and Discussion

Effect of surface coating on PDMS hydrophobicity over time

Polydopamine coating and air plasma treatment are two of the methods suggested to improve PDMS wettability for polar solvents (Chu et al., 2002; Chuah et al., 2015). Air plasma is most widely used for this purpose since it offers a good reduction in hydrophobicity in a rapid, straight-forward manner. Air contains oxygen, which oxidises methyl groups on the PDMS surface to hydroxyl groups that are highly hydrophilic (Waters, Finch, Bhuiyan, Hemming, & Mitchell, 2017). Nevertheless, this effect has been reported to be highly variable and unstable over time (Alves et al., 2008; Chu et al., 2002; Grace & Gerenser, 2003; Kim et al., 2014). Some researchers suggested exposing PDMS samples to air plasma at 70W for more than 5 minutes to obtain a more stable effect on wettability (Tan, Nguyen, Chua, & Kang, 2010). On the other hand, polydopamine is thought to offer a more constant wettability improvement (Chuah et al., 2015; Lee et al., 2007), but no actual studies of this effect over time have been found in the
Knowing which method resulted in the greatest wettability improvement – lowest contact angle – for the ink, and, more importantly, which method offered the most stable effect over time was necessary. For this purpose, sessile drop tests on PDMS surfaces modified with polydopamine coating, air plasma, and air plasma followed by submersion in distilled water (air plasma + W) at different time points were performed using water and Pt-precursor ink as the liquid deposited on the substrates. The effect of the different treatments on improving PDMS wettability was similar for both water and Pt-precursor ink. After surface modification (0h), polydopamine coating resulted in a contact angle reduction of approximately 50% when compared with the control group (untreated PDMS), while air-plasma resulted in a contact angle reduction of approximately 80% (Figure 5.A). These differences were statistically significant (p<0.001) and are shown in Figure 5.B and Figure 5.C. Consequently, air plasma offers the highest contact angle reduction, thus the highest improvement in PDMS wettability for both water and Pt-precursor ink.

<table>
<thead>
<tr>
<th>Liquid tested</th>
<th>Treatment</th>
<th>Contact angle (mean ± SD)</th>
<th>Contact angle decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Control</td>
<td>108.13 ± 3.71</td>
<td>–</td>
</tr>
<tr>
<td>Water</td>
<td>Polydopamine coating</td>
<td>58.6 ± 1.61</td>
<td>45.80%</td>
</tr>
<tr>
<td>Water</td>
<td>Air plasma</td>
<td>16.26 ± 6.72</td>
<td>84.95%</td>
</tr>
<tr>
<td>Water</td>
<td>Air plasma (+W)</td>
<td>16.63 ± 7.34</td>
<td>84.61%</td>
</tr>
<tr>
<td>Pt-precursor ink</td>
<td>Control</td>
<td>100.1 ± 1.85</td>
<td>–</td>
</tr>
<tr>
<td>Pt-precursor ink</td>
<td>Polydopamine coating</td>
<td>46.53 ± 6.02</td>
<td>53.51%</td>
</tr>
<tr>
<td>Pt-precursor ink</td>
<td>Air plasma</td>
<td>17.42 ± 3.88</td>
<td>82.60%</td>
</tr>
<tr>
<td>Pt-precursor ink</td>
<td>Air plasma (+W)</td>
<td>19.15 ± 2.89</td>
<td>80.87%</td>
</tr>
</tbody>
</table>

**Figure 5.** Effect of polydopamine coating and air plasma treatment on PDMS wettability for water and Pt-precursor ink. A reduction in contact angle (mean ± SD) translates to a higher wettability for the two different liquids. The decrease in contact angle right after treatment (t = 0h) is shown as the relative percentage change with respect to the control (A). The effect of
different treatments on PDMS wettability for water (B) and Pt-precursor ink (C) immediately after treatment (t = 0h) was compared (n=6, mean ± SD; *** p<0.001, one-way ANOVA).

Figure 6. Comparing the effect of polydopamine and air plasma on PDMS wettability for water and Pt-precursor ink over time. Higher wettability translates to lower contact angle and may change over time (A). The wettability for water (B) and Pt-precursor ink (C) was evaluated over
time and the effect of each treatment at 0h, 1h, 2h, 3h, 4h, 5h, 24h, 48h, and 72h was compared to the effect at 0h (n=6, mean ± SD; * p<0.05, ** p<0.01, *** p<0.001, two-way ANOVA).

When looking at how the reduction in contact angle changes over time, different trends were observed (Figure 6, Figure 7). Although the initial reduction in contact angle caused by the polydopamine coating was lower than the one caused by air plasma, it was more stable over time. Linear regression was performed on time-dependent contact angle data (Figure 7.D, Figure 7.H). The slopes (m in line equation y = mx + a) indicate the level of stability over time. The lower the slope is, the slower the increase in contact angle occurs, indicating a more stable effect over time. Air plasma treatment shows the highest slope, followed by polydopamine coating; on the other hand, air plasma treatment followed by keeping the samples in distilled water shows the lowest slope. Indeed, the reduction in water contact angle was stable for the first 24h, and higher contact angles indicating a partial loss of polydopamine-induced wettability did not appear until 48h after treatment. This increase in contact angle appeared at 24h in the case of the Pt-precursor ink. On the other hand, the improvement in wettability caused by air plasma treatment started reversing only 1 hour after treatment for water and 2 hours after treatment for Pt-precursor ink. Besides, the samples treated with air plasma and kept submerged in distilled water until the measurement was taken did show an improvement in the stability of the plasma-driven wettability. Although the contact angle significantly increased especially after 4h and 5h – for Pt-precursor ink and water respectively –, the average contact angle stayed lower until day 3 in contrast with the polydopamine-coating and air plasma counterparts. Razavi and Thakor (2018) suggested a combination of both methods, air-treating polydopamine-coated PDMS samples. They obtained a decrease in water contact angle of 35 ± 3% (p<0.05), which is lower to the decrease we obtained when using this two methods separately. Nevertheless, differences in equipment and measurement procedures may exist between their work and ours, therefore, this combination should be investigated further in our premises to be able to actually compare results in contact angle decrease. Moreover, the effect of this combined treatment over time must be evaluated.

These results differ from those reported by Tan and colleagues (2010), in which air plasma treatment led to an improved wettability that remained stable for 5h and up to 1 week when the samples were kept in distilled water in a vacuum environment. Their better result can be attributed to using higher power (70 W) and keeping the samples under vacuum in order to prevent any air bubbles to stay around the sample, while our treatment was performed at 18 W and the samples were not kept in a vacuum environment due to the facility constraints – although no air bubbles were observed. Higher power may result in a higher level of surface modification than lower power for the same duration of air treatment, therefore this difference must be considered as potential variation source.

Taking into account that PDMS samples would be used during the hours following treatment, a time span in which wettability is stable is needed to ensure the repeatability of the printed
patterns. For this reason, polydopamine coating is our preferred surface modification method to improve PDMS wettability for Pt-precursor ink. Besides, air plasma followed by water submersion seems noteworthy and further characterization following the suggested protocol (Tan et al., 2010) should be done in the future to assess its potential. Nevertheless, as it will be explained in the next section, such a large improvement in wettability would not be beneficial when printing patterns with high spatial resolution.

Figure 7. Behaviour of PDMS wettability for water (A, B, C) and Pt-Precursor ink (E, F, G) overtime after three different surface treatments. Contact angle was measured at 0h, 1h, 2h, 3h, 4h, 5h, 24h, 48h and 72h (n=6, mean ± SD; * p<0.05, ** p<0.01, *** p<0.001, two-way ANOVA). Linear regression of time-dependent data was performed (D, H).
**Reduction of Pt-precursor**

In order to observe the differences between reducing the Pt-precursor by exposition to air plasma or heat, Pt-precursor ink was drop-casted and reduced by either air plasma or heat (Figure 8). Reduced Pt appeared as grey, metallic films while unreduced Pt-precursor showed its characteristic amber colour (O’Connell et al., 2013). Heat seemed to be more efficient reducing the Pt-precursor than air plasma, since the material became grey and metallic and no amber residues were observable, as opposed to the plasma-reduced sample. The reduced areas conducted electricity, although quantifying the electrical conductivity was not possible due to the geometrical characteristics of the sample and its lack of homogeneity: as shown in figure 7, Pt films showed huge variations in density across the film surface, which affects the electrical conductivity of the film between different sets of two points. Taking all this into account, exposing the Pt-precursor ink to 160º for 15 min was the method chosen to reduce the printed Pt-precursor patterns.

![Figure 8](image)

**Figure 8.** Pt-precursor ink reduced by either air plasma (A) and heat (B). The metallic grey areas are deposited Pt-particles while the yellow areas correspond to unreduced Pt-precursor.

**Inkjet printing of Pt-precursor ink on PDMS substrates**

In order to be able to dispense ink droplets different voltage, pressure, and nozzle-opening waveform combinations were checked in order to find the set of parameters allowing the printer to dispense single Pt-precursor ink droplets. That set of printing parameters was a voltage of 80V, a pressure of -19.90 mbar, and a nozzle-opening waveform of 1.5 μs/8-10 μs/1.5 μs – all the prints shown were performed with a nozzle-opening waveform of 1.5 μs/8 μs/1.5 μs. In addition to this, the printing height (“Z” parameter on the printer) was optimized by printing patterns at different heights and comparing the printing fidelity. This “Z” parameter was set to 2.5 mm higher than the actual substrate height (measured prior to printing using a digital caliper).

Pt-precursor ink patterns have been created on PDMS and polydopamine-coated PDMS. A square design featuring straight lines in different directions (1 single layer, PDMS and polydopamine-coated PDMS) (Figure 9) and lines of 0.5 mm width (1, 3, or 5 superposed layers, only polydopamine-coated PDMS) or 50 μm width (1 single layer, only polydopamine-coated PDMS) were printed to assess printing fidelity along different axis, resolution, and electrical conductivity differences depending on the amount of ink deposited (the more layers, the more Pt-precursor present). All the images and measures included in this section were taken after reduction of the printed Pt-precursor patterns to Pt-nanoparticles. Due to technical issues
concerning the printer hardware and software, new files could not be loaded into the system and the straight lines and the square pattern showed in this work were the only available patterns to print. Otherwise, other designs more relatable to the CI electrode array would have been attempted.

Figure 9. Square Pt patterns with lines in multiple directions. The CAD file (A) was printed onto untreated (B) and polydopamine-coated (C) PDMS substrates and reduced. The Pt-precursor ink droplets stay individually when no surface modification is applied to enhance PDMS surface wettability for the ink resulting in a dotted Pt pattern (optical microscopy image) (B), while they extend and fuse creating a continuous pattern when the PDMS substrate has being coated with polydopamine (SEM image) (C). The bright circles appreciated on the optical microscopy image (B) correspond to air bubbles present within the transparent PDMS substrate and do not affect the homogeneity of the PDMS surface, as observed on the SEM image (C). Wrong “Z” values lead to faulty printed patterns (D, E).

The square pattern was printed onto both untreated PDMS and polydopamine-coated PDMS in order to assess the effect of polydopamine coating on the printed patterns. When the PDMS surface was uncoated and its wettability for Pt-precursor ink was low, the single dots were observed. The dots had a diameter of 39 ± 3 µm and were separated 100 ±12 m (measured from the centre; n=10). On the other hand, the ink droplets extended and fused to create a continuous Pt pattern when the PDMS was coated with polydopamine. The lines of the resulting pattern were 168 ±23 µm width (n=10), which is approximatively 4 times higher than the droplet diameter on the uncoated PDMS samples. Therefore, the improvement in PDMS wettability for
Pt-precursor ink necessary for printing homogeneous patterns leads to a loss of spatial resolution – as observed on the SEM image in Figure 9.C, where the lines on the left side fuse creating a solid triangle. For this reason, a different surface modification method that leads to a higher PDMS wettability than polydopamine coating such as the plasma air treatment evaluated on the previous section would result also in a greater loss of spatial resolution and, hence, is not recommended. Moreover, resolution could be improved by using a printed head with smaller nozzles, which would result in the deposition of smaller ink volumes that would extend less on the surface.

The printed lines were imaged by SEM (Figure 10) and the actual width of the “0.5 mm width” Pt lines was measured. Then, the increase in width with respect to the expected 0.5 mm width was calculated (Figure 10.D). All 1-, 3-, and 5-layers lines were wider than designed. Indeed, the number of layers printed have a significant effect on the width and how it increases. While no significant differences were observed between the 1- and 3-layers lines, they were found when printing 5 layers, suggesting that the greater the number of printed layers, the greater the effect. This is due to the fact that the more ink is deposited, the more area it covers when it extends on the PDMS surface.

![Figure 10. SEM images of Pt printed lines on polydopamine-coated PDMS. 1-, 3-, and 5-layers lines (from bottom to the top) (A) show different opacity due to differences in the amount of Pt deposited. A magnified detail of a 3-layers line (B) shows cracking on the printed pattern. And](image-url)
EDS image (C) of the same magnified area maps the presence of Pt (Pt M series). Actual width of printed lines and width increase with respect to the expected 0.5mm width (on polydopamine-coated PDMS) (D). The number of layers printed has an effect on the final width, the 5-layers lines being wider than its counterparts (n=12, mean ± SD; *** p<0.001, one-way ANOVA).

When the printed “50 µm width” lines were imaged (Figure 11), something unexpected was observed. The resulting pattern was not a line, but two parallel sequences of dots. The dot diameter was 67.5 ± 5.2 µm, close to the intended 50 µm width of the lines; and the sequences were 215.71 ± 9.15 µm apart. Additionally, the dots on both sequences were not aligned but they seemed to be intercalated. This suggest that the two sequences should have been printed aligned, so the intercalated dots would have created a continuous line with a width of 67.5 ± 5.2 µm (the dot diameter). Notice that this had not been observed when printing the square pattern or the other lines. Importantly, the lines on the square pattern were created by single sequences of dots (appreciable on the uncoated PDMS substrate) that extended and fused into a line (Figure 9). No complete explanation was found for this occurrence. As mentioned before, the printer used was malfunctioning which resulted in a multitude technical issues and this could simply be just a result of an operation error affecting this particular CAD file. Since the other lines were wider, more ink had to be dispensed and perhaps that promoted the extension and fusion of the droplets, explaining why it was not noticeable on those.

**Figure 11.** SEM image of a printed “50 µm width” line on polydopamine-coated PDMS. Instead of this, two parallel sequences of dots were obtained. The dots have a diameter of 67.5 ± 5.2 µm and both sequences are 215.71 ± 9.15 µm apart (mean ± SD).

Besides, the resulting Pt films were not even but they were cracked (Figure 10). Previous research shows how Pt films are composed by individual conducting Pt plates surrounded by non-conductive small non-reduced H₂PtCl₆ crystals(O’Connell et al., 2013), which explains the cracking observed. A 4-probe conductivity measurement was attempted, but due to the softness of the PDMS substrate, the electrodes penetrated the material and the conductivity could not be measured. Additionally, due to the small size of the printed features and the lack of visibility around the electrode setup, placing the 4 electrodes on the printed lines was very difficult. For
this reason, a 2-point resistance measurement using a multimeter was performed. Unfortunately, the printed patterns were not conductive. Despite the cracking being a plausible reason for this lack of conductivity, drop-casted Pt films with the same heterogeneous disposition are known to be conductive (O’Connell et al., 2013). The lack of conductivity of our samples may be due to the lower amount of Pt deposited by inkjet printing in comparison with drop-casting which, added to the cracking feature may result in conductive Pt plates that do not contact properly with each other. Additionally, H₂PtCl₆ is highly corrosive to metals (National Center for Biotechnology Information, n.d.) and it might have reacted with some of the metallic compounds within the printhead, which could have changed the overall ink composition and affected the formation of Pt-nanoparticles. The loss of conductivity of the resulting films completely hampers the use of inkjet printed Pt-nanoparticle patterns for any type of electrode and should be evaluated in detail to find the cause of the problem and a solution to overcome it.
Chapter 3

3D Printing of rLCGO/PDMS Coaxial Fibres

Introduction
In the previous chapter, we explored inkjet printing of Pt-precursors in order to obtain conductive patterns in situ. Nevertheless, conductivity of the patterns was not achieved. In this chapter, a different approach using an already conductive material was investigated: 3D printing of coaxial structures having conductive graphene fibres as core and insulating PDMS as outer layer. This structures resembled better the structure of electronics.

Electronic components are made of both conductive and insulating materials that are characterized by their strength and durability, in order to improve the resistance and extend the life-span of the devices they are integrated in. Nevertheless, when designing electrodes for clinical implantation softer, more flexible structures are desired, since they adapt better to the surrounding anatomy reducing the risk of injury and improving the electrode function (Atala, Lanza, Thomson, & Nerem, 2008; Blau et al., 2011; Murphy & Atala, 2014; O’Connell et al., 2013). Indeed, glial scarring has been reported after implantation of classic, rigid electrodes in the brain, which not only led to neuronal damage but also to the loss of the electrode function (Polikov, Tresco, & Reichert, 2005). Regarding the cochlear implant (CI), the flexibility of the final structure and the electrode array itself is crucial, since the implantation relies on this characteristic to make the electrode array adapt to the cochlea’s inner seashell-shape conduct while it is pushed through it (Clark, 2014; Clark et al., 1978; Wallace et al., 2012). For this reason, researchers are developing new conductive materials that are not only resistant to stress but also flexible (Agorelius et al., 2015; Apollo et al., 2015; Blau et al., 2011; Lind, Linsmeier, Thelin, & Schouenborg, 2010).

Graphene is a material consisting of a two dimensional, single-layer of sp²-hybridized carbon that creates a hexagonal pattern that resembles a honeycomb structure (Figure 12.A). Graphene is not only highly electrically conductive, mechanically resistant, and flexible but also biocompatible, what makes it a good candidate material to develop flexible electrodes for clinical implantation (Allen, Tung, & Kaner, 2010; Huang, Zeng, Fan, Liu, & Zhang, 2012; Sayyar et al., 2013). Oxidation of graphite to graphene oxide and its posterior reduction is currently the most commonly used method to obtain graphene (Gambhir, Murray, Sayyar, Wallace, & Officer, 2014). Graphene oxide can be dispersed in different solvents resulting in liquid crystalline graphene oxide (LCGO) dispersions, in which ultra-large graphene oxide nanosheets self-align with their longitudinal axis nearly parallel originating a nematic phase and exhibit an amphiphilic behavior (Figure 12.B) (Jalili et al., 2014). Particularly, LCGO can be wet-spun and reduced (rLCGO) resulting into highly resistant, conductive, and extremely flexible graphene
fibres (Figure 12.C, Figure 12.D) (Aboutalebi et al., 2014; Apollo et al., 2015; Jalili et al., 2013). Furthermore, graphene oxide has shown dose-dependent biocompatibility in different in vitro and in vivo studies, which can be assured by a complete reduction to graphene and successful coating (Kiew, Kiew, Lee, Imae, & Chung, 2016; Wang et al., 2011).

**Figure 12.** Molecular structure of graphene exhibiting a honeycomb-like structure (Papageorgiou, 2017) (A). Oriented graphene oxide sheets result in nematic liquid crystalline graphene oxide dispersions (Jalili et al., 2014) (B). SEM image of a wet-spun rLCGO fibre (C) and its cross-section showing different carbon sheets composing it (D).

Therefore, these fibres would not constitute an electrode on their own but would need a carrier material that would insulate them and protect them, while keeping them in place. As in the current CI, PDMS could be the carrier for such electrodes, due to its mechanical resistance, stability, biocompatibility, and flexibility. Moreover, the conductivity of these fibres could be further improved by coating them with biocompatible, highly-conductive metals such as Pt.

In order to create a rLCGO/PDMS hybrid construct that could be customized to the patient’s specific cochlear anatomy, 3D printing of coaxial rLCGO/PDMS composite fibres was explored. 3D printing is an automated fabrication process consisting in the deposition of materials in a layer-by-layer fashion to create a 3D object from a digital file. 3D printing techniques are characterized by a high spatial resolution that allows creating constructs with different morphological features with high accuracy (Malda et al., 2013; Zhang, Fisher, Leong, O’Brien, & Holmes, 2015). Extrusion printing is the most commonly used 3D printing technique. This method uses mechanical or pneumatic force to dispense continuous threads of materials through a nozzle in different directions along both X and Y axis (Figure 13.A). Additionally, the printer has mobility on the Z axis therefore multi-layered constructs can be printed (Murphy & Atala,
Unlike other 3D printing techniques such as inkjet printing, extrusion printing allows dispensing of high viscosity materials such as it is the case of PDMS (Bishop et al., 2017). Moreover, extrusion printing allows extruding coaxial structures when an adequate nozzle is used (Figure 13.B) (Cornock, Beirne, Thompson, & Wallace, 2014). Therefore, extrusion-based 3D printing was explored to produce coaxial structures made of rLCGO fibres and PDMS.

**Figure 13.** Extrusion-based 3D printing uses pneumatic and mechanical (piston, screw) forces to dispense continuous threads of material (Murphy & Atala, 2014) (A). Coaxial nozzle showing two different channels filled in with two different materials that concentrically converge resulting in the extrusion of coaxial structures (Cornock et al., 2014) (B).

A coaxial fibre is a cylindrical structure consisting of two or more different materials aligned concentrically along the fibre’s longitudinal axis (Cornock et al., 2014; Taylor, Beirne, Alici, & Wallace, 2017). Mimicking electric wires, the rLCGO/PDMS coaxial fibre would have a conductive core (rLCGO fibre) surrounded by an outer layer of insulating PDMS (Figure 14.A). The extrusion of such fibres next to each other and in multiple layers, where rLCGO fibres are arranged in parallel within a PDMS structure (Figure 14.B), would allow to fabricate a flexible, solid, conductive constructs with a multidimensional electrode array having the desired number of conductive channels.

A nozzle with two different channels that converge in one in a concentric way is necessary to extrude such coaxial fibres (Figure 13.B) (Cornock et al., 2014; Taylor et al., 2017). These channels are usually connected to a cartridge or barrel containing the different materials and attached to the printer’s extrusion system. In this approach, the only material that is extruded by the printer is the PDMS, the rLCGO fibre being pulled away by the PDMS flow due to adherence and friction forces and finally conforming the coaxial fibre. An adequate setup that includes a nozzle allowing the placement of a rLCGO fibre along the centre of the longitudinal axis of the coaxial fibre and ensures the attachment of the coaxial nozzle to the PDMS containing barrel and the printer has to be designed.
Furthermore, curing PDMS while printing might be necessary to make sure that PDMS retains its cylindrical shape to fabricate the required construct. For this, the use of a heated substrate and/or temperature-controlled jacket should be considered. Using a heated substrate to cure the PDMS upon contact may be preferred over heating the nozzle in order to avoid clogging. Nevertheless, warming up the PDMS in the barrel to a temperature below the curing threshold may be needed, in order to reduce the temperature difference between the material and the substrate and accelerate the curing process.

Taking all this into account, a setup that would allow 3D printing coaxial conductive fibres was designed and the printing process was optimized in order to obtain a 3D printed multilayer construct that was both flexible and conductive.

![Diagram of potential 3D printed coaxial construct for the CI. Coaxial fibres have a rLCGO fibre as conductive core and PDMS as insulating, outer layer. 3D printing these fibres would allow fabricating a flexible, solid construct with multiple parallel rLCGO fibres acting as an electrode array surrounded by PDMS.](image)

**Figure 14.** Potential 3D printed coaxial construct for the CI. Coaxial fibres have a rLCGO fibre as conductive core and PDMS as insulating, outer layer. 3D printing these fibres would allow fabricating a flexible, solid construct with multiple parallel rLCGO fibres acting as an electrode array surrounded by PDMS.

**Materials & methods**

**Materials preparation**

Medical grade PDMS silicon elastomer and curing agent (provided by Cochlea Ltd, AUS) were mixed 10:1 (w/w) on a planetary centrifugal mixer (Thinky Mixer ARE-250, THINKY, Japan) according to the following program: mixing, 30 seconds, 800 rpm; mixing, 2 minutes, 2000 rpm; defoaming, 1 minute, 2200 rpm.

Since the fabrication of rLCGO fibres is part of the unpublished work of a colleague, the detailed method cannot be disclosed. The fabrication of these fibres consists in injecting a LCGO dispersion in a saline coagulation bath that contains a reducing agent as well. This bath is kept at
an elevated temperature overnight. Then the fibres are hanged and let dry at room temperature. The platinization of the fibres is done by sputter deposition.

Coaxial nozzle and printing setup design

A coaxial nozzle having an upper vertical channel for the positioning of the rLCGO fibre and a channel inclined at 45 ° degrees for the PDMS flow was designed (Figure 15). Both the vertical and the inclined channel lead into a common vertical channel on the lower portion of the nozzle. The inclined channel would be fastened with a screw to a printing barrel containing the uncured PDMS and connected with the pneumatic pumping system of the 3D printer. The vertical, upper channel has been designed to fit and lock in place a 30G needle tip (0.15 mm inner diameter, 0.31 mm outer diameter, 12.7 mm length) (Precision Tip, 7018433, Nordson EFD, US). This tip would be attached to a 10 ml plastic syringe tube that would collect the rLCGO fibre by a Luer-Lok system. The rLCGO fibre would go through the syringe and the tip until its final location centred along the longitudinal axis of the nozzle’s lower vertical channel. When the PDMS is pumped, it will go through the nozzle and fill in the lower vertical channel, surrounding the rLCGO fibre and pulling from it, resulting in a coaxial fibre being extruded. In addition to this, a piece acting as an adaptor to attach the printing barrel containing PDMS to the printer in a 45° angle was designed. This adaptor allows holding the barrel within a temperature-controlled jacket in case heating the material is needed. The nozzles were printed in a high temperature resistant resin (High Temp Resin, Formlabs, US) using a stereolithographic 3D printer (Form 2, Formlabs, US) and following the default settings for such material. The piece to attach the barrel to the 3D printer was cut out from a plastic plank using a laser cutter (ILS12.150D, Universal Laser Systems, US). 3D models were designed using the 3D computer-aid design Solidworks (Dassault Systemes, France).

Figure 15. Sketch of the longitudinal section of the nozzle showing the internal architecture (A) and 3D model of the nozzle (B).
3D printing of rLCGO/PDMS coaxial fibres

Coaxial fibres were extruded through a printed nozzle using a manual pump (LEGATO 180 Syringe Pump, KdScientific, US) set at 100% force and manually patterned on a glass slide. A 5 ml syringe was used to contain the PDMS.

A KIMM SPS1000 Bioplotter pneumatic extrusion system (Korea Institute of Machinery and Materials, South Korea) was used in conjunction with a heating block on which the substrate (glass slide) was placed and heated to 120 °C. Proprietary KIMM Bioplotter software (Korea Institute of Machinery and Materials, South Korea) was used to generate the G-code of the different printing patterns (described in the “results and discussion” section). This G-code was manually modified to generate more complex features. A non-platinized rLCGO fibre was passed through a blunt 30G needle tip (0.15 mm inner diameter, 0.31 mm outer diameter, 12.7 mm length) (Precision Tip, 7018433, Nordson EFD, US) and then through a syringe tube that collected it. The syringe tube was attached to the needle tip and the whole was fitted on the upper vertical channel of a 3D printed nozzle (Figure 21.A). The used nozzles had an inner diameter of either 400 µm or 700 µm (Appendix 1: Sketches of the nozzles). The liquid PDMS was loaded into a stainless-steel barrel connected to the pneumatic pump of the printer in a temperature-controlled jacket. The nozzle was screwed to the end of the printing barrel. The printing pressure and velocity was optimised for each nozzle to deliver continuous and homogeneous coaxial fibres and was set at 200 kPa and 40 mm/min for the 700 µm nozzle and 400 kPa and 100 mm/min for the 400 kPa nozzle. The coaxial fibre was extruded onto an untreated, heated (120 °C) glass slide, secured to the heating block with Kapton tape.

Characterization of electrical, rheological, and morphological properties

Both uncoated and platinized rLCGO fibres were extended on glass slides and conductive silver paint (High Purity Silver Paint, SPI Supplies, US) was added at different points, dividing the fibre into various segments and creating an electrical contact (Figure 16). After drying, the electrical resistance of each fibre segment was measured using a multimeter (287 True RMS Multimeter, FLUKE, US). Each segment was imaged using an optical microscope (Leica M205 A, Leica Microsystems, Germany) equipped with a camera (Leica IC90 E, Leica Microsystems, Germany) to measure the segment length using ImageJ software (Rasband, 2016; Schneider et al., 2012). A total of 15 segments per fibre type were evaluated. This software was also used to measure the average diameter of fibres. Knowing the length and electrical resistance of each segment and the average fibre diameter, the average electrical conductivity (σ) of each type of fibre was calculated according to the following equation:

\[
\sigma = \frac{l}{R \times A}
\]
Where $l$ is the length of the segment, $R$ is the electrical resistance of the segment, and $A$ is the cross-sectional area of the fibre.

Both ends of the printed rLCGO/PDMS structures were clean cut with a scalpel to expose the fibre in the fibre section. Electrical resistance along the fibre was measured using the multimeter. Silver paint was applied at the ends of the fibre to create an electric contact but this contact did not always provide good conductivity measures due to poor attachment to the PDMS in the fibres. A Shimadzu mechanical tester (EZ-L, Shimadzu, Japan) was used to subject the 3D printed constructs to 100 cycles of bending to 30% of the construct length – measured from the end of the clamps that hold it in place. Electrical resistance of the constructs was measured before and after the test to evaluate the effect of mechanical bending on the electrical resistance of the construct.

![Image](image.png)

**Figure 16.** Optical microscopy image showing one fibre segment delimited by silver paint acting as electrical contacts.

PDMS rheological behaviour with respect to temperature was evaluated. Uncured PDMS was placed in a rheometer (AR G2 Magnetic Bearing Rheometer, TA Instruments) and subjected to a temperature ramp step from 25 °C to 150 °C (5°C/min) followed by a time sweep step at 150 °C for 10 minutes. The oscillation was set to at 1% strain. Storage ($G'$), loss ($G''$) moduli, and temperature were measured versus time.

An optical microscope (Leica M205 A, Leica Microsystems, Germany) equipped with a camera (Leica IC90 E, Leica Microsystems, Germany) and a reflex digital camera (EOS 700D, Canon, Japan) have been used to take images of the nozzles and the printed coaxial fibres. Measurements have been done using ImageJ software (Rasband, 2016; Schneider et al., 2012).

Graphpad Prism 7 (Graphpad Software, US) was used for statistical analysis. T-test was performed to compare the diameter and the electrical conductivity of rLCGO and Pt-rLCGO fibres.
Results and Discussion

Characterization electrical properties of rLCGO and Pt-LCGO fibres

The diameter, length and electrical resistance of rLCGO and Pt-rLCGO fibre segments were measured. Then, the electrical conductivity of the fibres was calculated.

Platinizing the fibre did not affect the final fibre diameter (Figure 17.A), but it caused a remarkable improvement in the electrical conductivity of the fibres (Figure 17.B) Indeed, the electrical conductivity increased significantly from 155.20 ± 20.35 S/m on the rLCGO fibres to 3575.75 ± 1168.89 S/m on the Pt-rLCGO fibres, a 23-fold increase.

In this way, platinizing the fibres appears as a great approach for increasing fibre conductivity. Other work on wet-spinning LCGO fibres reported a much higher native electrical conductivity of the rLCGO fibres, such as 2.5 x 10^3 S/m (Aboutalebi et al., 2014) and 2.5 x 10^4 S/m (Xu & Gao, 2011), however, their method of preparation was different from what was adopted to prepare fibres for this work. Aboutalebi and colleagues (2014) used acetone as coagulation bath and reduced the fibres by overnight annealing at 220 °C under vacuum, while Xu and Gao (2011) injected the fibres into a NaOH/methanol coagulation bath in a 1.5 MPa nitrogen environment and reduced them in a solution of hydroiodic acid at 80°C for 8h. Due to the higher electrical conductivity reported by Xu and Gao (2011), their method should be adopted in the future to produce high-conductive rLCGO fibres that could be used as conductive core for coaxial fibres. Additionally, platinizing those fibres to further increase their conductivity should be attempted and evaluated in order to try to obtain a conductivity closer to that of Pt metal (9.43 x 10^6 S/m (Lazarev & Shaplygin, 1978; Serway & Jewett, 2015)).

Figure 17. Characterization of rLCGO and Pt-rLCGO fibres. While platinizing the fibre did not affect fibre diameter (A), it did lead to a dramatic increase in electrical conductivity (B).
**PDMS rheology**

PDMS cures at high temperatures. The PDMS provider (Cochlear Ltd, Australia) recommended curing it at 140 °C for 4 to 5 hours, nevertheless other works reported different sets of temperature and time to cure PDMS, PDMS being cured between 60 °C and 80 °C for 30 to 120 min (Blau et al., 2011; Ding et al., 2017; Liu & Choi, 2009). In order to study the effect of temperature on curing the PDMS, a dynamic rheological test was performed. The test consisted in a temperature ramp step from 25 °C to 150 °C followed by a time sweep step at 150 °C (Figure 18). Shear storage ($G'$) and shear loss moduli ($G''$) were measured during the test. $G'$ measures the stored energy by the material when deformation occurs, representing the elastic portion of the material, while $G''$ measures the energy dissipated as heat, representing the viscous portion of the material (Meyers, Marc. Chawka, 2007). $G'$ surpassed $G''$ when the temperature reached 108 °C ($t = 16.5$ min), indicating that PDMS shows an elastic, solid-like behaviour when the temperature is higher than this. $G'$ and $G''$ kept increasing until the temperature is 124 °C ($t = 20$ min), and then reached a plateau. Indeed, $G'$ showed a minimal steady rise over time, while the temperature kept rising, suggesting that times longer than 20 min are needed to reach a fully cured state. Concerning the printing process, the extruded PDMS on the coaxial fibres needs to be cured before a second layer is printed. Taking into account the obtained results, printing on a hot plate heated at 120 °C or higher and waiting 15 minutes may well be sufficient to allow printing a second layer on top. The final construct should be placed in an oven at temperatures higher than 125 °C for some hours to ensure a complete curing.

**Figure 18.** Temperature dependence of PDMS rheological behaviour. The dynamic test comprised a temperature ramp step from 25 °C to 150 °C (5 °C/min) (0 – 25 min) followed by a 10-minutes time sweep step (25 – 35 min). $G'$ and $G''$ were measured over time. $G'' > G'$ at $t = 0$ min meaning that PDMS shows a viscous behaviour. $G'$ becomes larger than $G''$ at $t = 16.5$ °C, when the temperature is 108 °C, therefore PDMS shows an elastic behaviour at temperatures above that.
Coaxial nozzle fabrication and extrusion of rLCGO/PDMS fibres

Coaxial printing nozzles were designed and produced using a stereolithography 3D printer (Figure 15, Figure 19). As mentioned in the “materials and methods” section, the nozzles had an upper vertical channel that fitted a 30G needle tip which would allow the rLCGO fibre to pass through it and be positioned in the lower vertical channel in a concentric manner; and a 45° inclined channel through which the PDMS would flow. Both channels converged (Figure 19.B, Figure 19.C) to create the lower vertical channel in such a way that the flowing PDMS would surround the rLCGO and pull from it along the channel, resulting in the extrusion of a fibre consisting of a PDMS outer layer and a concentrically aligned rLCGO fibre (Figure 14). The diameter of the orifice at the end of the lower vertical channel was 350 µm, a bit smaller than the expected 400 µm. The nozzles had an inner diameter of either 400 µm or 700 µm gauge (Appendix 1: Sketches of the nozzles). In order to achieve the concentric alignment of the rLCGO fibre, the upper and lower vertical channels must share the same longitudinal axis. Microscopy images taken from the bottom of the nozzle show how the vertical channels are perfectly open and aligned (Figure 19.D). Moreover, when the needle tip is inserted, its end is perfectly visible when focusing in a deeper plane in the bottom view (Figure 19.E).

**Figure 19.** Image of one printed nozzle (A) and a partially sectioned nozzle with an inserted 30G needle tip showing the inner architecture (B). Optical microscopy image of the sectioned nozzle’s lower section showing the fusion of the vertical channel – for the rLCGO fibre – and the inclined channel – for the PDMS (C). Bottom view of the nozzle showing the vertical channel open (optical microscopy) (D). Bottom view of a nozzle with an inserted 30G needle tip; the needle tip can be observed through the nozzle’s channel when focusing a deeper plane (E), what ratifies the perfect alignment of the lower vertical channel with the needle tip.
The nozzle has a bi-layered structure (Figure 19.B) that creates an inner air chamber that isolates the PDMS flowing along the channels from the environmental temperature. This may have two opposite goals. When using a hot substrate, the heat irradiates towards the nozzle and could cure the PDMS in the nozzle tip. In this case, the isolation chamber would protect the PDMS from overheating. On the other hand, a thermal jacket may be used to warm up the PDMS within the barrel to temperatures below the curing point (e.g., 50 °C) to reduce the temperature difference between the printed PDMS and the hot substrate and promote a faster curing process. In this situation, the isolation chamber would help keeping the PDMS at a constant temperature from the moment it leaves the warm barrel until it is printed on the hot surface, isolated from both the colder room air around the nozzle and the irradiated heat to the tip.

As a proof of concept, a manual pump –directly controlled by the user, contrary to an extrusion printer which is controlled by a computer– was used to extrude coaxial fibres on a glass slide. A non-platinized rLCGO fibre was passed through the needle tip and positioned within the vertical channel of a 700 µm diameter nozzle. A syringe containing PDMS was attached to the nozzle’s inclined channel and connected to the pneumatic pump. Coaxial fibres were successfully deposited manually on a glass slide (Figure 20), confirming the possibility to create rLCGO/PDMS coaxial fibres using an adequate coaxial nozzle and an extrusion system.

![Figure 20. Manually placed rLCGO/PDMS coaxial fibres using a extrusion pump.](image)

When depositing any material, high spatial control and an adequate printing velocity are fundamental to obtain a good, reproducible result. Placing the lines by hand, as if drawing, does not allow controlling these parameters and for this reason the resulting coaxial structures did not show good linearity or concentricity. Using an extrusion 3D printer to extrude such fibres could solve this issue and result in homogeneous fibres with a good concentricity. Additionally, the PDMS extended on the surface quickly, resulting in flat, wide structures. This could be solved by curing the PDMS while it is extruded. Nevertheless, curing within the nozzle must be avoided since solid PDMS could not be cleaned out of the nozzle due to its inner architecture. For this reason, extruding the fibres on a heated substrate that cures the PDMS upon contact must be
implemented. As explained on the previous section, a substrate heated to 120 °C should be enough to cure the fibre.

3D printing rLCGO/PDMS coaxial structures

Non-platinized rLCGO fibres were used for all the printing optimization described in this work since they are easier and cheaper to fabricate. Platinized fibres should be introduced once the printing is optimized to obtain constructs with high electrical conductivity. The components needed to 3D print the rLCGO/PDMS coaxial fibre were assembled as shown in Figure 21. The hot plate was heated at 120 °C and the thermal jacket was deactivated. Its presence helped keeping the PDMS within the barrel at room temperature and prevented it from heating due to the heat irradiated from the hot plate.

Figure 21. Printing setup. The rLCGO fibre (1) is passed through the needle tip (2) and collected in a syringe tube (3) attached to the tip (A). The loose end of the fibre is passed through the nozzle (4) vertical channel by inserting the needle tip into it. The cartridge attaching system is positioned in a 45 ° angle thanks to the printed adaptor piece (5) (B). The nozzle (4) is attached to the metal barrel (6) that contains the PDMS (C). This barrel is connected to the printer pneumatic system (7) and placed within a thermal jacket (8) in case warming up the material is necessary. The substrate (9) is placed on a hot plate (10).

Both 700 µm and 400 µm diameter nozzles were used to print individual, straight lines. Coaxial fibres were not achieved at first, since the PDMS flowed but it did not pull the rLCGO fibre out enough and it dragged within the printed PDMS line. Shear stress within the printed coaxial fibre should help keeping the rLCGO fibre in place and pull from the fibre that is in the nozzle as it moves, but high printing velocity may overcome this effect and drag the printed rLCGO fibre.
Different sets of extrusion pressure and printing velocity were tried until one was found that allowed producing coaxial fibres. When the printing velocity was too slow and/or the pressure too high, the rLCGO was extruded with the PDMS but too much PDMS accumulated along the fibre causing a loss of spatial resolution. On the other hand, when the printing velocity was too high and/or the pressure too low, a thin, homogeneous PDMS line was obtained but the fibre was not extruded at a correct rate and dragged along the printed PDMS. Good coaxial fibres were obtained at 200 kPa and 40 mm/min for the 700 µm diameter nozzle and 400 kPa and 100 mm/min for the 400 µm diameter nozzle (Figure 22). The diameter of these fibres was 3.44 ± 0.58 mm for the 700 µm diameter nozzle and 1.28 ± 0.08 µm for the 400 µm diameter one. Since the smaller the coaxial fibre, the higher the spatial resolution, the 400 µm diameter nozzle offered better results than the 700 µm diameter counterpart and was the design used from then on.

When a line was finished, the fibre was cut with small scissors to be able to print a new, independent line. During this process, the fibre was sometimes pulled, what resulted in the rLCGO fibre slightly dragging towards the end of the print, the beginning of it being composed by PDMS only (Figure 22.B). The lines quickly started curing on the slide. After the print was finished, the slide was placed in the oven at 120 ºC for 3h. After this, the fibres cooled down and the ends were cut clean using a scalpel to remove any PDMS at the beginning of the fibre (when dragging occurred) and to obtain a section of the coaxial structure where the rLCGO fibre was exposed (Figure 22.A). Exposing the rLCGO fibre is important to measure the electrical resistance along it. Since rLCGO fibres had been extruded through PDMS, its surface was always covered by a thin, insulating PDMS layer. A clean, conductive surface can be obtained by cutting the fibre.

Although the rLCGO fibres never broke during the printing process, internal changes in structure due to shear stress along the metallic needle tip may have occurred. Therefore, the electrical conductivity of the printed fibres before and after printing was analysed in order to evaluate if the rLCGO fibre structure was intact. If the fibre were somehow damaged, its conductivity would be highly compromised. Silver paint was placed on the section of the coaxial fibre to create an electric contact that allowed measuring the electrical resistance along the printed structure. The length of the fibre was measured and the electrical conductivity calculated as explained on the previous section. The resulting electrical conductivity was 153.30 ± 21.20 S/m, very similar to the 155.20 ± 20.35 S/m exhibited by the native rLCGO fibres. This suggest that the printing did not affect the fibre integrity and conductivity.

In order to replicate the architecture of the current CI, 3D printing a construct consisting of rLCGO/PDMS coaxial fibres arranged in such a way that they create a PDMS prism with parallel conductive rLCGO fibres running along its longitudinal axis (Figure 14) should be attempted. Such a simple structure is not a challenge by itself when extruding liquid polymers.
The difficulty arises when printing a coaxial structure containing a solid, rLCGO fibre. As shown in (Figure 22.C), the rLCGO fibre drags towards the centre of the structure when the direction of printing is inverted. This could be due to changes in the direction of the stress vectors acting on the fibre (mainly shear stress within the printed PDMS structure and nozzle moving and pulling from the fibre). The velocity at which the corners are printed was reduced up to 20% compared to the original, but no improvement in regard to dragging was observed.

![Figure 22.](image)

Figure 22. Printed rLCGO/PDMS coaxial fibres through the 700 µm (A) and 400 µm (B) diameter nozzles. The end of the fibres was cut clean (A) to eliminate any defect due to dragging of the rLCGO fibre (B) and to expose the rLCGO fibre on the section. 3D printed continuous rLCGO/PDMS fibre showing dragging of the rLCGO fibre in the corners (C). 3D printed continuous rLCGO/PDMS fibres with a circle of radius 3 mm at the corners. Dragging of the rLCGO fibre stopped after the first loop (D).

Although this issue deserves further characterization to find a way to stop it from happening, there are two ways that would allow mitigating the issue when it does. Firstly, printing much longer lines next to each other and cutting away the faulty ends after curing. Secondly, printing a corner displaying a higher radius, therefore making the change in direction to be less abrupt and more gradual and perhaps preventing the dragging from happening. The resulting loops at the corners would have to be cut out after curing as well; but if they were not too big, they may help saving rLCGO fibre and printing time. This is important because every time a new rLCGO fibre must be loaded a new clean nozzle must be used – since the previous one is full of PDMS, which prevents the rLCGO fibre from passing through it –, and because a single fibre must be used when printing a single layer in a straight run to ensure the continuity of the rLCGO fibre in the final coaxial structure. To assess how the placement of loops at the corners would affect the dragging of the rLCGO fibre, a circle with a radius of 3 mm was printed at the end of each line.
(Figure 22.D). Although dragging of the rLCGO fibre kept happening after the first loop, it stopped after the second loop. Even if the result was not optimal, this shows how creating such loops could prevent the dragging issue. To achieve this, further characterization of the phenomenon and optimization of the process to find the best combination of loop radius and printing velocity at the corners is needed. Knowing which radius is the smallest that can be printed with the rLCGO/PDMS coaxial fibre would also be beneficial. This could be evaluated by simply attempting to print circumferences of various radius with different printing velocities.

Due to the limited project time performing all these characterizations could not be achieved in this study. Nevertheless, obtaining a construct with more than one layer and several conducting rLCGO fibres that could be bended without them being damaged and affecting the electrical conductivity was a priority, since such a construct would constitute a proof of concept of a 3D printed flexible electrode using the rLCGO/PDMS coaxial approach. In order to be able to measure the electrical resistance along individual rLCGO fibres, these should project from the structure. The G-code that defines the printing process was modified to print 4 individual lines with a 1 mm separation – so they would contact each other and cure together into one solid structure) – and different lengths to create a stepped alignment at one end, resembling the CI structure in which the individual channels within the electrode contact the cochlea at different lengths. Two layers were printed, letting the first layer cure on the hot plate (120 ºC) for 15 min. The rLCGO fibre was cut and manually pulled out of the nozzle before and after printing each line to get some fibre to stand out of the printed structure. This manual process involved manually changing the position of the nozzle to have space to cut and pull from the fibre and repositioning again to print the next line, which affected the spatial disposition of the fibres and resulted in a poor morphology, especially on the second layer (Figure 23).

Measuring the electrical resistance along individual fibres was attempted but they did not conduct electricity. This was due to the fact that they had been pulled through PDMS, and even though the PDMS was “removed” using tweezers both before and after curing, it may well have left a thin film around the fibre. For this reason, the ends of the construct were cut clean using a scalpel so the clean fibres would be exposed on the section (Figure 23.B, Figure 23.C). In the future, portions of the fibres could be exposed at desired points on the construct’s surface using a laser cutter to remove with high control the PDMS on top. The section shows that fibres flattened upon contact with the substrate, keeping a semi-circular shape. Nevertheless this is not a crucial issue since the coaxial fibres contacted each other and created a single structure, which was the goal. The only way to prevent that flattening from happening would be curing the PDMS as it is extruded from the nozzle but that is highly likely to result in clogging of the nozzle. Nevertheless, warming up the material within the barrel would reduce the temperature difference between the extruded PDMS and the substrate, which would likely result in a faster curing process and less flattening of the fibre.
Measuring the electrical resistance along individual fibres was not possible due to the proximity between them, making it impossible to know which fibres were in contact with the multimeter electrode. For this reason, silver paint was applied all over the ends to measure the electrical resistance along the whole construct (8 conducting fibres). The resulting electrical resistance of the construct was 379.7Ω. In order to assess the quality of the contact between the silver paint and the fibres and obtain an even more accurate resistance value, the contact resistance between the silver paint and the fibres in the construct should be measured. Contact resistance refers to the percentage of the total resistance of a particular circuit attributed to two the connection between two contacting interfaces (Weis, Lin, Taguchi, Manaka, & Iwamoto, 2010). Nevertheless, since the rLCGO fibres were covered by PDMS, this could not be addressed.

Figure 23. Top view of a 3D printed two-layer rLCGO/PDMS construct with a stepped alignment of the fibres (A). Portions of the fibres stand out of the construct. Given the transparency of the PDMS, the perimeter of the construct has been marked in red to help visualizing. Top (B) and section (C) view of the 3D printed two-layer rLCGO/PDMS construct after cutting out the ends. Due to differences in light reflection across the curvy PDMS surface,
some of the rLCGO fibres cannot be properly visualized. 2 layers with 4 coaxial fibres each were printed. The morphology of the second layer was very poor.

In order to evaluate the flexibility of the construct and how bending affects the structure and therefore the conductivity of the construct, a bending test was performed using a mechanical tester. Measuring the electrical resistance of the construct online using the multimeter integrated in the mechanical tester system while the construct undergo many bending cycles was intended in order to align both electrical and mechanical information and see how the resistance changed with respect of the number of bending cycles. Silver paint was applied on the ends and they were wrapped in aluminium foil to create a contact where two alligator-style electrodes were clamped to apply current across the construct. Nevertheless, the silver paint did not stay attached to the construct when this one was manipulated or bended. The same happened when carbon-paste was used instead. Therefore, a successful electric contact that would allow to connect the multimeter to the construct was not achieved and the electric resistance could not be measured simultaneously with the bending test, but it had to be measured before and after the mechanical test.

![Bending Test](image)

**Figure 24.** Bending test. The construct underwent 100 cycles of bending.

The mechanical test consisted in 100 cycles of bending the construct by reducing the distance between the two clamps by 30% (Figure 24). Minimal changes in the relation between force and displacement occurred between cycles (the lines do not perfectly overlap), which means that deformation of the construct along cycles was marginal. This is not a critical issue towards its potential application to the CI since the electrode will only undergo deformation during implantation and stay in its position within the head. Silver paint was placed on the construct after the test and the electrical resistance was measured when the paint was dry. The electrical resistance of the construct was 351.8 Ω, not dramatically different to the 379.7 Ω obtained before
the bending test, therefore it can be concluded that the 100 cycles of bending did not damaged the rLCGO fibres nor it reduced the electrical conductivity of the construct. Nevertheless, if the measured electrical resistance was due mostly to contact resistance, small variations in the intrinsic resistance of the construct (8 fibres) would be overlooked. Therefore, finding a method that allows measuring such contact resistance must be achieved in the future, in order to obtain a more accurate value of the construct’s electrical resistance.
Chapter 4

Conclusions

We have explored two different approaches to produce biocompatible flexible electrodes that could be useful for the development of a new generation CI by 3D printing techniques.

The first approach consisted in inkjet printing a Pt-precursor ink (10% H₂PtCl₆ (w/w) in EG) on an insulating flexible PDMS surface to create a pattern that would be exposed to heat (160 ºC) to reduce the Pt-precursor to Pt-nanoparticles in situ. The resulting Pt patterns were expected to be highly conductive, so this approach could be used to print high resolution parallel lines or microchip-like structures that could become part of the CI electrode array. Reducing the wettability of the PDMS substrate for the Pt-precursor ink was necessary to allow the printability of the material, therefore the effect of polydopamine coating and air plasma treatment on PDMS wettability was evaluated. Polydopamine coating offered the most stable effect, ensuring a span of at least 5h in which the wettability of the material did not change hence allowing to print patterns on top in a repeatable manner. Additionally, since it did not result in a very high wettability improvement, the loss of printing resolution due to the ink extending more on the PDMS surface was minimized. Although this approach might be feasible in the future, we encountered a great challenge: the lack of electrical conductivity of the resulting printed patterns. Further morphological, chemical, and electrical characterization of the resulting reduced Pt patterns will be needed to understand the reason of this and be able to solve it.

In order to avoid such an issue again, we decided to use a material that was already conductive in our second approach. This method mimics more the actual CI structure since conductive rLCGO fibres were arranged within an insulating PDMS frame creating a flexible conductive construct. We designed a coaxial nozzle that allowed us to 3D print coaxial fibres displaying a conductive core (rLCGO fibre) and an insulating outer layer (PDMS). Although printing layers in a single run needs more optimization, a prototype construct having two layers with four parallel rLCGO fibres each was created and used to show how bending it does not affect its electrical properties. The fibres used for the development of the printing process were not highly conductive, although their conductivity could be dramatically increased by platinization, as shown in this work. Nevertheless, other rLCGO fibres exhibiting higher conductivity could be used instead. The data shown in this work is still very preliminary, but promising. Optimization of the 3D printing process must be the next step towards the development of this technology for the CI.
Bibliography or List of References


Bullion by Post UK. (n.d.).


https://doi.org/10.1017/CBO9781107415324.004


Appendices

Appendix 1: Sketches of the nozzles

![Sketch of the nozzles](image-url)
KIMM Coaxial Part Intersect - 700um needle