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Electrokinetic devices from polymeric materials

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Cover: Mandalas with different tools used and parts designed and constructed during my time as a PhD student. Design: Katarina Bengtsson

Abstract

There are multiple applications for polymers: our bodies are built of them, plastic bags and boxes used for storage are composed of them, as are the shells for electronics, TVs, computers, clothes etc. Many polymers are cheap, and easy to manufacture and process which make them suitable for disposable systems. The choice of polymer to construct an object will therefore highly influence the properties of the object itself. The focus of this thesis is the application of commonly used polymers to solve some challenges regarding integration of electrodes in electrokinetic devices and 3D printing.

The first part of this thesis regards electrokinetic systems and the electrodes' impact on the system. Electrokinetic systems require Faradaic (electrochemical) reactions at the electrodes to maintain an electric field in an electrolyte. The electrochemical reactions at the electrodes allow electron-to-ion transduction at the electrode-electrolyte interface, necessary to drive a current at the applied potential through the system, which thereby either cause flow (electroosmosis) or separation (electrophoresis). These electrochemical reactions at the electrodes, such as water electrolysis, are usually problematic in analytical systems and systems applied in biology. One solution to reduce the impact of water electrolysis is by replacing metal electrodes with electrochemically active polymers, e.g. poly(3,4-ethylenedioxythiophene) (PEDOT). Paper 1 demonstrates that PEDOT electrodes can replace platinum electrodes in a gel electrophoretic setup. Paper 2 reports an all-plastic, planar, flexible electroosmotic pump which continuously transports water from one side to the other using potentials as low as 0.3 V. This electroosmotic pump was further developed in paper 3, where it was made into a compact and modular setup, compatible with commercial microfluidic devices. We demonstrated that the pump could maintain an alternating flow for at least 96 h, with a sufficient flow of cell medium to keep cells alive for the same period of time.

The second part of the thesis describes the use of 3D printers for manufacturing prototypes and the material requirements for 3D printing. Protruding and over-hanging structures are more challenging to print using a 3D printer and usually require supporting material during the printing process. In paper 4, we showed that polyethylene glycol (PEG), in combination with a carbonate-based plasticizer, functions well as a 3D printable sacrificial template material. PEG2000 with between 20 and 30 wt% dimethyl carbonate or propylene carbonate

have good shear-thinning rheology, mechanical and chemical stability, and water solubility, which are advantageous for a supporting material used in 3D printing.

The advances presented in this thesis have solved some of the challenges regarding electrokinetic systems and prototype manufacturing. Hopefully this will contribute to the development of robust, disposable, low-cost, and autonomous electrokinetic devices.

Populärvetenskaplig sammanfattning

Polymera material finns överallt omkring oss; våra kroppar är uppbyggda av dem, plastpåsarna och burkarna vi förvarar vår mat av består av dem, våra kläder och andra ting som finns i vår vardag är uppbyggda av olika typer av polymerer. En polymer är uppbyggd av en repetitiv sekvens av identiska grupper, de kan liknas vid en mönsterrapport vilken är den minsta del som man behöver repetera för att få mönstret. Beroende på hur rapporten ser ut så förändras utseendet av mönstret. Hos en polymer påverkar sammansättningen av den repetitiva gruppen (rapporten) egenskaperna av materialet och polymerer kan vara allt från hårda och robusta, till flexibla och elektriskt ledande. Arbetet som presenteras i den här avhandlingen berör hur funktionen av olika system påverkas av att man använder sig av polymerer istället för konventionella material.

Första delen av avhandlingen handlar om integrering av elektronik i system som innehåller vätska. När vätskor, laddade partiklar, molekyler och joner rör på sig på grund av ett yttre elektriskt fält, så kallas detta för elektrokinetik. Detta kan användas för att pumpa vätska i kanaler som är mindre än 0.2 mm, genom så kallad *elektroosmos*, samtidigt kommer molekyler med olika laddning att börja separera, så kallad *elektrofores*. Elektroosmos används inom t.ex. analytisk kemi för injektion och transport av vätskor. Elektrofores används inom bl.a. rättsvetenskap och molekyllärbiologi för att separera makromolekyler, så som DNA och proteiner, med avseende på deras storlek och laddning. I dessa system använder man sig oftast av metallektroder.

När en spänning läggs till ett par metallektroder som är i kontakt med vatten kommer den huvudsakliga reaktionen att vara spjälkning av vatten, så kallad vattenelektrolys. Spjälkning av vatten innebär att det bildas väte- och syrgas samt att pH börjar ändras. Gaserna som bildas kan bryta kopplingen mellan elektroderna och därmed stoppar strömmen, så som sker när man drar ut sladden för t.ex. en elvisp. Förändringar i pH kan t.ex. påverka biologiska prover negativt, så som proteiners funktion och kan leda till celledöd, men kan också minska flödena en elektroosmotisk pump kan generera. Det finns flera olika sätt hur man kan hantera vattenelektrolys i system med metallektroder, så som användning av en pH-buffer. Arbetet i den här avhandlingen visar vad som händer om man ersätter metallektroder med elektriskt ledande plastelektroder. I detta fall har metallektroder ersatts av den elektriskt ledande polymeren PEDOT vilket resulterar i att , där man istället för generera gas och pH-

förändringar, så förflyttar man joner mellan elektroden och omgivande lösning. Ledande polymerer är billiga och enkla att tillverka vilket gör dem lämpliga för engångssystem.

I den här avhandlingen visas följande exempel där metallektroder ersatts av ledande plastelektroder: Gelelektrofores (separation av proteiner i en gel), (se paper 1), tyg som kan pumpa vatten (plan elektroosmotisk pump, se paper 2) och en kompakt pump som inte är större än ett kaffemått, som enkelt kan kopplas till befintliga sprutkopplingar och som kan användas för att kontrollera flödet över t.ex. celler (se paper 3).

Andra delen av avhandlingen handlar om 3D skrivare och hur materialval påverkar utskriften och designen. 3D skrivare är ett bra alternativ för att snabbt och billigt kunna producera prototyper och funktionella individanpassade objekt i varierande storlekar. 3D skrivare kan beskrivas som en avancerad spritsmaskin där material läggs lager på lager för att bygga upp det slutgiltiga objektet utifrån en datorgenererade 3D model. Detta förändrar helt hur man designar objekt och vilka möjliga strukturer och material man kan använda sig av jämfört än då man till exempel använder sig av svarv eller fräs för tillverkning. Det finns flera olika typer av 3D skrivare, t.ex. smältplastskrivare (den typ som man kan se i flertalet affärer idag) och den variant som använts i den här avhandlingen, en sprutbaserad. En sprutbaserad 3D skrivare kan hantera många olika typer av material så länge dessa kan fyllas i en spruta och tryckas ut genom en nål. Det färdiga resultat kan därmed bli mycket olika beroende på vilka material som använts.

Överhängande och utstickande strukturer kan vara komplicerade att skriva ut med en 3D skrivare. Utskrift av dessa strukturer kan underlättas genom att man skriver ut en temporär struktur i ett annat material, ett offermaterial. Offermaterialet fungerar som en mall eller stöd till det slutgiltiga objektet och tas bort (offras) när övriga delar av objektet är klara. I den här avhandlingen beskrivs hur ett offermaterial baserat på polyetylen glykol (PEG, vanligt förekommande i t.ex. schampo och läkemedel) och en mjukgörare kan anpassas för att fungera tillsammans med en sprutbaserad 3D skrivare (se paper 4) för att skriva ut strukturer från 0,2 mm och uppåt.

Arbetet i den här avhandlingen visar användningen av den ledande polymeren PEDOT i ett elektroforessystem och en elektroosmotisk pump. Detta kan förhoppningsvis underlätta utvecklingen av dessa system till att bli mindre, smidigare, snabbare och billigare. Den andra delen presenterar ett vattenlösligt, PEG-baserat material som kan användas som stöd till andra material i sprutbaserade 3D utskrifter för att underlätta tillverkningen av 3D utskrivna objekt.

Preface

This thesis is the result of my doctoral studies in the Transport and Separations Group, within the Division of Surface Physics and Chemistry at the Department of Physics, Chemistry and Biology at Linköping University between November 2011 and June 2017.

During the course of the research underlying this thesis, I was enrolled in Forum Scientum, a multidisciplinary doctoral program at Linköping University, Sweden.

Parts of the results are published in scientific journals and the introductory chapters are based on my licentiate thesis *Additive Manufacturing Methods and Materials For Electrokinetic Systems*, Linköping Studies in Science and Technology, Thesis No.1724 (2015).

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Included papers

Paper 1

Conducting polymer electrodes for gel electrophoresis

Katarina Bengtsson[†], Sara Nilsson[†], Nathaniel D. Robinson
PLOS ONE, 2014. doi:10.1371/journal.pone.0089416

Author contribution: A large portion of the experimental work including construction and evaluation of large PEDOT electrodes which were then incorporated into gel electrophoretic systems. Demonstrated separation equivalence with traditional methods.

Paper 2

An all-plastic, flexible electroosmotic pump

Katarina Bengtsson, Nathaniel D. Robinson
Manuscript

Author contribution: Planned, conducted and analyzed all experiments, including construction evaluation of the electroosmotic pump's characteristics. Wrote the majority of the manuscript.

Paper 3

A clip-on electroosmotic pump for oscillating flow in microfluidic cell culture devices

Katarina Bengtsson[†], Jonas Christofferson[†], Carl-Fredrik Mandenius, Nathaniel D. Robinson.

[†] These authors contributed equally to the work.

Manuscript

Author contribution: Planned, developed, and modified the electroosmotic pump to suit the integration and application of microfluidic system, conducted and analyzed experiments regarding the electroosmotic pumps' characteristics when integrating them with microfluidic devices. Wrote the main part of the manuscript

Paper 4

Plasticized polymeric sacrificial materials for syringe-based 3D-printing

Katarina Bengtsson, Jonas Mindemark, Daniel Brandell, Nathaniel D. Robinson
Submitted

Author contribution: Majority of experimental work including development of the sacrificial material, analysis of rheology and DSC measurements as well as testing the material's properties in the 3D printer and removal via dissolution. Structured manuscript.

Manuscripts not included in this thesis

Patterning highly conducting conjugated polymer electrodes for soft and flexible microelectrochemical devices

Alexandre Khaldi, Daniel Falk, Katarina Bengtsson, Ali Maziz, Nathaniel D. Robinson, Daniel Filipini, E.W.H. Jager

An internal control method to reduce variability in platelet flow chamber experiments

Kjersti Claesson*, Katarina Bengtsson#, Lars Faxälv*, Nathaniel D. Robinson#, Tomas L. Lindahl*

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Electroosmotic characterization of several membranes in an all-plastic fabric pump

Sara Ulloa Valero, Katarina Bengtsson, Nathaniel D. Robinson

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Abbreviations

DEC	Diethyl carbonate
DMC	Dimethyl carbonate
EDL	Electrical double layer
EOF	Electroosmotic flow
EOP	Electroosmotic pump
PC	Propylene carbonate
PEDOT	poly(3,4-ethylenedioxythiophene)
PSS	Polystyrene sulphonate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
PEG	Polyethylene glycol

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1 Introduction

Polymers are molecules in which a subunit, also called monomer, is repeated to form a polymer (this word is derived from the Greek, *polu* = many and *meros* = part). Polymers are all around us and can have very different functions and applications depending on what the monomers in the polymer actually look like. Polymers were viewed as non-conductive materials, useful for insulation, until 1977, when it was discovered that pi-conjugated polymers have conductive properties. It was demonstrated that conductance of the semi-conducting pi-conjugated polymer *trans*-polyacetylene could be improved when using halogens during production, which can be considered to be the start of the research field of conductive polymers.¹ Heeger, MacDiarmid, and Shirakawa were awarded the Nobel prize in chemistry in 2000 for their work on conducting polymers.

The focus of this thesis is centered on the use of well-known polymers in new applications, e.g. gel electrophoresis, 3D printing, and electroosmotic pumps. The use of alternative polymer materials in these systems aid in the development of smaller and more disposable units, something that currently proves challenging. The first part of this thesis concerns the electrochemical reaction at the electrodes in electrokinetic systems. Electrokinetic systems, further described in chapter 3, are systems where motion of liquids and charged species and particles are controlled by an electric field, frequently used in microbiology, biochemistry and analytical chemistry as a tool for analysis. One advantage with electrokinetic systems, is that they can be made very compact, as for example when used as electroosmotic pumps in microfluidic devices. They are easily controlled and parallelized, since they are operated using well-developed electronics. One challenge with electrokinetic systems is the electrochemical reactions at the integrated metal electrodes. The dominating electrochemical reaction is often water electrolysis (further described in chapter 2.1), which can negatively impact the function of the device, e.g. by disrupting flow due to trapped gas and changes in surface chemistry and damage of biological samples due to large pH changes. The impact of water electrolysis can be reduced by replacing the metal electrodes with electrochemically active pi-conjugated polymers, which alter the electrochemical reactions occurring at the electrodes, further described in chapter 2.2.

The second part of this thesis describes the influence the choice of material can have on 3D printing, specifically syringe-based 3D printing. Compared to more traditional manufacturing methods, e.g. milling, where material is removed to form the desired shape, 3D printing builds an object bottom-up, by adding materials layer-by-layer according to a computer generated 3D model, further described in chapter 4. Structures that are partially free-hanging with no contact to the printing table, (e.g. protruding and over-hanging structures) have been shown to be challenging to print using simple and cheap desktop 3D printers. To simplify the printing process and reduce the limitations of printable structures, a sacrificial material can act as a temporary support during the printing process and then be removed from the final object.

1.1 Aim

In short the aim of this thesis, was to investigate the potential use of certain polymers to simplify manufacturing and miniaturization of electrokinetic devices. The papers included in this thesis can briefly be summarized as follows:

- Replacing metal electrodes with conducting polymers to reduce the impact of water electrolysis in electrokinetic systems - see papers 1, 2 and 3.
- Implementing a modular electroosmotic pump to control flow in a cell-culturing device - see paper 3.
- A sacrificial material's impact on 3D printing - see paper 4.

2 Electrochemical reactions at the electrodes

An electrokinetic system is controlled and influenced by an electric field, as in the case of e.g., electrophoresis or electroosmosis, further described in chapter 3. The electric field is maintained by electrochemical (Faradaic) reactions at the electrodes, meaning that charge, e.g. electrons are transferred at the electrode-electrolyte interface. According to Faraday's law, the rate of electrochemical reaction at the electrodes is proportional to the electric current. These electrochemical reactions can generate by-products which can be problematic in electrokinetic systems. The predominant electrochemical reaction at the electrode-electrolyte interface can be influenced by the choice of electrode material, further discussed in papers 1 and 2. The following chapter will briefly describe the dominating electrochemical reactions in an aqueous solution that can dominate both at metal electrodes and when using conjugated polymers, and how these reactions impact electrokinetic systems.

2.1 Electrochemical reactions at metal electrodes*

A simple electrochemical cell consists of two electrodes immersed in an electrolyte, as seen in Figure 1. Oxidation (reaction at the anode) and reduction (reaction at the cathode) have to occur at the electrode-electrolyte interface to allow a continuous current through the cell and to maintain the electric field. The oxidation and reduction of different species at the electrode-electrolyte interface result in an electron transfer between the electrolyte and electrode. In water-based electrokinetic systems, the dominating electrochemical reaction is often water electrolysis (see table 1), which produces byproducts that can cause problems. The by-products change pH, which can alter surface chemistry, damage biological sample, and create gas bubbles that can disconnect the electrochemical circuit.

*The following chapter is based on chapter 2 and chapter 3 in my licentiate thesis: *Additive Manufacturing Methods and Materials for Electrokinetic Systems*, Linköping Studies in Science and Technology, Thesis No.1724 (2015). doi: 10.3384/lic.diva-121252

Figure 1 General electrochemical cell with metal electrodes immersed in electrolyte connected via e.g. a capillary. The coulombic forces ($\vec{f}$) on the ions in the electric field ($\vec{E}$) induces a motion of ions in the electrolyte towards the oppositely-charged electrode. The electric field in the electrochemical cell is maintained by electrochemical reactions (Faradaic reactions) at each electrode-electrolyte interface where O and R represent an oxidized and reduced species, respectively. Reduction occurs at the cathode (left-hand side) and oxidation at the anode (right). These half-reactions effectively transduce a current between the electronic and ionic portions of the electrochemical cell.

Water electrolysis, as seen in table 1 consumes water in multiple ways where the generated species can be detrimental to electrokinetic systems. The three first reactions in table 1 are the most common reactions for water electrolysis to occur. The oxidation of water at the anode is producing $O_{2(g)}$ and hydrogen ions (H^+ , protons) and at the cathode water is reduced yielding $H_{2(g)}$ and hydroxyl ions. Oxidation of water at a metal electrode, e.g. Pt, will have an onset potential of 1.229 V vs standard hydrogen electrode (SHE). The reaction at the cathode is usually reduction of hydrogen ions (that have dissociated from the water molecules) to hydrogen gas which is the reaction of the SHE (see table 1, half-reaction 2), which is defined as 0 V.² The potential difference between the half-reactions of oxidation and reduction of water defines the electrochemical stability window of the electrolyte (water). For water electrolysis to occur at two identical metal electrodes as shown in Figure 1, an applied potential of at least 1.23 V (across the system), but usually more is required to drive a Faradaic current through the system.

Table 1 Possible half-reactions occurring during water electrolysis.

	Reaction	E^0 vs SHE [V]
1)	$2 \text{H}_2\text{O} \rightarrow \text{O}_{2(\text{g})} + 4\text{H}^+ + 4\text{e}^-$	- 1.229
2)	$2 \text{H}^+ + 2\text{e}^- \rightarrow \text{H}_{2(\text{g})}$	0.000
3)	$2 \text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{H}_{2(\text{g})} + 2\text{OH}^-$	- 0.828
4)	$2 \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}_2 + 2\text{H}^+ + 2\text{e}^-$	- 1.763
5)	$\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O}_2$	0.695
6)	$\text{O}_2 + \text{H}_2\text{O} + 4\text{e}^- \rightarrow 4\text{OH}^-$	0.401

Water electrolysis in large electrokinetic systems, such as the gel electrophoresis setup in paper 1, are usually less problematic than in smaller systems, such as the electroosmotic pump in paper 2, since the generated byproducts are more easily handled. For example, the generated gas usually dissipates quickly in large systems, either through dissolution in the electrolyte or bubbles that effervesce into the surrounding (open) environment. In small systems, for example systems containing microchannels and capillaries, the generated gas is more likely to disconnect the electrodes from each other in for example, the form of a trapped bubble, separating the electrolyte into separate units. Large-scale electrokinetic systems also have room for generous amounts of buffered electrolyte to stabilize the pH of the system. As an example, changes in pH can disrupt the electroosmotic flow, since many materials' surface chemistry changes with the pH due to protonation of the channel surface. Maintaining a constant/physiological pH is especially important in electrokinetic systems used for biology and biochemistry. Many biological samples are influenced by fluctuations of the pH (acidic $\text{pH} < \text{pH} = 7.4 < \text{basic pH}$), where organisms have mechanisms to keep the pH stable around 7.4. Even small variations of the pH can have a huge impact on a biological samples, changing the proteins functionality and can cause cell stress, while more extreme pH (very acidic, $\text{pH} < 4$ or basic, $\text{pH} > 8$) can denature proteins or cause cell death. A protein's charge, in most cases depend on the surrounding electrolyte's pH. As seen in chapter 3.2.1, the

migration rate of a protein during electrophoresis depends on the protein's charge. Changes in pH can alter the migration speed, or even direction, of a protein through the gel — a property used in IEF setups (further described in chapter 3.2.1.2), while in other methods it negatively influences the separation, for example, by reducing the separation (resolution) of the sample's constituents. SDS-PAGE separations, as used in paper 1, are largely unaffected by changes in pH, since the proteins that are separated are already denatured by SDS, resulting in proteins with a negative net charge, independent of the charge of the native protein. However, electrolytic consumption of water (drying of the gel) and gas generation (reduction of active-electrode area and disruption of electrolyte-connection to the gel at the electrode-electrolyte interface) limit the minimum size of a SDS-PAGE. Paper 1 further discuss the impact of the electrode material on the gel electrophoretic setup.

2.2 Electrochemical oxidation and reduction of pi-conjugated polymers*

An alternative to metal electrodes are electrodes made out of pi-conjugated polymers, which are often themselves electrochemically active. This reactivity means that a Faradaic reaction other than electrolysis of water can occur at the electrodes, which in most cases result in reduced applied potential and produced byproducts. Briefly, a pi-conjugated polymer has a carbon backbone with alternating double- and single-bonds, see Figure 2. The neighboring pi-bonds (one of the bindings in a double-bond) partially overlap. The overlap allows electrons to move between different pi-bonds along the pi-conjugated backbone of the polymer. This conducting pathway conducts current within the polymer similar to the way a metal does. Throughout this thesis, the terms conductive polymer and conducting polymer are used interchangeably. There are several examples of conductive polymers for example polypyrrol, polyacetylene, and polythiophenes, specifically poly(3,4-ethylenedioxythiophene) (PEDOT), which can be seen in Figure 2.

* Parts of this section has previously been published in my licentiate thesis, *Additive Manufacturing Methods and Materials for Electrokinetic Systems*, Linköping Studies in Science and Technology, Thesis No.1724 (2015), doi: 10.3384/lic.diva-121252

Figure 2 Examples of conductive polymers and their chemical structures

Many conductive polymers are electrochemically active, meaning that they can be oxidized or reduced when a potential is applied and ions are present. The oxidation for a general pi-conjugated polymer follows reaction scheme 1, where P^0 is the neutral and undoped form of the conjugated polymer and X^- is a counter ion. Applying an anodic potential oxidizes the polymer into P^+ , which results in the formation of a complex with the counter ion, stabilizing the oxidized form of the polymer. Conducting polymers with such a positive charge are said to be *p*-doped. There are also polymers that can be reduced to have a negative net charge, *n*-doped, with a positive counter ion instead, although these are less common. Reduction follows the reverse path of the half-reaction 1.

The electrochemical reactions at the electrodes of an electrochemical cell with polymer electrodes will differ from an electrochemical cell with metal electrodes. A general schematic of an electrochemical cell with general pi-conjugated polymer electrodes can be seen in Figure 3. The dominating electrochemical reactions at the electrodes will usually be that of the polymer electrode, provided that the oxidation/reduction of the polymer occurs at lower potentials than electrolysis of water. The oxidation/reduction of the polymer results in ions moving between the electrolyte and polymer film in order to balance/neutralize the change in charge of the polymer (see Figure 3, where negatively charged ions move between electrolyte and polymer film). The cell potential of a cell with two identical polymer electrodes, in the same state, will be zero, since the dominating reduction at the cathode is the reversible reaction of the oxidation reaction at the anode (see Figure 3). A similar electrochemical cell with two identical metal electrodes instead (as described in chapter 2.1), will have a non-zero cell potential, since the electrochemical reaction at the cathode differs from that at the anode, as in the case of water electrolysis. An electrochemical cell with polymer electrodes will

therefore be able to generate a much higher current at potentials close to zero compared to an electrochemical cell with metal electrodes.

The transport of the counterion, between the electrolyte and electrode itself, during oxidation in half-reaction 1, shows that a conjugated polymer film can function as an electron-to-ion transducer. The transfer of electrons during oxidation/reduction leads to ion movement between a polymer film and an electrolyte (see Figure 3). The movement of ions in conjugated polymers can therefore be used as a delivery system of biomolecules or ions to e.g. cells in biology applications.³⁻⁵ The movement of ions also causes the polymer to swell, since the ions draw water into the film, which is applied in polymeric actuators.⁶⁻⁸

In addition to the ability to act as an electron-to-ion transducer, conducting polymers have other benefits when compared to most metal electrodes: they are flexible, cheap, easily manufactured and modifiable, and many are biocompatible.^{3,9,10} These properties make conductive polymers useful in various applications, e.g. solar cells, electrodes in microsystems, antistatic coatings, and actuators.^{6,11} Biological applications benefit from flexible materials that are less rigid than e.g. metals and have a consistency similar to tissue and cells. Methods for depositing conductive polymers are, e.g. solution processes and electropolymerization. Solution process deposition includes several techniques such as spray-painting, drop-casting, spin-coating, and printing such as inkjet, screen- and 3D printing.

Figure 3 An example of an electrochemical cell with polymer electrodes immersed in electrolyte.

Oxidation and reduction of the conducting polymer material, P, permits a current through the electrochemical cell. The polymer oxidizes at the anode where anions move in to the polymer film from the electrolyte, (see right-hand side of the picture) while the polymer electrode functioning as cathode is reduced and anions move into the electrolyte from the polymer film (see left side of the picture).

The conjugated polymer poly(3,4-ethylenedioxythiophene) (PEDOT) in combination with the counter ion polystyrene sulfonate (PSS) was used to replace metal electrodes in papers 1, 2, and 3 to reduce the amount of water electrolysis. PEDOT is a highly hydrophobic polymer, which is difficult to dissolve in most solvents. PEDOT is usually sold as an aqueous dispersion denoted PEDOT:PSS, where PSS stabilizes the dispersion.^{12,13} PEDOT:PSS is a frequently used polymer blend and the final films properties such as high conductivity and electrochemical stability in its oxidized state, (PEDOT⁺), biocompatibility^{3,9}, and electrochromic activity¹⁴⁻¹⁶. It is also relatively easy to process and manufacture.¹³ Oxidation of PEDOT is shown in half-reaction 2, where PEDOT⁰ is the neutral and reduced form of the conjugated polymer. M⁺ is any cation which can move between the polymer film and the electrolyte. Note that this cation serves the same functionality as the anion X⁻ shown in figure 3, but moves in the opposite direction. PEDOT electrodes initially consist of a mix of both doped and undoped polymer, allowing the electrode to function as either cathode or anode.

2.3 Limitations with integrated electrochemically active electrodes

As mentioned in the previous section and papers 1, 2 and 3, conducting polymer electrodes can replace metal electrodes in electrokinetic systems, and offer several advantages. However, the conducting polymer electrodes can only be used to limit water electrolysis to the extent that the system is designed to accommodate, i.e., such that the dominant electrochemical reaction is that of the polymer rather than water electrolysis. Conducting polymer electrodes have a limited charge capacity, which follows from the finite number of monomer units available for oxidation and reduction. A polymer electrode, e.g., consisting of PEDOT:PSS, electrode will initially consists of both charged and uncharged polymer chains, allowing the electrode to function as either anode or cathode. However, when all the electrochemically active groups in the polymer electrode are either oxidized or reduced, the polymer electrode will behave as an electrochemically-inactive metal electrode, driving a current through the circuit via another Faradaic reaction, e.g. water electrolysis, generating undesired byproducts (as described in chapter 2.1). Water electrolysis is reduced (or even eliminated) as long as there are polymer chains that can be oxidized and reduced. The columbic capacity of polymer electrodes is thus proportional to the mass of the pi-conjugated polymer. Exceeding this capacity, i.e., depleting the electrochemically active groups in the polymer electrodes, can be avoided through proper device design, and by tracking the transported charge through the system during operation. For example, in paper 1, the polymer electrodes were designed to contain enough PEDOT:PSS to handle a current of 1 mA for 20 minutes (i.e., corresponding to 1.2 C).

Conjugated polymer electrodes, particularly polythiophenes, are also subject to over-oxidation, which renders the polymer non-conductive.¹⁷ Over-oxidation describes an electrochemical process that oxidizes a polymer beyond the p-doped state. Once over-oxidized, the conjugated backbone is damaged, and its conducting properties vanish and cannot be regained. Generally, this occurs when an excessive potential is applied to the conductive polymer. Any electrokinetic system with polymer electrodes should therefore be designed in such a way that the potential drop across the polymer electrode and electrolyte interface is below that of over-oxidation. In the case of PEDOT, that would be below 1.5 V vs a Ag/AgCl electrode.^{15,18,19} It has also been shown a dependence between over-oxidation of PEDOT and pH of the electrolyte.²⁰ Apparently, PEDOT is more susceptible to over-oxidation when the electrolyte has a pH>10. However, PEDOT can still be used to apply potentials up to 250 V in a gel electrophoretic setup, as shown in paper 1, without being over-

oxidized, since the major potential drop occurred across the gel (presumably the largest resistance in the system) This results in that the remaining potential drop across the gel/electrode interface was smaller than the over-oxidation potential.

3 Electrokinetic systems

“Electrokinetics” refers to motion of a liquid, charged particles and/or ions induced by an electric field. The motion of charged species relative the liquid/stationary phase is called *electrophoresis* and the liquid motion is referred to as *electroosmosis*, both of which will be further described in coming sections.

3.1 Basic concepts regarding electrokinetic systems

In the context of this thesis, electrokinetic systems consist of electrodes connected via an electrolyte through, for example, a channel, porous structure, or gel (see Figure 1, p. 4). An applied potential creates an electric field, which drives a current through the system since the electric field induces a motion of ions, induced due to the Coulombic forces exerted on the charged species (ions).²¹ The Coulombic force f is proportional to the charge number (z) and the electric field ($\vec{E}$) as seen in equation (1), where e is the elementary charge.

$$\vec{f} = ze\vec{E} \quad (1)$$

In an electric field, all anions (negatively charged species) move towards the anode, which is the positively charged electrode, and all cations move toward the cathode.²² To maintain the electric field across the system, and thereby the current through the system, Faradaic (electrochemical) reactions have to occur at the electrolyte-electrode interface to transduce the ionic current (through the electrolyte) into an electric current (through the electrodes and electronic-conducting wiring, see Figure 1,p.4). This results in a transfer of charge, e.g. transfer of electrons, from the electrolyte to electrode by oxidizing or reducing some species at the electrode surface. Without Faradaic reactions at the electrodes, the ions would accumulate at the interface between the electrolyte and the electrode and shield the bulk of the electrolyte from the electric field.

Coming sections in this chapter will briefly describe the interfacial region between a solid surface immersed in electrolyte, electrophoresis (focus on gel electrophoresis), and electroosmosis.

3.1.1 Interfacial region

An electrokinetic system always has at least one interfacial region, a region where a charged solid surface is in contact with the electrolyte, for instance, the interface between the electrode and the electrolyte where oxidation or reduction occurs. A thin layer of ions forms at the surface of a charged solid in contact with an electrolyte, the so called electrical double layer (EDL) (see Figure 4).^{2,21,23,24} In the case of an electrode in contact with an electrolyte and when no Faradaic reactions occur, ions are attracted to the surface until they neutralize the surface net charge. The unbalanced ions in the EDL form a non-zero net charge density (ρ_E) compared to the electroneutral bulk, which result in an electric potential drop (φ_0) between the surface and the bulk of the electrolyte.

Figure 4 Schematic of the interfacial region of a negatively charged surface immersed in an electrolyte where the electric double layer (consisting on positively charged ions), described according to the Gouy-Chapman-Stern model.^{2,21,25} The Stern layer is a single layer of ions that are strongly adsorbed to the surface. Just outside the Stern layer, a diffuse layer of ions forms to further neutralize the surface charge density. Together, these layers give rise to an electrical potential drop (φ_0). The net charge density approaches zero with the distance from the surface into the considered electroneutral bulk (no net charge). The electrical double layer has a thickness between 1 nm to 100 nm depending on salt concentration and surface charge density.

There are several models describing the EDL and the interaction between ions in an electrolyte and a charged surface, the most frequently used is the Gouy-Chapman-Stern (GCS) model.^{2,25} The GCS model describes the EDL as having an inner layer (the Stern layer) and an outer diffuse layer to neutralize the surface. The inner layer is described as a single

layer of ions that strongly adsorb to the surface. The Stern layer is followed by the diffuse layer of ions, in which the ions are more free to move. The thickness of the diffuse layer depends on the surface charge density and the concentration of ions in the electrolyte. Decreasing ion concentration or increasing the surface charge density results in an increased diffuse layer thickness. The thickness of the EDL is often referred to as the Debye length (λ_D), which is defined according to equation 2, where ε is the electrical permittivity, R is the universal gas constant, T is the temperature, F is Faraday's constant, and c is the ionic concentration.^{21,25} The surface charge density and impact of the EDL will be further discussed in the aspect of designing an electroosmotic pump, see chapter 3.3.1.

$$\lambda_D = \sqrt{\frac{\varepsilon RT}{2F^2 c}} \bigg|_{bulk} \quad (2)$$

3.2 Electrophoresis

Electrophoresis describes the motion of charged species, relative to the solvent/electrolyte, driven by an electric field. The motion is caused by Coulombic force ($\vec{f}_{Coulomb}$) on the ions in the electric field.²¹ The force exerted on the charged species depend on its charge and size, resulting in differing migration rates for different species. The electrophoretic mobility of each species i , ($\mu_{EP,i}$), is the proportionality constant between the migration rate ($\vec{u}_{EP,i}$) and the applied electric field ($\vec{E}$) (see equation 3). An electrophoretic separation, e.g. gel electrophoresis, uses the difference in $\mu_{EP,i}$ between various species which depends on their size and charge, resulting in different migration rates. The $\mu_{EP,i}$ also give an indication of how easily two different species will separate.

$$\vec{u}_{EP,i} = \mu_{EP,i} \vec{E} \quad (3)$$

The total motion of charged species in the electrokinetic systems is also influenced by the net flow of the electrolyte, characterized by the electroosmotic mobility, μ_{EO} (further described in section 3.3). The total migration rate is thereby influenced by the net electrokinetic mobility, $\mu_{EK,i} = \mu_{EO} + \mu_{EP,i}$, times the applied electric field (see equation 4).

$$\vec{u}_{tot} = \mu_{EK,i} \vec{E} \quad (4)$$

3.2.1 Gel electrophoresis*

One frequently used application of electrophoresis is gel electrophoresis, where charged macromolecules, such as proteins, DNA, RNA, and amino acids, are separated in a gel across which an electric field is applied, schematically shown in Figure 5.^{26–28} Gel electrophoresis can be used as a purification step, e.g., after PCR, in order to improve the efficiency of subsequent analysis. This technique is a standard analytical method in biochemistry, molecular biology, and forensic science, and there are different setups as briefly described in the coming sections. The setup is simply described as two electrodes, typically made of platinum, that are placed in buffer at opposite sides of a gel, e.g. an agarose gel or polyacrylamide gel (see Figure 5). The gel functions as a sieve, where larger molecules move more slowly and smaller molecules move more swiftly. The applied electric field drives the motion of species through the gel at a rate proportional to its electrophoretic mobility, $\mu_{EP,i}$ (the gel effectively prevents electroosmosis from transporting the species). Gel electrophoresis is either run at a constant applied potential or at a constant current. The migration rate of the species is kept constant by running the process at a constant current, where the potential increases with time during the separation, as can be seen in paper 1. The separation in paper 1 was performed using a constant current which resulted in an applied potential between 60 V to 250 V across a 4 cm long polyacrylamide gel.

*The following chapter regarding gel electrophoresis and following two subchapters have previously been published in my licentiate thesis, *Additive Manufacturing Methods and Materials for Electrokinetic Systems*, Linköping Studies in Science and Technology, Thesis No.1724 (2015). doi: 10.3384/lic.diva-121252

Figure 5 Schematic setup for gel electrophoresis, where differently charged species have been separated in the gel depending on their size and charge. The sample, a mixture of the charged species e.g. proteins, is placed in the middle of the gel (dashed line) from which the charged species will start to migrate through the gel towards the oppositely charged electrode. Cations (positively charged ions) move towards the cathode (negatively charged electrode) and anions (negatively charged ions) move towards the anode (positively charged electrode) with different migration rates.

3.2.1.1 *Types of gels in gel electrophoresis*

The most common gels used in electrophoresis are agarose and polyacrylamide. The gels are optimized for different types of samples and procedures. Agarose is usually appropriate for large proteins, and DNA, while polyacrylamide gels are used for smaller proteins, fragments of DNA, and peptides.²⁸ The choice of gel also influences what can be done with the sample after the separation (e.g., blotting). For example, agarose gels allow for easy retrieval of the sample by simply melting the gel. When making agarose gel, there is little control over the gel's porosity, while the porosity/density of a polyacrylamide gel can be reliably and repeatedly determined.^{26,28}

A polyacrylamide gel is formed by mixing acrylamide and bisacrylamide together using a radical to initiate polymerization. Varying the concentration of acrylamide and bisacrylamide, influences the resulting gel density: the higher the concentration of acrylamide, the higher the density of the gel and the more suitable it is for separation of smaller sample constituents. The same goes for agarose gel, where higher agarose concentration leads to a denser gel, but dense agarose is not as dense as polyacrylamide. The choice of gel material and the porosity of the gel also affect how well the bands/proteins can be resolved/separated in the gel. Optimizing

the gel in this manner is outside the scope of this thesis. A commercially available polyacrylamide gel with a density gradient was used in paper 1.

3.2.1.2 Isoelectric focusing (IEF) and Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

There are different methods of employing gel electrophoresis. Isoelectric focusing (IEF) uses a gel with pH gradient, where native proteins are separated in respect to their isoelectric point, the pH at which the protein's net-charge is zero. When the potential is applied, the electric field starts electromigration of the ions and creates a pH-gradient in the gel. The sample components migrate in the gel until they each reach an environment where the pH matches the component's isoelectric point, rendering it uncharged and thereby stops. This type of gel electrophoresis maintains the native structure of the protein for further analysis or use.

Another method is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which separates denatured proteins depending on their mass. Dodecyl sulfate denatures proteins, creating a negative net-charge. SDS-PAGE separations are therefore always run from cathode to anode. The amount of SDS that interacts with a protein is proportional to the number of amino acids.²⁷ The ratio is approximately one molecule of SDS per two amino acids. The mobility of the denatured protein will, for the most part, be logarithmically inversely proportional to the mass of the protein, which can result in poor resolution on a uniform gel. A gel with a density gradient improves the separation resolution of the proteins in a shorter gel, as used in paper 1.

3.3 Electroosmosis

The motion of a liquid generated by an applied electric field is called electroosmosis. Coulombic forces ($\vec{f}$) on the ions in the diffuse layer of the EDL, result in a motion that drags the surrounding liquid along with a mean flow velocity, ($\vec{u}_{EO}$), proportional to the external electric field ($\vec{E}$) and the electroosmotic mobility (μ_{EO}) (see equation 5).²¹ The electroosmotic mobility is influenced by the concentration and surface charge density of the channel surface. This simplified model can be used to describe electroosmotic flow in most microchannels, assuming that they are made out of a single material in which the EDL is thin (approximately 0.01 of the channel diameter) and there is no pressure difference present.²¹ μ_{EO} can be used to compare different materials (considering that the electrolyte is the same, e.g. concentration and pH) where higher values result in increased electroosmotic flow (EOF), desirable for efficient electroosmotic pumps (see section 3.3.1). The zeta potential, ζ , or the electrokinetic potential, can also be used to compare materials for the use in electroosmotic systems. ζ is the potential drop across the diffuse layer of the EDL and can be indirectly measured by the electroosmotic mobility or the streaming potential generated by a pressure-driven flow.²¹

$$\vec{u}_{EO} = \mu_{EO} \vec{E} \quad (5)$$

A setup for measuring the electroosmotic mobility is to place a pair electrodes in a set of reservoirs connected via a microfluidic channel. The velocity of the liquid through the channel generated by electroosmosis can then be estimated by measuring the accumulation of transported liquid (e.g., by mass), or following the movement of an optically active tracer, (e.g. a neutrally-charged fluorescent dye); or analyzing the time for the current to reach steady-state as the channel is filled with an electrolyte with a different ionic concentration.^{21,29,30} The velocity obtained, and the electric field are then used in equation 5 to calculate μ_{EO} , provided that there is no pressure-driven flow through the system. ζ is then calculated according to equation 6 where, η_{bulk} , and ϵ_{bulk} are the viscosity and the electric permittivity of the bulk.

$$\zeta = \frac{\mu_{EO} \eta_{bulk}}{\epsilon_{bulk}} \quad (6)$$

The zeta potential can also be inferred from the streaming potential. The streaming potential refers to the potential difference occurring when a pressure-driven flow creates a

motion of the ions in the EDL.^{21,25} The measured potential difference, ΔV , is then used to estimate the zeta potential according to equation 7, assuming that the EDL is thin and uniform, where Δp is the pressure difference used to generate flow, σ is the conductivity of the electrolyte, ε is the electrical permittivity, and η is the fluid viscosity. Streaming current can be measured similarly to streaming potential, however any polarization of the electrodes can lead to large estimation errors when inferring the zeta potential. Preferably, more than one method of measuring zeta potential is used to improve the accuracy of the parameter's estimation.

$$\zeta = \frac{\Delta V}{\Delta p} \frac{\sigma \eta}{\varepsilon} \quad (7)$$

3.3.1 Electroosmotic pumps

A device that pumps liquid via electroosmosis is referred to as an electroosmotic pump (EOP) also known as an electrokinetic pump. An EOP has no moving mechanical parts, can be very compact, and is relatively easy to integrate, e.g., into the micrometer-sized channels that in this thesis is referred to as microfluidic devices. An easy way to construct an EOP is to connect two electrodes using an electrolyte-filled glass capillary. The generated flow in an EOP is described as plug flow, since the velocity profile is uniform and there is no turbulence, resulting in for example little spreading and mixing of a sample transported through the microchannel. Also, since EOPs are controlled using electronics, it is relatively easy to employ multiple EOPs in parallel. The construction and material of the microfluidic device and the electrolyte composition are important factors that influence the flow rate and pressure the EOP can generate.

The function of the microfluidic channel, which connects the electrodes, is to effectively generate an electroosmotic flow, where the dimensions of the channel and the channel's surface charge influence the electroosmotic velocity. The microfluidic device can be more than a single capillary, as in the case of using a porous silica frit, a capillary packed with microparticles or porous membrane (microfluidic device used in papers 2 and 3), where each "pore" functions as an EOP, resulting in multiple, parallel EOPs which all contribute to the overall generated flow rate.^{31–37}

The surface chemistry/surface charge density of the material that the microfluidic device consists of impacts the electroosmotic velocity, since it together with the electrolyte affects

the diffuse layer of the EDL.²⁵ An increased electrolyte concentration in combination with a low surface charge, result in a thinner EDL compared to a higher surface charge and lower electrolyte concentration. A thinner EDL generates a lower electroosmotic velocity than a thicker EDL (assuming that the pore diameter is the same). The electrolyte's concentration was used in paper 3 to increase the generated flow rate. In paper 3, the application for the EOP was to control the flow in a microfluidic cell culturing device to expose the cells to shear stress. Initially, the pump was intended to pump cell medium (concentration of about 150 mM) with a flow rate of at least 60 $\mu\text{l}/\text{min}$. This ionic concentration in the pump would have reduced the thickness of the EDL to about 1 nm, reducing the generated flow rate induced by the motion in the EDL. By using a non-physiological concentrated buffer in the pump, the thickness of the EDL increases and with that the overall flow rate. However, the non-physiological buffer in the pump should not come in contact with the cells in the microfluidic device, since it damages the cell (seen in experiments but not shown). Therefore the electrolyte in the pump was separated from the cell medium in the device by an air bubble. Another benefit with separating the pump's electrolyte from that in the cell culturing chip is that any residues, e.g. pH changing species, or residues from the polymer electrodes, with possible negative impact on the sample/cell assay, are also separated from the assay itself. The cell medium can also be problematic in an EOP, due to surface interactions (fouling of the channel surface) between the constituents in the cell medium and the microfluidic network changing the surface chemistry or clogging of the channels, which can alter the flow rate.

The surface charge of the microfluidic device can also depend on the pH of the electrolyte. For example, a channel with negative surface charge density (zeta potential < zero), as in the case of glass, an increased pH will reduce protonation of the surface, increasing the surface charge density, which increases the thickness of the EDL, resulting in a greater electroosmotic mobility. The pH of the electrolyte can change due to the electrochemical reactions at the electrodes (see chapter 2.1). The pump in papers 2 and 3 is expected to maintain a constant pH, even when used without pH-buffer, due to the use of conducting polymer electrodes.

The generated volumetric flow rate for a given applied potential can also be influenced by the thickness/length of the microfluidic device (e.g., capillary). The potential drop across the channel, results in an effective electric field dependent on e.g., the thickness of the membrane. In the case of a thin membrane (thickness 10 – 30 μm , as in the case of papers 2 and 3), a large electric field (range kV/m – MV/m) is achieved using small potentials (0.3 – 5V), which increases the electroosmotic velocity according to equation 5.

The pressure, Δp , that an EOP can deliver depends on the size of the microfluidic network and the electroosmotic mobility of the material, as seen in equation 8, where ΔV is the potential drop across the channel, and R is the channel radius of e.g. a cylindrical capillary.^{21,36,37} Notice that the smaller the pore size is, the greater is the pressure generated for a given potential difference. The delivered pressure also increases with increased μ_{EO} .

$$\frac{\Delta p}{\Delta V} = -\frac{8\mu_{EO}\eta}{R^2} \quad (8)$$

An EOP that can generate high flow rates and a high pressure should, according to the theory describe in this chapter, consist of a microfluidic network, with high porosity, small channel dimensions and a high surface charge density. The concentration of the electrolyte used in the pump should be low to increase the thickness of the EDL, increasing the electroosmotic mobility, as well as a pH that increases the surface charge density. The electroosmotic velocity is further increased by increasing the electric field applied across the EOP. An EOP fulfilling all these criteria would generate a high flow rate and produce a high pressure.

4 Additive manufacturing / 3D printing*

3D printing, as an additive manufacturing method, has rapidly become a useful technique for making small quantities of individual systems, customized prototypes, or prostheses.³⁸ 3D printers use a bottom-up approach, where material is added layer-by-layer to an object.^{38,39} 3D printers have decreased the time required to convert a virtual 3D model into an actual object, allowing testing of different designs and prototypes during the same time it previously took to make a first prototype.^{38,40} Over time, 3D printers have evolved from expensive tools available only to a few experts to low-cost machines accessible to everyone.^{38,40–42} There are several types of 3D printers with different deposition methods, described in the following chapter. The quality of the final printed object depends on the material, the calibration of the deposition tool's parameters, and the type of printer used. Lower quality objects are made quickly. However, with increased demands on quality and refined structures, come an increase in production time and higher demands on calibration. Open-source and cheap printers e.g., RepRap and Fab@home, usually produce lower-quality objects, usually sufficient for prototyping and research.^{41,42} These types of printers allow a lot of freedom in terms of the use of various (commercial and non-commercial) materials and the possibility to modify the printer to suit specific applications. The dimensions of the printed object can be in the range of a few millimeters up to 25 cm for a cheap desktop printer.

*The following chapter is has previously published in my licentiate thesis *Additive Manufacturing Methods and Materials for Electrokinetic Systems*, Linköping Studies in Science and Technology, Thesis No.1724 (2015), doi: 10.3384/lic.diva-121252

4.1 Basic principles of 3D printing

The basic principle of additive manufacturing is the building of an object by adding material (layer-by-layer) to replicate a digital 3D model. Figure 6 illustrates a 3D printer where the deposition tool is a syringe filled with building material. The deposition tool translates in the x- and y-directions (horizontal plane) while depositing material by translating the piston in the syringe. To create multi-layered structures, either the table or the deposition tool is moved in the z-direction, creating space for the next layer.

Figure 6 Basic principle of 3D printing, illustrated with a syringe filled with the construction material as the deposition tool. Movement of a piston extrudes material while the syringe moves in the x- and y-direction to form the shape of the object. For each layer, the table moves down (in the z-direction).

4.2 Deposition tools for 3D-printing

The choice of deposition tool varies depending on the material for the final object. There are 3D printers that melt materials by sintering, polymerizing materials with laser- or UV-light, or adding material by extrusion.^{38,39,43} Sintering-based printers form an object by printing a glue, solvent or by melting a powder into the desired 3D structure. The structure is formed embedded within the powder, which supports the object while it is being printed. Sintering-based printers provide more freedom when designing overhanging and protruding structures compared to other 3D printers, since the object is surrounded by the powder which supports printed structures. Materials for sintering-based printers must be compatible with the glue or melting process, which makes many biomaterials unsuitable. UV/laser-based printers

require a material that is photo-curable into a solid. The structure is built by directing light in the shape of the structure into a basin of photo-curable material. Extrusion-based 3D printers add material bottom-up to an object, and can have various extrusion tools that are often specific to a certain type of material. Thermoplastic printers usually extrude plastic filament heated to 60 °C or more. The increased temperature precludes the use of materials that are easily destroyed at these temperatures, including many biological materials. This is the type of printer most familiar to the general public, mainly because it represents the largest group of cheap and open-source printers available, such as RepRap⁴¹, which evolved into the commercial printer MakerBot. The plastic filaments consist of, e.g., poly lactic acid (PLA), acrylonitrile butadiene styrene (ABS), and polyvinyl alcohol (PVA). Another type of additive deposition tool is the inkjet printer, which uses a deposition head like those commonly found in commercial desktop document printers. This type of deposition tool requires “ink” with low viscosity.⁴⁴ In comparison, syringe-based printers can extrude both high and low viscosity material.⁴² Syringe-based printers extrude material through a tip attached to a syringe barrel, where the pressure on the piston can be controlled. Examples of materials printed with this type of printer are silicones, biomaterials including cells, frosting, and conductive polymers.^{38,42,45–47} An object composed of several types of materials can be constructed during a single printing run by simultaneously using multiple syringe barrels. The limits on the size of the object printed depend on the tip size, the material properties, and the accuracy of the printer. In paper 4, a material was developed for the syringe-based printer in Figure 7.

Figure 7 Syringe-based 3D printer (Fab@home Model 3, Seraph Robotics Inc), photo by K. Bengtsson.

4.3 Printing parameters

There are several parameters that govern extrusion during 3D printing, e.g., the size of the nozzle, ambient temperature, material viscosity, nozzle translation speed, extrusion rate, which all influence the final product. Three settings adjusted during syringe-based 3D printing are: deposition rate, initial pressure increase, and the final release of pressure on the material. These parameters must be adjusted and optimized for each set of materials in order to achieve an even, straight structure with a cross-section similar to the nozzle without any gaps in the printed lines. The deposition rate is the rate of (continuous) flow of material during printing, and is usually specified relative to the translation rate of the nozzle. To initiate extrusion, the initial pressure created by the piston is increased (hereafter referred to as pushout), and the pressure is released (later referred to as suckback) to halt the flow. To initiate and stop flow of a viscous material, a larger pushout and suckback are required, compared to that required for a low viscosity material. The deposition rate and tip diameter influence the shear rate that a material is exposed to during printing, which can be used to estimate shear rates that the material is exposed to during printing (see paper 4).

4.4 Material requirements for 3D printing

A material suitable for 3D printing should be compatible with the deposition tool as well as the application. Material properties suitable for syringe-based printing and applications in biology and biochemistry are described in table 2. These were the characteristics considered when choosing a sacrificial material to be further characterized in paper 4.

Table 2 Material properties desirable for syringe-based printing for applications in biology and biochemistry.

<i>Property</i>	<i>Definition</i>	<i>Need</i>
Biocompatibility	A material that can coexist with living tissue and cells, which has low or no toxic effect in the biological application.	Devices used in biology and biochemistry typically need to perform their functions without disturbing the tissues, cells, or biomolecules under study.
Chemical stability	The material has a consistent chemical structure and is inert when in contact with other materials.	Predictable behavior while printing and handling. Possibility to combine multiple materials in a device without undesired reactions.
Mechanical stability	Sufficient consistency for the material to maintain its form when printed, including the support of subsequent layers and structures. Viscosity > 20 Pa·s	3D printing requires multiple layers to be patterned, where underlying structures support additional layers. High mechanical stability of the final printed object allows it to be handled without being deformed or otherwise damaged.
Thermal stability	The material should be stable and have consistent viscosity in the relevant temperature range during fabrication, use and handling.	Minimal changes of viscosity in the temperature range of 20±5 °C. Predictable behavior while printing and handling without temperature control. Many biological applications require temperature stability near 37 °C.

4.5 Sacrificial template materials

A sacrificial material is a temporary support that allows for manufacture of more complicated printed structures in combination with other materials. It can, for example, facilitate the bottom-up construction of over-hanging, protruding and/or hollow structures, that otherwise are difficult to make with certain 3D printers, e.g., syringe-based printing. A sacrificial material should be easy to remove, for example by dissolution in water, but also maintain its printed structure during all steps until removal. For example, it must not dissolve or collapse when acting as a support during molding or during the addition of layers of another material. Sacrificial template materials for biological applications are preferably water-soluble with biocompatible residues. Sugars⁴⁸, poloxamer^{49,50} and polyethylene glycol (PEG)⁴⁶ are examples of materials that have been used as sacrificial template materials in biological applications.

The polymer polyethylene glycol, PEG, (also known as polyethylene oxide, PEO, see Figure 8) is available in a range of molecular weights. The consistency of the polymer ranges from liquid to crystalline (wax-like) with increasing chain length. The mechanical stability and printing properties can therefore be altered by choosing an appropriate molecular weight. The choice of molecular weight, i.e. the number n in Figure 8, can also be optimized for dissolution of the printed PEG structure. Lower molecular weight PEG is more easily dissolved than higher molecular weight varieties. The dissolution of PEG can be improved by increasing the temperature, where shorter chains are affected more due to their lower melting temperature. PEG has been used in many different kinds of applications such as plasticizers⁵¹, hydrogels⁵², batteries⁵³, cosmetics, laxatives and drug delivery systems.^{54,55} PEG is also used as a precipitant in DNA and protein purification as well as for concentrating viruses. PEG is soluble in water, and in many organic solvents, such as ethanol, toluene, chloroform, and acetone.⁵⁴ Paper 4 characterized plasticized polyethylene glycol with the required properties for use as a sacrificial material suitable for syringe-based 3D printing.

Figure 8 Polyethylene glycol (PEG)

A plasticizer typically reduces the degree of crystallinity, making the polymer more flexible. Addition of plasticizer to PEG decreases the viscosity and thus enables printing of PEGs with higher molecular weight, as shown in paper 4. 100 % PEG with molecular weight above 2000 g/mol (PEG2000) is mechanically stable enough to function as a support, but too rigid to extrude through a syringe tip at room temperature without the addition of a plasticizer. The addition of a plasticizer also makes the viscosity of the PEG2000 blend less susceptible to small temperature changes (in the range of $20 \pm 5^\circ\text{C}$) than PEG with lower molecular weight (for example ≤ 1000 g/mol). This simplifies handling without risking deformation, as in the case of PEG1000, which melts at body temperature when handled. PEG combined with organic carbonates, such as diethyl carbonate (DEC), dimethyl carbonate (DMC), and propylene carbonate (PC), as plasticizer have previously been used as electrolytes in Li polymer batteries⁵⁶ and other ion-conducting applications.⁵³ These carbonates were used as plasticizers in paper 4 to make high molecular weight PEG suitable for syringe-based printing. The chemical structures of DEC, DMC, and PC can be seen in Figure 9. As PEG and PC are both non-toxic and water-soluble, the combination is a suitable choice as a sacrificial material for biological applications.

Figure 9 Chemical structures (from left to right) of diethyl carbonate (DEC), dimethyl carbonate (DMC), and propylene carbonate (PC)

5 Concluding remarks and future works

Polymeric materials can have very different properties and applications, useful when constructing electrokinetic devices. The work in this thesis demonstrates the application of previously known polymers in areas and techniques where other materials usually are used. This work shows the applicability of electrochemically active electrodes in electrokinetic devices. In this case PEDOT electrodes are used, which can be made into soft and flexible electrodes that also reduce water electrolysis. The electrochemically active polymer, PEDOT, utilized in papers 1, 2, and 3, has been shown to have the capacity to replace metal electrodes, thereby simplifying the development of smaller, cheaper, and disposable electrokinetic devices. In these papers, we show that PEDOT electrodes can replace metal electrodes in a gel electrophoretic setup and in electroosmotic pumps. In paper 4 we demonstrate that a PEG-based material (PEG2000 plasticized with e.g., propylene carbonate) can be used to print self-supporting structures down to 200 μm in diameter using a syringe-based 3D printer. The consistency of this PEG-based material can be tuned with the amount of plasticizer and it can be used in both macroscopic and microscopic applications.

The work in this thesis started with the aim to make a simple electrokinetic system on a microscope slide, bottom-up using a simple syringe-based 3D printer. The primary concept involves programming the 3D printer to assemble the electrokinetic device (electrodes and microfluidic network) from materials with different properties. This could be achieved by using the electrochemically active polymer PEDOT for electrodes, a temporary material for a channel network, which was then molded by another polymer (e.g., polydimethylsiloxane (PDMS), commonly used in soft lithography) for the permanent structures. The result of this work is the development and characterization of the PEG-based sacrificial material presented in paper 4. In comparison to many other temporary materials e.g., PVA, this PEG-based material can be printed on and in proximity to materials that are sensitive to temperatures above 40 $^{\circ}\text{C}$. However, the dimensions (minimum dimensions achieved in this work: 200 μm) and the surface roughness of the printed structures out of the PEG-based material molded in PDMS, were too large to result in a well-functioning electrokinetic device. A syringe-based 3D printer is a convenient tool for assembling various kinds of materials, and can give a reasonable printing accuracy in the mm-scale. The material developed in paper 4 can easily be used to make mm-sized channels using a basic soft lithography process, for applications where surface roughness/smoothness is of less importance. A soft lithography process, using a

soluble master, open up the possibility of using more rigid polymers that are difficult to employ in the more traditional soft lithography process, where the mold has to be removed from the master (silicon master and PDMS).

Water electrolysis can be reduced as long as there are sufficient amounts of PEDOT that can be oxidized/reduced in the electrode, also referred to as the coulombic capacity of the electrode. The applications presented in this thesis benefit from having PEDOT electrodes with a large coulombic capacity; however, many available PEDOT emulsions contain a low percentage of PEDOT, resulting in tedious manufacturing processes for making electrodes with large coulombic capacity, as is the case in papers 1,2 and 3. The development of the future electrokinetic systems would benefit from the development of PEDOT emulsions that simplify the manufacturing process, increase the coulombic capacity of the PEDOT electrodes, and reduce the number of additives that can alter the surface charge in the microfluidic device.

The calculated electroosmotic mobility of the polycarbonate membranes used in papers 2 and 3 differs from previously reported values.^{34,57} Previously reported data shows that the EOP using a polycarbonate should be able to produce even higher flow rates than what we reported; it was not determined if the difference in electroosmotic mobility was due to the coating of the membrane with residues from the PEDOT electrodes or whether it was caused by altering pH in the measuring chamber.

The EOP in paper 3, was a first prototype of how this kind of EOP can be integrated to a microfluidic device and this prototype could be further improved. The electrodes exit the syringe filter holder between the threads, at which point there is a risk of tearing the electrodes or leakage of electrolyte. The connection to the electrode and reduction of leakage could be achieved by integrating the connection of the electrodes into the holder itself. Also, the maximum generated flow rate by this type of pump could probably be increase by modifying or replacing the membrane that separates the electrodes, for example, a porous glass frit would likely increase both the generated pressure and flow rates.

To verify the function of the pump, we applied the pump to a cell culturing system, where we exposed the cells to shear stress of about 5 dyn/cm². The preliminary results from the cell study in paper 3 showed some unexpected results regarding the alignment of the cells. We were not able to detect or verify the cell alignment and it seems that the cell response in an oscillatory flow might differ from the cell response in a continuous flow. However, we noted

that the surface coverage and area of the cell nucleus increased and the shape index (value of a cell's circularity) decreased, when culturing cells under higher flow rates. Future studies could use this kind of setup to further study the cells response, e.g., integrin expression and surface coverage, to varying oscillatory flow and different flow rates and shear stresses, where this clip-on, modular pump is a simple alternative to control the flow in a cell culturing device. The setup presented in paper 3 allows for easy programming of the generated flow rates and parallelization of experiments.

6 Summary of included papers

6.1 Paper 1

Conducting polymers for gel electrophoresis

In paper 1, we evaluated the use of the pi-conjugated polymer PEDOT as an electrode material in gel electrophoresis. We were able to incorporate the polymer electrodes in a commercially available gel electrophoretic system, the PhastSystem from GE Healthcare, where the resulting gel image from a separation with polymer electrodes was very similar to a separation run with platinum electrodes. The PEDOT electrodes showed good compatibility with the gel, buffers, and separation protocol, in which they were used to apply a potential up to 250 V across a 4 cm long gel. We also showed that the generated currents through the gel when applying a constant potential within the electrochemical stability window of water is much higher when applying the potential using PEDOT electrodes compared to platinum electrodes. We expect that by replacing expensive electrode materials, e.g. Pt, with a cheaper, more likely disposable electrode material, will result in decreasing reuse of electrodes and thereby the risk of cross-contamination between successive runs. Additionally, by using conducting polymer electrodes, byproducts from water electrolysis will be reduced. By replacing metal electrodes with conducting polymer electrodes, which are cheaper, flexible and electrochemically active, we expect that we are one step closer to developing smaller and more disposable gel electrophoretic systems with incorporated conducting polymer electrodes.

6.2 Paper 2

An all-plastic, flexible electroosmotic pump

In paper 2, we present an all-plastic, flexible electroosmotic pump that can be made into a large-area, fabric-like pump with potential applications in e.g., garments that can control water (sweat) transport. The pump is constructed from commercially available materials: a porous polycarbonate membrane which is placed between two electrodes made of the electrochemically active polymer PEDOT. We demonstrate that water can be actively transported from one side to the other by applying a potential, equal to that of a small battery. We measured a water mass flux of up to 23 mg/min per cm² polycarbonate membrane (porosity: 12 %), at an applied potential of 5 V, and a limiting operating pressure of

0.3 kPa per V. This paper also reports the benefits of using an electrochemically active polymer as an alternative electrode material, resulting in reduced water electrolysis and required applied potentials to generate flow.

6.3 Paper 3

A clip-on electroosmotic pump for oscillating flow in microfluidic cell culture devices

Paper 3 presents a small, modular pump for microfluidic cell culture devices. Controlling flow in a microfluidic device can be challenging, e.g. connecting the pump to the chip and the size of the pump. We developed a clip-on, electroosmotic pump in the same size as many commercial microfluidic devices that can be connected directly to existing Luer connectors. This pump also consists of a porous polycarbonate membrane and PEDOT electrodes (further described in paper 2), which are incorporated in a syringe-filter holder. Volumetric flow rates up to 350 $\mu\text{l}/\text{min}$ were observed when driving 5 mA using applied potentials between 0.6 V and 3.5 V. An oscillatory flow was employed to reduce the volume in the reservoir as well as to improve the operability of the pump to be within the columbic capacity of the electrodes, to reduce water electrolysis. This pump was used to keep cells alive up to 96h in a microfluidic device and was able to generate a sufficient volumetric flow rate to expose endothelial cells to shear stress (up to 5 dyn/cm^2). These initial cell experiments exhibit some interesting results regarding how cells appear to align due to elevated shear stress and oscillatory flow. We think that this first clip-on, low-cost electroosmotic pump that is easily parallelizable can improve and simplify the way flow is controlled in microfluidic devices.

6.4 Paper 4

Plasticized polymeric sacrificial materials for syringe-based 3D printing

In this study, we characterized and evaluated a polyethylene glycol (PEG)-based material combined with a plasticizer (diethyl carbonate, dimethyl carbonate or propylene carbonate) for syringe-based 3D printing. We intended to use this material as a temporary support in additive manufacturing to facilitate printing of more advanced structures, such as overhanging and protruding models. Syringe-based 3D printing requires a material that can be easily extruded through a nozzle, while still maintaining its shape in the final object. The sacrificial material should also be strong enough to support surrounding structures without collapsing as well as being easily dissolved/removed. In this paper, we demonstrate a PEG-based material,

which can be adjusted to suit different applications, where the consistency can be altered by the choice of polymer length and amount of plasticizer. Since, PEG is known to be water soluble and biocompatible, it is a suitable solution for printing objects for biological applications.

In this paper, we tested and characterized PEG plasticized with three different plasticizers; dimethyl carbonate, diethyl carbonate, and propylene carbonate. We recommend a blend of 25-35 wt% propylene carbonate in PEG2000, which results in a mixture that is easily extruded through a syringe, is self-supportive, has increasing mechanical stability over time after printing and is easily dissolved in warm water. Viscosity measurements showed that the viscosity increases with storage time after blending PEG and plasticizer. Differential scanning calorimetry shows that the phase-transition temperatures stay the same regardless of storage time of the material, up to 3 weeks.

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Papers

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