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TECHNIQUES TO FABRICATE THIN MEMBRANES WITH CONTROLLED PERMEABILITY

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ABSTRACT

Kalle Nurmi: Techniques to fabricate thin membranes with controlled permeability
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Membranes are an essential tool in many biomedical applications whether they are used as an implant or as a substrate for cell cultures. Permeability of different molecules such as nutrients is an important property do determine the functionality of a membrane in that task. This text goes through various membrane manufacturing methods used with various material types and examines possibilities of these methods.
In the first chapter, different membrane properties and their characterization methods are discussed starting with properties of the material and continuing to biocompatibility, thickness, porosity, and permeability. Properties of the material consist of physiological aspects, such as chemical structure, thermal properties and weight distribution of molecules and their characterization methods vary from microscopic methods to different physical methods. Characterization of biocompatibility is heavily tied to the chemical properties of the material which is why samples of the membrane material is used in different characterization methods to provide either quantitative or qualitative information on the biocompatibility. Thickness of the membrane is often characterized with materials optical properties using e.g. spectrophotometry. Porosity characterization can also be done with similar methods, but also different intrusion methods are possible. Likewise, with permeability characterization different intrusion methods are popular but all these methods are considered experimental.
In the second chapter, varying methods for porous membrane manufacturing are discussed with some other film manufacturing methods which showed some interesting properties considering cell cultivation. These film manufacturing methods could not be used as they were but combined with some pore producing method, such as etching, they could be useful. Other methods such as salt leaching methods, electrospinning or lithographic methods produced complete porous membranes.
Finally, in conclusion all discussed methods were collected to a table with different thicknesses and porous sizes that could be produced with these methods. Also, the most important aspects of the methods were collected to advantages and drawbacks.

Key words: membrane, porosity, films, permeability

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Ohuita membraaneja käytetään laajasti erilaisissa biolääketieteen tekniikan sovelluksissa, oli kyseessä sitten kirurgiassa käytetyt verkkoimplantit tai solunäytteiden kasvatusalusta. Oleellisia ominaisuuksia membraaneille usein ovat membraanin paksuus ja läpäisevyys. Läpäisevyys erityisesti on tärkeä ominaisuus membraaneille, sillä se mahdollistaa eri ravintoaineiden läpipääsyn solukasvatuksessa. Tämä teksti käy läpi useita eri metodeja näiden membraanien valmistukseen ja tutkii niiden erilaisia käyttömahdollisuuksia.
Ensimmäinen kappale käy läpi membraanin eri osa-alueiden karakterisointimetodeja alkaen materiaalin ominaisuuksista ja siirtyen sitten biokompatibiliteettiin, paksuuteen, reikäisyyteen ja permeabiliteettiin. Materiaalin ominaisuuksiin, kuten kemialliseen rakenteeseen ja painon jakautuvuuteen löytyy useita eri metodeja, kuten mikroskooppiset menetelmät. Materiaalin kemialliset ominaisuudet ovat myös hyvin vahvasti sidottuja aineen biokompatibiliteettiin, joten biokompatibiliteetin karakterisointi tehdään erilaisin materiaalinäytetestauksin, jotka tuottavat joko kvalitatiivista tai kvantitatiivista tietoa biokompatibiliteetista. Membraanin paksuuden karakterisointiin käytetään usein materiaalin optisia ominaisuuksia, joista pystytään myös tuottamaan tietoa membraanin reikäisyydestä. Reikäisyyden arvioimiseen kuitenkin käytetään useimmin, joko jonkin kaasun tai elohopean tunkeutumista membraanin läpi. Samoin permeabiliteetin karakterisoinnissa usein käytetään eri aineiden tunkeutumista kappaleen läpi, mutta permeabiliteetin arviointiin ei ole vakiomenetelmää.
Toisessa kappaleessa eri valmistusmetodit käytiin läpi ja niiden hyötyjä ja haittoja pohdittiin. Osa metodeista käy läpi eri filmien valmistusmetodeja joidenkin niiden muiden ominaisuuksien takia. Nämä metodit vaativat jonkin muun metodin reikäisyyden valmistamiseen kalvojen pintarakenteisiin, kuten eri etsausmenetelmät. Menetelmät, kuten suolan uutto, sähkökehräys ja eri litografiset menetelmät pystyvät tuottamaan valmiita membraaneja.
Viimeisessä kappaleessa eri menetelmien tärkeimmät piirteet, kuten niiden paksuus ja reikien halkaisija, on kerätty taulukkoon ja niiden hyödyistä on keskusteltu. Myös metodien muita tärkeitä ominaispiirteitä on taulukoitu ja niiden hyödyllisyyttä pohdittu.

Avainsanat: Membraani, huokoisuus, kalvot, permeabiliteetti

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1. INTRODUCTION

Fabrication of membranes and coatings is necessary in many different fields, from electronics to microbiology. In microbiology these membranes are an essential tool for growing cell and tissue cultures, and they can also be used for separation of different tissue layers and for electrical measurements.

Thin membranes can be fabricated from several different materials with different properties, but this research is mainly focused on polymers as fabrication material. This research mainly focuses on the purpose of finding a way to give tissue cultures an optimal support membrane which the tissue can grow on. This support membrane needs to have certain set of properties to function in this task. It needs to be as thin as possible while having controlled permeability evenly across the membrane which is needed for nutrient and metabolic product exchange through the membrane. The material also needs to have positive biocompatibility so that cells can attach to the membrane and grow on it.

In the characterization methods chapter different methods used on membrane characterization are introduced and discussed, such as assessment of molecular structure of the membrane, its biocompatibility, thickness and porosity evaluation. In the second chapter different fabrication techniques for membranes are examined with their strength and restrictions such as possible pore concentration and distribution differences between techniques. In conclusion essential features of fabrication techniques are collected into tables, and the chapter also discusses the results.

2. CHARACTERIZATION METHODS

2.1 Material

The characterization of any material contains assessment of different physiological properties of the observed material. Such properties are often molecular weight distribution, thermal properties, and molecular structure [1]. All of these material properties require their own characterization methods. These methods vary greatly from each other and are grouped into chromatographic, thermal, spectroscopic, microscopic, rheometric, and mechanical methods. [2] These methods are essential to confirm the wanted morphology and purity of the used material [1]. Different methods for the characterization of membrane properties are also required. These characterization methods also vary greatly but are mostly similar with methods described here and in other characterization chapters. These methods can be grouped into spectroscopic, microscopic, chemical, and physical methods.

Chromatographic techniques are analytical techniques meant to separate molecules to different phases in mixtures. There are several different chromatographic techniques such as liquid chromatography, size-exclusion chromatography, interaction chromatography. [2] The liquid chromatography is considered important characterization method for molecular weight, its distribution, and structure for synthetized polymers. It is especially good for molecules that are heterogenous. The size-exclusion chromatography on the other hand is mostly known for weight distribution analysis. It separates different molecules according to their polymer chain size by utilizing the conformational entropy change. The polymer needs to be linear since the size-exclusion chromatography is not suitable for polymers with branching chains or different functional parts. For this reason, interaction chromatography is considered better choice for being able to separate copolymers from each other according to their chemical composition. It does this with enthalpic interaction such as adsorption and partition. [3,4]

Thermal analysis methods are a group of techniques that are an essential way to collect accurate thermoanalytical measurements of materials physical behavior in a function of temperature. For biomedical application, techniques are also necessary for measuring thermal resistance of biopolymers with functional groups. The most common analysis techniques are thermal gravimetric analysis and differential scanning calorimetry. In thermal gravimetric analysis the loss of mass is measured in function of sample temperature.

Differential scanning calorimetry on the other hand measures the energies which the sample absorbs and emits in controlled temperatures. [2]

Spectroscopic analytical techniques, such as nuclear magnetic resonance (NMR), and infrared spectroscopy (IR) are important methods of assessing functional groups in polymers. In NMR, the magnetic fields are used to measure the energy absorbance of the material. This allows assessment of molecular structures, stability, and the degradation of macromolecules. [5,6] The information collected through NMR is quantitative, and makes it possible to assess polymers by their functional groups which is not possible through other means [2]. For membranes spectroscopic analytical techniques are also used to provide information on the surface and structural properties [7].

2.2 Biocompatibility

Characterization of biocompatibility is an essential part of deciding on the used material in thin membrane fabrication. Positive biocompatibility in this means that the material has wanted reaction with the intended tissue culture. Before using the material, it is necessary to understand the chemical composition of the material and if it can have harmful reactions with grown tissues. This means that the characterization of biocompatibility is heavily tied to characterization of the material. [8] What type of testing is used depends on the sample's properties, where and how it is meant to be used [9]. This chapter goes through some standard testing's concerning in vitro cytotoxicity.

Tests of in vitro cytotoxicity can be divided into extract, direct contact, and indirect contact tests. Depending on the test they can give quantitative or qualitative information on the degree of cytotoxicity in cells. The qualitative information is observable changes in cell structures and integrity of the cell membrane. These observations can be made with microscope, but they do not give measurable information on the grade of the cytotoxicity. Quantitative tests on the other hand give measurable information on the focus of the test. Such targets of measurement can be for example cell damage, cell growth or some aspect of cellular metabolism. When the viability of cells is reduced by 30% the reaction is considered cytotoxic. Since the quantitative test methods give more precise information, they are preferable test method. [9]

Extract tests requires extraction of part of the test material which is then but in contact with a cell culture. This method can give both qualitative and quantitative information about the degree of cytotoxicity. In direct contact tests the cell culture comes into contact

directly with the material. Similarly, with extract tests this gives both qualitative and quantitative information about the cytotoxicity. Sterilization of tested materials and equipment's is essential since contamination can produce false information of the cytotoxicity. The indirect method can be used to get qualitative assessment of cytotoxicity. It uses for example filter with certain small pore size or thin agar layer to separate the test sample from cell culture. [9] This allows chemicals to diffuse through the layer while protecting the cell culture from possible mechanical damage from test sample. This also allows assessment of cytotoxicity of leaching chemicals. The material must be able to diffuse through the separating layer. [9]

2.3 Thickness

Characterization of thickness can be achieved with different ways. Some of the most common methods are spectrophotometry and ellipsometry [10]. These methods measure different optical properties of the material to calculate its physical properties such as thickness or porosity. All materials can reflect, transmit or absorb light. Spectrophotometry uses the substances ability to absorb radiation to determine various properties of the substance. Spectrophotometry is quantitative method that uses wavelengths to measure film thickness as shown in Image 1. The measurement can be done by inspecting wavelengths interference pattern when the substance is radiated with electromagnetic radiation. Before the measurement can be done, the angle of incidence and the refractive index of the substance should be measured. [11] The absorption of wavelength is directly proportional to the thickness of a sample. This makes measurement of film thickness possible.

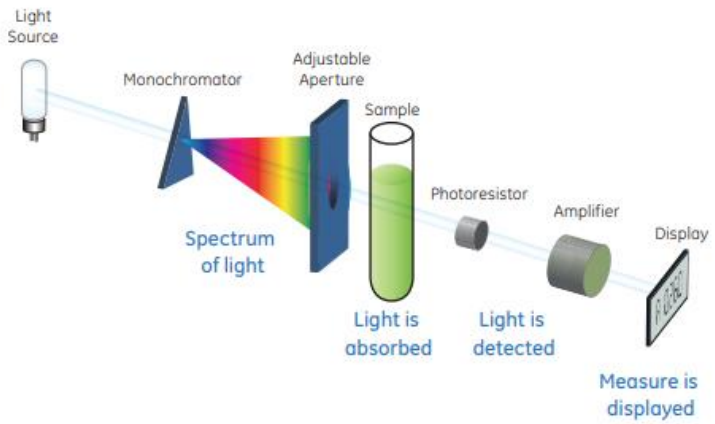

Figure 1. *Description of spectrophotometry [12]*

Ellipsometry is another crucial method of film thickness characterization. It does this by measuring the change of polarization between the initial and reflected light. This change

can be seen as the ratio of amplitude and the phase difference. The measurement instrument is called ellipsometer. The ellipsometry measurement happens in five steps. Generation of polarized light beam, oblique reflection of the light beam from the measured object, analyzation of change in polarization, determination of reflection parameters and finally determining the parameters of the measured object such as film thickness. [13] This process is demonstrated in Figure 2. Ellipsometry is very precise method which can detect extremely small changes in the surface thickness. These measurements are also possible in any medium which is transparent to the used light beam. [14] Since scatter does not affect the measurement that much, measurements are also easily reproducible. Optical properties of the thin membrane surface are affected by several different material properties so ellipsometry can also provide information on those. These properties can be for example conductivity and chemical composition. [15]

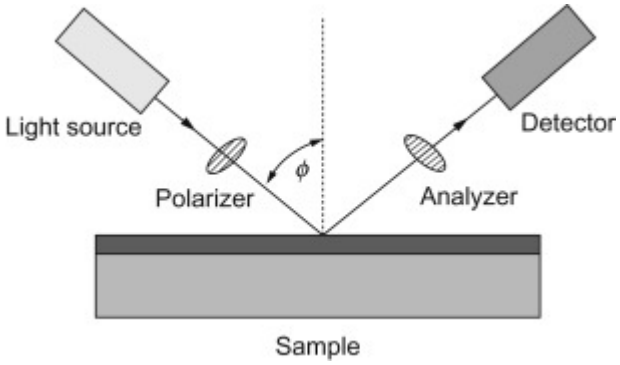

Figure 2. *Description of ellipsometry [15]*

2.4 Porosity

There are several methods for porosity characterization which depend on different way of analyzation. They are still experimental and underdeveloped [16,17]. Some of the most common methods for measurement of porosity are an x-ray computed tomography, a gas pycnometry and mercury intrusion porosimetry [16]. These methods are used in references to fiber mats made by electrospinning, but they could be reliable with other types of membranes as well.

A mercury intrusion porosimetry describes a method of evaluating the porosity and pore size distribution by pushing mercury through the pores with high pressure. This method however can cause changes in the structure morphology due to high pressure. [17] Mercury is pushed into larger pores first in low pressure. When the pressure is increased smaller and smaller pores are filled. Pore diameters can be calculated with the Washburn

equation which shows relation between applied pressure and the pore diameter by using physical properties of mercury. This method requires use of a porosimeter. [18] This method seems to be applicable for materials which can handle high pressures without large chances on their morphology such as tricalcium phosphate.

A gas pycnometry is a similar method to a mercury intrusion porosimetry by injecting the porous structure with monoatomic gas such as helium. It is usually used to determine density or volume of porous or non-porous materials. There is some variation in methodology of the technique, but it generally works as such. It happens by first filling the constant volume reference chamber with helium gas and then letting the gas expand into second chamber which has a sample of the test material inside it. The change of pressure is then measured and with it the volume and density of the sample can be calculated. [19]

An X-ray computed tomography or CT describes method where visual information of a sample density distribution is collected by rotating the sample 180 to 360 degrees while exposing it to x-ray radiation [20]. The x-ray CT can be divided into different specific methods and the technology has been greatly developed in the past 15 years. Some of these specific methods are able to provide spatial resolution of 100 nm [21].

2.5 Permeability

Permeability describes the ease in which gas or liquid travels through pore spaces in the porous material. It depends on various factors considering the medium passing through the material and physical properties and structure of the material. The properties of the flowing medium that affect the permeability are viscosity, surface tension and the angle is flow. Also, conditions of the test process have an effect on the measurement such as the temperature and the pressure which the medium is injected. The physical properties of the material that affect the permeability are the porosity, the shape of the structure and its flexibility. [22]

Permeability can be divided into unsaturated and saturated permeability since turbulence of the flow also has a great effect to permeability. Both of these permeabilities require different kind of experiments but since there is no set standard method in permeability characterizations, described methods are considered experimental. Methods also differ depending the dimensions of the system since the permeability also depends on the

direction of the flow. Described methods are also done with liquids so characterization methods differ from gas permeability. [22]

In unsaturated flow experiments the liquid is injected a mold containing the porous material which does not yet contain any of the liquid. This kind of experiments can be done with two different methods, either constant pressure injection or constant flow rate injection. In saturated flow experiments on the other hand the measurements of flow rate and change of pressure are taken when the porous material is completely filled with the liquid. There is also radial flow experiment where the liquid is injected through one point in the porous material. Advantage of this method is that the permeability variables can be measured from the surface area of the material. [22] Some studies show that permeability is prone to significant scatter. The most of this scattering is considered to be caused by human factors although the measured permeability varies by the tested specimens. [23]

3. FABRICATION TECHNIQUES

3.1 Floating-on-water fabrication method

In floating-on-water fabrication method liquid solution is placed on top of water layer where it solidifies to a thin membrane. This technique has been shown usable for Polydimethylsiloxane (PDMS) solution which is PDMS base and PDMS curing agent in the ratio of 10:1. The methods is decently fast, producing membrane in one hour, and is able to produce wide variety of membrane thicknesses and sizes by controlling the amount of solution, solution temperature and water temperature. Handling of the finished membrane is also rather easy since adhesion forces between PDMS and water substrate are weak, and the membrane can handle decent amount of strain depending on the thickness of the membrane. [24]

PDMS solution is lighter material than water so when solution is poured on the water it floats and starts to spread and create the membrane very quickly on top of the water layer. The membrane is formed in under 30 seconds and heating the system in 40-80 Celsius hardens the PDMS into solid form. The solution tries to spread to the entire surface area of the water so the container must be small enough to ensure consistent PDMS layer. Too large container causes formation of holes in some parts of the membrane.
Since the fabrication happens on the surface of water, the surface conditions of the water side of the membrane are different from the air side of the membrane. The roughness of the water side is much larger from the air side due to possible thermal fluctuation, density and movement of water and possible evaporation. [24]

Control of membrane thickness happens by controlling the initial temperature of the solution and the temperature of water substrate but mainly with the amount of solution used. Since the solution spreads to the entire water layer the amount of solution is directly linked to the thickness of the formed membrane. [24]

Strength of this fabrication technique is that it is very cheap method which does not require any expertise or special equipment. It also allows great size and thickness variation. [24] However, this method is not very well researched, so restrictions of this method are still mostly unknown. Method has also only been studied with PDMS material so its usefulness with other materials is not known. Generally, this method can only be used with materials which are lighter than water and have appropriate viscosity. Produced

PDMS membranes have generally good gas permeability but need to go through some etching process to have good enough permeability for nutrients.

Permeability of the membrane is greatly affected by the ratio of curing agent to the base of the solution. Increase on the amount of base in the solution causes the elastic modulus of the membrane to rise which allow micropores in the membrane to change easier. [24] This allows greater number of molecules to pass through the membrane. Permeability of PDMS material is heavily dependent of the thickness on the membrane when thickness is tens of micrometres [25].

3.2 Spin coating method

Spin coating method is one of the most common ways to create thin films and it is used with several different materials and substrates. The process starts by spreading the solution to the substrate. After this the substrate is spun at high speed causing creation of thin layer of the solution on top of the substrate. It is then heated at an appropriate temperature to finalize the film. [26] The thickness of the film depends on the viscosity and surface tension of the solution and parameters of the spinning such as speed, acceleration and time spun [26, 27].

Before the fabrication process can be started, the surface of the substrate must be prepared by cleaning. It can be done by washing the substrate with appropriate solution which removes unwanted particles from the surface for example acetone or piranha solution. After this the cleaning can be finalized with drying the substrate with clean air and heating it to remove all moisture from the surface. For a wafer substrate the heating is done in 120 Celsius for 15 minutes. [27]

If the membrane needs to be removed from the substrate after the process, sacrificial layer must be formed before the actual formation of the membrane. This can be done by spin coating a layer of appropriate material on the substrate which makes the removal of the actual membrane easier. [27]

The solution is poured on top of sacrificial layer and the substrate is spun. The spinning causes the solution to form planar layer with certain thickness depending on the spinning parameters and the viscosity of the solution used. The spinning parameter are acceleration, speed of spinning and time. The layer is then heated which hardens the solution and forms the membrane. [27]

Result of spin coating method is relatively planar surface which is the reason it is often used. It is well researched method which can be used in variety of materials from polymers to ceramics. [26] With PDMS it can result in film thickness of 1-10 micrometres [28]. Spinning and heating process can cause evaporation of solution [26]. Since the produced film does not have manufactured pores, the sufficient porosity for nutrient permeability must be produced with some other means such as etching. Nonetheless, the planarity of the produced film makes this method attractive for cell cultivation.

3.3 Langmuir–Blodgett method

Langmuir-Blodgett (LB) method is a method of producing ultrathin membranes with controlled layer structure. Materials suitable for this method are usually molecules with long hydrophobic hydrocarbon chain such as stearic acid and arachidic acid. Appropriate molecules can be attached to the hydrocarbon chain to make the molecule compatible to the method and provide different properties to the membrane such as electrical conductivity. The attached molecule works as a hydrophilic group and the hydrocarbon chain as a hydrophobic group in the initial material. [29]

The fabrication of LB films happens with a specific equipment called LB film deposition instrument. It happens on air-water interface where solution made from compatible material and water insoluble medium is slowly poured. Water used in the process must be distilled and deionized or some other appropriate liquid can be used in its place. When the solution is spread on the water surface the insoluble medium starts to evaporate leaving the wanted material on the interface. Molecules float on the water surface separate from each other with hydrophilic group in the water and hydrophobic group in the air. This state where the material has a lot of room to move around is called gas phase of the fabrication. [29]

The liquid phase is formed by moving dispersed molecules closer together by compressing the molecular area with movable barrier. In the liquid phase the movement of molecules is much more restricted. When this process is continued the solid phase is formed where molecules do not have any freedom of movement and a monolayer is formed. This is known as Langmuir film. Langmuir film deposited on top of solid substrate by dipping the substrate vertically into the bath is known as Langmuir-Blodgett film. [29]

When the dipping of the substrate is done horizontally the film is referred to as Langmuir-Schaefer film. [30]

The Langmuir-Blodgett method produces highly organized and flawless membranes with extremely small thickness. The thickness of the membrane can be highly controlled by repeating the process and producing multi-layered film. However, the process is expensive since it requires special equipment and trained users. Process can also be time consuming since the material must be synthesized and purified before it. [29] Since the thickness of the film is very small the gas permeability through the film is significant. The film on the other hand is pore free so the permeability of bigger molecules is small. For this reason, manufacturing of a porous membrane requires some other technique to apply pores on the film. Still, the extreme thinness of produced membrane can be used to effectively mimic tissue surfaces by using collagens in the membrane production [31].

3.4 Photolithography

Photolithography is very prominent technique in a biomedical field and a foundational in development of other more advanced lithography methods. It is a top-down method of producing patterned coatings on a substrate layer which is managed by first coating the substrate with light-sensitive material to a desirable thickness and then through patterned mask exposing it to a light source. This causes the light chemical and physical changes in the coating layer. [32] The light sensitive material, which is called photoresist, can be either type positive or negative. The type positive solution becomes more soluble when it is exposed to the light source and on the contrary the type negative hardens. [33] These reactions cause accurate and highly predictable patterns to form on the substrate surface and making it possible to create membranes with even porosity.

The method is usually differentiated by the type of light that is used in the process since the solutions usually are affected only by certain wavelengths or the light has some other specific property. For example, making patterned coatings with different proteins is commonly done with infrared or near-infrared light since lower energy of the infrared causes lesser damage to the protein structure. Infrared light can also penetrate thicker coatings better. [32] Patterning produced by this method can be very precise being at their smallest in tens of nanometres. [33]

Photolithography is popular method in biomedicine. For example, it is commonly used to manufacture three-dimensional polymer structures as drug carriers. [34] The method also provides a way to make various types of structures which do not necessarily require the supporting substrate after the structure is finalized. Arayanarakool et. al. produced two-dimensional polymer membrane with evenly patterned holes of 2-4 micrometers. The membrane consisted of silicone monoxide and silicone dioxide layers and had a thickness around 860 nm. The membrane was set up in cylindrical formation meant to simulate microvasculature and showed promise as an in vivo implant due to its good biocompatibility. [35]

This method is fundamental and well researched method which has led into production of other lithography techniques which do not share weaknesses of photolithography. The main problems with photolithography are the environmental factors since foreign particles, temperature changes, vibrations, humidity, air pressure and lighting have significant effect on quality of the final product. For this reason, using photolithography requires cleanroom facilities where these environmental factors can be controlled. [36] Nonetheless, this method allows great control over variables of the final product and the design of the patterns that are created. The porosity of the membrane can be designed to be extremely uniform with even pore sizes across the membrane. [37] Photolithography does also work with several other electrically conductive polymers such as polyaniline [38].

3.5 Salt leaching

Salt-leaching is a casting method which produces porous sponge like structures with a use of water-soluble salt crystals. [39] It firstly requires production of solution consisting of the wanted casting material. This solution is poured on salt bed which is then placed inside of a vacuum to extract the solvent. After the extraction of solvent, the residual cast contains the salt crystals in it. This residual cast is then placed in high temperature water which dissolves the salt particles from the cast leaving behind a sponge like structure. The temperature should be chosen so that it will not damage the morphology of the membrane. This structure is then washed with distilled water to remove possible residual salt particles. [40] The properties of the produced final scaffold can vary greatly depending on the properties of the used salt and scaffold material.

SLUP or salt leaching using powder is a different variation of salt-leaching method where using solvent in not necessary. It uses PCL (polycaprolactone) and NaCl powders which are mixed together in certain ratio and then heated to 80 Celsius to melt the PCL powder. This causes the NaCl salt particles to remain in particle form inside the forming PCL coating. After the PCL has solidified, it is then soaked in disinfected water which then dissolves the NaCl particles. This leaves behind porous PCL membrane. The NaCl particles can be around diameter 350-400 micrometer leaving similar size pores on the membrane. Membranes made by SLUP have been shown to be better at cell cultivation compared to conventional salt-leaching method. [41]

Gas-foaming method uses gas instead of salt particles to produce the desired porosity of the membrane. The most common gas used in this method is carbon dioxide. Similarly, with SLUP method it does not require any organic solvents. The process can also be done in low temperatures, so it is more suitable to temperature sensitive materials. [42] Casts produced by normal salt leaching have dense surface layer and weak connectivity between macropores. This causes poor distribution of cells on the surface of the membrane [43]. This problem was solved by e.g. Nam and Park by using ammonium bicarbonate which produces gas when dissolving causing more interconnected pores to form when using PLLA paste [44]. Similar process was used have been used with PLGA solution and citric acid, carbon dioxide and ammonia in the gas production. [43] Figure 3 demonstrates surface properties of membranes produced with combination of salt leaching and gas foaming done with carbon dioxide and natrium chloride salt.

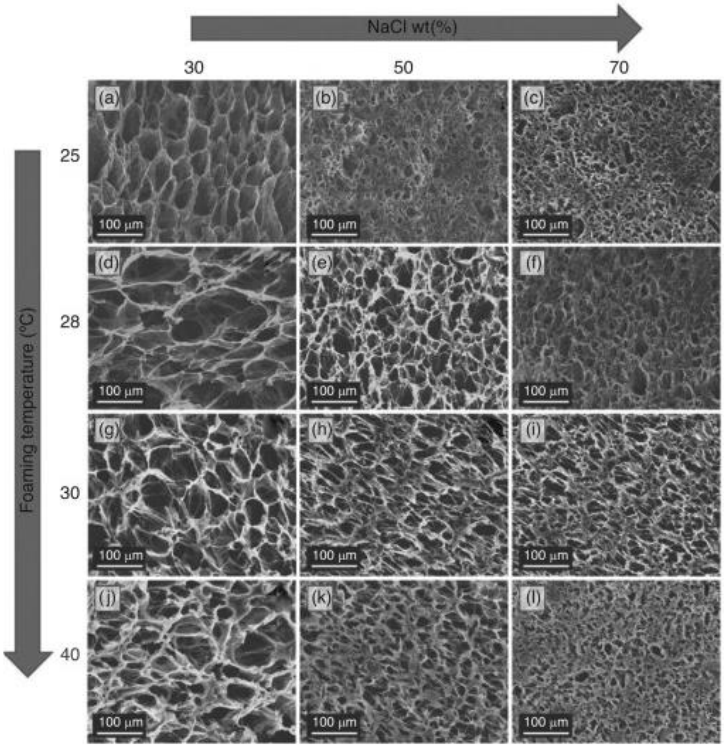

Figure 3. *Surface properties in combination of salt leaching and gas foaming process [42]*

Salt leaching method allows production of various structures with controlled porosity [43]. These structures can be shaped into different dense structures which do not need any supportive framework depending on the used material [40]. The pore sizes and porosity in the produced structures can directly be altered by altering the concentration and the size of the used salt particles [45]. Similarly, the concentration of gas producing agent affects directly to the pore concentration in gas-foaming. These methods are possible to use with various material with different properties. The processes allow production of membranes with thicknesses around 50 to 60 micrometers [46]. Salt leaching method also gives great control of the porosity and pore size of the produced membrane but had problems with cell cultivation. These problems can be effectively minimized with SLUP method or with gas foaming.

3.6 Electrospinning

Electrospinning is a complicated method of producing long thin nano fibres with electrically charged highly viscous polymer solution. These fibres are produced by stretching the polymer solution with strong electric fields. Therefore, the solution must be highly conductive to produce enough electrical force for the polymer solution to start stretching. This causes electrically charged solution to jet in controlled direction and the solvent to

evaporate. This jet can be used to produce different porous matrixes such as thin membranes. Products of electrospinning are used in variety of fields such as water filtration, tissue engineering and medicine. [47] The solution can also contain natural proteins with polymers. Natural protein fibre such as Bombyx Mori silk has been used to create scaffolds with good mechanical properties and biocompatibility. [48]

These natural fibre and silk scaffolds show great promise in wound healing applications or as cell-growth scaffolds because of good biocompatibility and cell adhesion [49]. Similarly, biodegradable polyurethane fibres have been used to create scaffolds for cell growth. Electrospinning has grown in popularity since the produced membranes and scaffolds mimic cells extracellular fibrous properties. [50] The orientations of the manufactured fibres are not controlled in the traditional electrospinning. For this reason, methods to produce aligned electrospun fibres were developed. [51]

Several parameters have a great effect on the properties of the final product. Many of these parameters are caused by the machine. Basic setup for electrospinning consists of jet needle which works also as an electrode, power supply and a collector which is usually grounded. The design of the jet tip and its distance from the collector have a significant effect on the finished product. Likewise, the strength of the produced electric field and the flow rate of the solution have a great effect. The properties of the solution also affect the diameter of the produced fibre and the structure of the final product. Such properties are the solution viscosity, temperature, concentration, and the molecular weight. Also, the ambient properties such as humidity have been shown to cause variations in the structure of the final product. To able to control the production of desired fibre formation, it is also important understand how the formation of the nanofiber from the solution happens. [47] When using different parameters, it is possible to create surprisingly varying surfaces and microstructures with electrospinning such as nano webbings and beads formations as demonstrated in Figure 4 [47, 52].

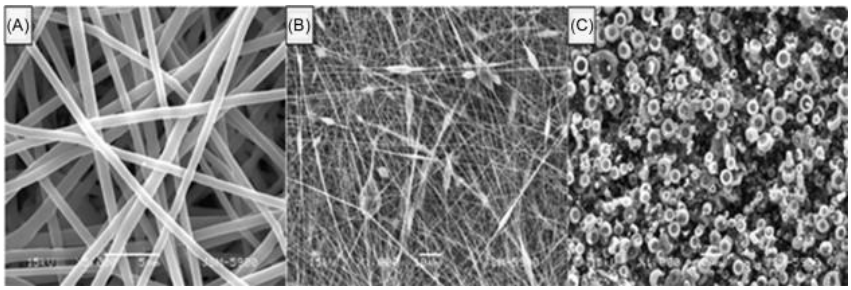

Figure 4. *Fibre variation produced with electrospinning. [47]*

Electrospinning shows great variety and benefits in thin membrane manufacturing and is still clearly important technique in many fields. It can be used to produce polymer fibre continuously which allows great production volume. It also allows control of porosity and fibre size by several different parameters. [47] Similarly, the uniformity of porosity across the membrane depends on many variables. The method is highly adaptable to different materials which allows the produced membranes to have other desired properties such as electrical conductivity by using highly conductive materials. Method is also usable with some metals, ceramics, and glasses. [47] Properties are also easy to modify more desirable after manufacturing the membranes by functionalizing them for example chemical vapor deposition, plasma modification or with chemical modification like cross-linking [52]. The mechanical properties of the manufactured membranes are also greatly affected by fibre alignment. For example, when the fibres are aligned at same directions the tensile strength of the membrane is increased. [48, 53] It also allows creation of three-dimensional structures [53]. Electrospinning can create porous membranes with various thicknesses the thinnest being around 140 µm with polyvinylidene fluoride. [54]

3.7 Soft lithography

Soft lithography is a group of different techniques which use elastomeric stamps or moulds in creation of patterns on a substrate [55]. Implantation of these patterns can be done on different types of substrates such as soft or flexible or curved substrates. These techniques also allow use of various different materials. [56] They extend the possibilities of photolithography since they use soft materials like the name suggests. In soft lithography the most common material used to produce membranes is PDMS. The manufacturing process requires master mould or stamp made from elastomeric material which is the negative or positive of the desired membrane shape depending on the technique used. Epoxy and PDMS are the most common materials used in the manufacturing the master mould and often it is manufactured with photolithography or with some other soft lithographic method. Manufacturing of this master mould can be expensive, but it allows multiple usage. [57] The soft lithography method has many different variations but the most commonly used are microcontact printing (µCP), replica moulding (REM) and solvent-assisted micromoulding (SAMIM) [56].

Microcontact printing is one of the first invented soft lithography methods and it is very often used to produce necessary structures in different biotechnology applications [56]. For example, it has been used to produce precise patterns of molecules to guide chick retinal ganglion cell axon growth [57]. µCP happens in three steps. First the membrane

material or ‘ink’ is transferred on a PDMS master stamp which in this technique is the positive of the desired pattern. After the ink has dried, the stamp is brought in direct contact with the substrate. The ink binds to the substrate and when the stamp is removed, the ink leaves desired imprint on the substrate. Used ink and substrate must be selected so that the necessary bonding is possible between substrate and ink. [56]

Replica moulding is another technique used for example to build PDMS microfluidic structures [58]. The manufacturing process starts by mixing the solution and pouring it into the mould where it is then cured. The possible air bubbles can be removed from the solution by exposing the solution to a vacuum. [59] After the removal of possible air bubbles the solvent is removed by heating the solution at an appropriate level for several hours. For example, PDMS solution is heated around 70 celsius for 24 hours to make sure all the solvent has dissipated. After this the finished membrane is carefully removed from the mould and placed on the substrate. This process is demonstrated in Figure 5. The surface of the mould is often treated with coating of some other chemical to lower the surface energy, making the removal of the membrane easier. When PDMS is used, the substrate must be treated with plasma to cause bonding between PDMS surface and glass substrate. [60]

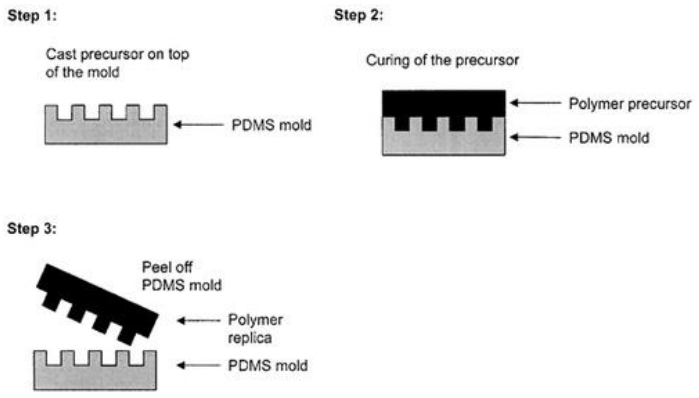

Figure 5. *Description of replica moulding [61]*

Solvent-assisted micromoulding has similar features with replica moulding. The mould in SAMIM is covered with solvent which does not affect the mould itself. The mould is the brought into direct contact with a film of desired membrane material. The solvent then dissolves the surface of the film causing it to conform to the desired membrane shape. When the solvent evaporates, the structure of the membrane hardens forming the negative of the used mould. After the solvent has evaporated, the mould can be removed. [61] SAMIM has shown some problems with forming desired sharp rectangular shaped surface structures. This problem can be minimized by distributing the solvent nonuniformly. [62]

Soft lithography provides low cost and easy set up way to produce membranes with extremely precise patterns. The pattern resolution depending on the used method can be ranging from nanometres to micrometres. [55] This allows creation of uniformly porous membranes with thickness of 4 to 5 micrometre [58]. Since the pore size and uniformity can be controlled by mould design, also permeability of nutrients can be highly controlled, and creation of several identical membranes is possible. On the other hand, soft lithography method is always dependent on creation of the master mould which requires some other lithographic method such as photolithography.

3.8 Solution casting method

The solution casting method is very simple technique where the polymer is dissolved with solvent and poured on to the supporting substrate. The solvent is then removed by drying in a vacuum or by heating in appropriate temperature leaving behind solid polymer film. Additional layers of different materials can then be added with similar method to create multi-layered film with different properties. [63,64] This method is used in various industrial processes for example in photography film production. Using the solution casting method in thin film fabrication requires using small concentrations of the wanted polymer. This type of fabrication is prone to defects, so it requires easily vaporizing solvent and careful filtration of solution to avoid any unwanted particles to produce defect-free film. Using low concentration solutions in film production has high risk of causing holes in the final film. [63] This method also describes a film production, so the method requires either modification for example by polymer casting or some kind of after-process, such as etching to produce the final thin membrane [65].

Solution casting method has been used to produce film thicknesses ranging from 150 to 12 micrometres by Carestream company by using several different materials such as polymer films and polyurethane films. [64] This method gives huge freedom of choice in used materials and solvents. This gives great control of final film properties. The structure and porosity of the produced film can also be altered by adding for example polymer particulates (polymer casting) or gas producing agent (gas foaming). The method has been used in production of porous composite scaffolds by using polymer casting. This method allowed a production of 50 to 1000 micrometre pore sizes in PLGA and PLA scaffolds. [66] Other method which uses solution casting in more specific way is honeycomb membrane production.

Honeycomb membrane fabrication is a technique produced by Fujifilm where thin membrane with highly organized pore patterns are produced. Pores in the membrane form honeycomb pattern as seen in Figure 6. giving the technique its name. This structure is achieved by placing condensed water drops with same diameter in hexagonal formation in the solution casting process. When the solvent is removed leaving behind the polymer membrane, water drops have caused formation of pores with same diameter inside the final membrane. These pores can range from 5 to 20 micrometres in diameter and the membrane thickness can range from 5 to 10 micrometres. The final membrane also has some unique properties caused by its structure. The membrane adheres clearly much more strongly to water-existing surfaces than normal films because of capillary forces. Also depending the material which the membrane is produced its oil or water absorbance and repellence can be altered. The membrane also has ability to grip on water droplets. [67]

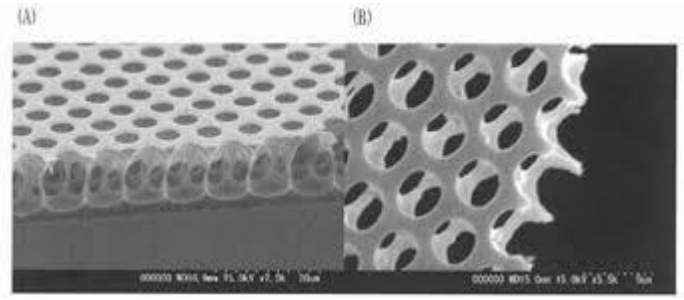

Figure 6. *Structure of honeycomb membrane [67]*

All in all, the solution casting method is a simple method which does not require any special equipment and is very applicable to various ways of use which also make production of porous membranes possible. It can be used with many different materials the most popularly with polymers such as PDMS. The electric conductivity should also be possible with conductive materials but there is not much specific information about these. The method has risk of defects and holes in thin film production because use of low concentration solutions which can be minimized with careful filtration and choosing of used material and solvent.

3.9 Sintering

Sintering is a broad description for methods which use heat to compact powders into a thin solid mass. This method is often used with ceramic or metallic powders but can be used with some polymers as well. [68] In the formation process powder particles start to fuse together because of atomic diffusion forming a membrane. This fusing causes pores between particles to get smaller which enhances characteristic such as membrane strength, electrical and thermal conductivity but reduces permeability of the membrane. The process always uses heat, but different variants can be differentiated by the application of pressure and liquids [69]. Variations concentrated in here are solid-state sintering, liquid phase sintering and pressure assisted sintering.

Solid-state sintering is a very well understood group of sintering techniques which do not apply external pressure at the material in the sintering process. It can be divided into mixed phase and single-phase techniques which are distinguished by different materials are used in the process. As the name suggest, single-phase sintering uses only one material in the process while mixed phase uses multiple. [69] Heat is often added to cause more motion between atoms in the powder since solid-state sintering methods rely on lowering of surface energy in the material [70]. Mixed phase method is used to produce composites and alloys. In composite production, one material often forms the matrix phase. Homogenization of the two materials can also happen if two materials are soluble with each other. [69]

Liquide phase sintering uses liquid phase to assist the sintering rate. It is often applied with material such as alloys and composites which melt on large range of temperature. This means that the powder can be partially in a liquid phase in the process to assist the formation of the sinter. In the sintering process, parts that are still in solid phase get pulled together by capillary forces that form with the liquid phase. The solid material also becomes softer, making the bonding between particles stronger. Porosity of the final sinter is low while the mean pore size is large compared to the particle size, since the liquid phase covers the small pores in the structure first. [71]

Pressure assisted sintering covers many different sintering techniques such as hot isostatic pressing and spark sintering [69]. All of these techniques use external pressure to assist the sintering process. Hot isostatic pressing is a method which uses gas to inflict external isotropic pressure on the material during the heating process. This way the densification of the material is achieved by combined effort of temperature and pressure.

[72] Spark sintering or spark plasma sintering describes methods where high intensity, low voltage, pulsed current is used with assist of external uniaxial pressure to produce coatings [73].

As discussed, sintering methods provide variety of ways to produce membranes or coatings with controlled average pore size and concentration. Different methods are used depending on desired properties and the material. Nonetheless, simple solid-state sintering method is able to produce porous ceramic membranes of 30 micrometers with the average pore size of 0.6 micrometers [74]. Problems with sintering methods may rise with need of similar size membranes and with controlling surface textures of the material.

4. CONCLUSIONS

Many different membrane producing methods were inspected, and their different benefits and drawback were discussed. The purpose was to broadly go through wide variety of methods and possibly find a suitable manufacturing method for a porous membrane which is able to permeate nutrients for growth of a cell culture. These methods have been summarised below with the most important properties that were found. Some discussed methods produce thin films instead of complete porous membrane which makes implementation of some pore producing method such as etching necessary.

Many of these methods showed some other important properties considering viability for cell cultures, such as Langmuir-Blodgett methods excellent gas permeability due to extreme thinness, which is the reason they are taken into the account in this work. Similarly, electrospinning method is able to produce different nanostructures, such as nano webbing which could possibly have some effect on cell growth.

Method	Thickness	Permeability	Benefits	Drawbacks
Floating-On-Water Method	1-10 µm	-	Easy and cheap	New and not widely researched
Solution Casting	5-10 µm	5-20 µm	Very simple	Prone to defects
Langmuir-Blodgett	Single molecule	Excellent gas permeability	Extremely thin	Expensive and complicated
Spin coating	1-10 µm	-	Relatively planar surfaces	Pores must be produced separately
Photolithography	860 nm	2-4 µm diameter pores	Control over final product	Requires cleanroom
Soft lithography	4-5 µm	Patterns between nm and µm	Low cost Great uniformity	Dependent on other methods
Salt leaching	50-60 µm	350-400 µm diameter pores	Great control of porosity	Some problems with cell cultivation
Electrospinning	140 µm	Excellent permeability	Great variability	Depends on many variables
Sintering	30 µm	0.6 µm diameter pores	Easy to produce	Uniformity of pore concentration

Table 1. *Summary of researched methods*

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