Tampereen yliopisto

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RHEOLOGICALLY MEASURED VISCOSITY PARAMETERS AS INDICATORS FOR 3D PRINTABILITY OF HYDROGELS

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TIIVISTELMÄ

Vilma Lehtola: Reologisesti mitatut viskositeettiparametrit hydrogeelien 3D-tulostettavuuden indikaattoreina
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3D-tulostus on ainetta lisäävä valmistusmenetelmä, missä kolmiulotteinen rakenne valmistetaan kerros kerrokselta. Hydrogeelit ovat ristisilloittuneista polymeereistä muodostuneita kolmiulotteisia verkostoja, jotka kykenevät absorboimaan suuren määrän vettä. Lääketieteellisiin käyttötarkoituksiin 3D-tulostus mahdollistaa hydrogeelien monipuolisen valmistuksen kustomoiduilla muodoilla ja suurella tulostuslaadulla. Tulostettavat rakenteet voivat olla soluvapaita tai sisältää soluja.

Tässä työssä esitellään hydrogeeleille sopivia tulostusmenetelmiä ja käydään läpi reologisia testimenetelmiä, joilla voidaan saada informaatiota materiaalin käyttäytymisestä, kun se on kuormituksen alaisena. Käytetty tulostusmenetelmä asettaa rajoituksia käytettäville tulostusmateriaaleille. Yksi merkittävimmistä ominaisuuksista on reologinen käyttäytyminen, jota voidaan tutkia reologisilla testimenetelmillä, kuten rotaatio- ja kapillaarireometreillä. Kapillaarireometri sopii materiaalin käyttäytymisen tutkimiselle suurilla leikkausnopeuksilla ja oskilloivalla mittauksella saadaan selville materiaalin viskoelastisia ominaisuuksia. Työssä lisäksi käydään läpi, miten reologiamittauksia voidaan hyödyntää hydrogeelien 3D-tulostuksessa.

Musteiden käyttäytymistä ja hydrogeelirakenteen ominaisuuksia tutkitaan, jotta tulostusprosessi olisi onnistunut ja valmistettava tuote sopisi käyttötarkoituksiinsa mahdollisimman hyvin. Reologisilla mittauksilla saadaan selville materiaalin tärkeitä ominaisuuksia, joita voidaan hyödyntää tulostusprosessissa. Matemaattisten mallien avulla voidaan mallintaa materiaalien käyttäytymistä kuorman alaisena. Lisäksi niiden avulla voidaan myös määrittää viskositeettiparametrejä, joita voidaan hyödyntää 3D tulostuksessa ja siten arvioida, ovatko musteen ominaisuudet sopivia tulostukseen.

Viskositeetti on ratkaiseva tekijä musteen sopivuudessa tulostusprosessiin. Ekstruusiotulostuksessa voidaan käyttää suurempia viskositeettejä, kun taas pisaratulostuksessa, laser-indusoidussa menetelmässä ja stereolitografiassa viskositeetin pitää olla riittävän matala. Kaksifotoniabsorptio menetelmässä viskositeetilla ei ole rajoituksia. Sopiva viskositeetti ei kuitenkaan kaikissa tapauksissa ole riittävä määrittämään musteen sopivuutta. Materiaalin viskositeetin muutoksella, geeliytymisellä ja mekaanisilla ominaisuuksilla on vaikutusta tulostusprosessin aikana ja tulostuksen jälkeen, jotta mahdollisimman muototarkkoja rakenteita saataisiin tulostettua.

Avainsanat: Hydrogeeli, 3D-tulostus, reologinen käyttäytyminen, viskositeettiparametrit

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ABSTRACT

Vilma Lehtola: Rheologically measured viscosity parameters as indicators for 3D printability of hydrogels
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3D printing is an additive manufacturing technique, where a three dimensional structure is manufactured layer by layer. Hydrogels are three dimensional networks formed from crosslinked polymers that are capable of absorbing large amounts of water. For medical applications, 3D printing enables the versatile production of hydrogels with customized shapes and high printing quality. The printed structures can be cell-free or contain cells.

In this thesis is presented suitable 3D printing methods for hydrogels. There is also propound rheological test methods, which can provide information about the behavior of the materials when they are stressed. The utilized printing method sets limitations on the printing ink that can be used. One of the most significant properties is the rheological behavior, which can be studied with rheological test methods, such as rotational and capillary rheometers. The capillary rheometer is suitable for studying the behavior of materials at high shear rates and the oscillatory rheometer is suitable for studying viscoelastic properties of the material. In addition, in this thesis is also discussed how rheology measurements can be utilized in 3D printing of hydrogels.

The behavior of inks and the properties of the hydrogel structure are studied so that the printing process is successful and the manufactured product is as suitable as possible for its intended use. Rheological measurements reveal important properties of the material that can be utilized in the printing process. Mathematical models can be used to model the behavior of materials under load and can also be used to determine viscosity parameters that can be utilized in 3D printing. The results gives information about the properties of the inks and tells are they suitable for printing.

Viscosity is an important factor in the suitability of the ink for the printing process. Higher viscosities can be used in extrusion printing, while in droplet, stereolithography and laser-induced forward transfer printing, the viscosity should be relatively low. In the two-photon absorption printing there are no limitations on viscosity. However, a suitable viscosity is not the only important property to determine the suitability of the ink. The change in viscosity, gelation and mechanical properties of the material have an effect during and after the printing process, in order to print structures with the most accurate shape possible.

Keywords: Hydrogel, 3D printing, rheological behavior, viscosity parameters

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PREFACE

This bachelor's thesis was completed in the Faculty of Medicine & Health Technology in the Biomaterials & Tissue Engineering Group at Tampere University. I want to thank my supervisor Janne Koivisto for his patience, support, guidance and advice during the writing process.

Tampere, on 23 January 2025

Vilma Lehtola

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LIST OF SYMBOLS AND ABBREVIATIONS

3D	Three dimensional
CIJ	Continuous inkjet
CSD	Controlled shear deformation
CSR	Controlled shear rate
CSS	Controlled shear stress
DOD	Drop-on-demand
ECM	Extracellular matrix
LIFT	Laser-induced forward transfer
PEG	Polyethylene glycol
PLA	Polylactic acid
SLA	Stereolithography
TPP	Two-photon polymerization

A	area
E	elastic modulus
F	force
G'	storage modulus
G''	loss modulus
h	height
K	consistency index
n	flow index
Oh	Ohnesorge number
t	time
v	velocity
η^*	complex viscosity
$tan\delta$	loss factor
ω	angular frequency
ε	deformation
τ	shear stress
γ	shear strain
$\dot{\gamma}$	shear rate
σ_E	tensile stress
η	shear viscosity
η_∞	viscosity at an infinite shear rate
η_B	plastic viscosity in Bingham model
η_c	plastic viscosity in Casson model
τ_0	yield stress

1. INTRODUCTION

3D printing is a branch of additive manufacturing technique where a 3D structure is made layer by layer through computer-aided design. With this technique it is possible to manufacture customized complex three dimensional structures from different materials like polymers, metals, glass or ceramics. The advantage of 3D printing is its flexibility and cost-efficient thus it is less material wasteful, requires less tooling and machining and does not need molds. Because of these, 3D printing it is a good manufacturing technology to easily make prototypes or small batches for personalized medicine, and is economically favorable. [1]

One material group that can be 3D printed is hydrogels, that are 3D networks that have ability to absorb a large amount of fluid, typically water. Usually, the network is formed by a crosslinked polymer. Hydrogels can be made from natural or synthetic polymers. [2], [3] There is wide range of hydrogels and their physical, chemical and biological properties are tailorable. This makes possible to use hydrogels in various different applications. One of these is biomedicine. Hydrogels have several advantages that make them suitable for tissue engineering, regenerative medicine, in vitro tissue modeling, soft robotics and flexible wearable electronics. [1] In addition to modifiable properties, hydrogels are good materials for biomedicine due to their high hydrophilicity, biocompatibility, cell-friendly properties and ability to mimic ECM [4]. Hydrogels are the base materials in the 3D printing process and can be printed as such or with cells or other biological molecules [3].

The rheology of hydrogels is one of the most important material properties in 3D printing of hydrogels. Rheology is the study of material's flow and deformation under the influenced forces. Rheology can be used to model material behavior, what can be utilized in manufacturing for example in material selection, choosing a processing method or deciding on processing parameters. [5] In 3D printing of hydrogels rheology is needed to making sure that the printing process is successful and the product is of high quality. [6] The technology used for measurements is called rheometer. There is many rheometers that are usable for measuring hydrogels, but the choice of method is influenced by the desired characteristics. [7]

In this bachelor thesis will be reviewed how to 3D print hydrogels, how rheological properties of materials are studied, and how the obtained results can be utilized in 3D printing of hydrogels.

2. 3D PRINTING OF HYDROGELS

2.1 3D printable hydrogels

Hydrogels are one of the most promising materials in biomedical 3D printing [1]. Hydrogel is a non-fluid colloidal or polymer network that can absorb large amounts of water. Usually, the network is formed by polymers. Hydrogels or hydrogel precursors are used as printing materials, i.e. inks in 3D printing. [8] The precursor, i.e. a pre-hydrogel solution is non-crosslinked monomer or polymer solution that forms a hydrogel when chains attach to each other by crosslinking. Crosslinking results to gelation of the precursor into hydrogel. [9] Crosslinking can be made due physical or chemical crosslinking and can occur before, during or after the printing process, for example due to heat or UV light. Crosslinking type and density has an impact on the hydrogels properties and printability. [1], [10]

The polymers used in hydrogels can be from natural or synthetic origins. Natural polymers are derived from living organisms including human, animals, plants and bacteria. Alginate, cellulose and gelatin are examples of natural polymers. Synthetic polymers are chemically produced from their monomers. Polyethylene glycol (PEG) and polylactic acid (PLA) are two example of a synthetically produces biomaterials [11]. Both natural and synthetic polymers are used in hydrogels in biomedical applications, but there are some differences in their properties. [1], [3] The advantages of natural polymers are excellent biodegradability, biocompatibility and the bioactivity [11]. However natural polymers are generally weaker compared to synthetic polymers, which limits their usability [3], [12]. That is why some times it is used semi-synthetic polymers that are modified natural polymers. Polymers can be modified to improve the physical and mechanical properties but at the same time some properties can deteriorated. [1], [12]

In hydrogel-based printing there is possibility to print only hydrogel material or hydrogel material combined with living cells. In these cases, different terms are used for inks. If only the hydrogel material is printed and the ink does not contain living cells, i.e., it is cell-free, the ink is called biomaterial ink. If the ink contains living cells in the matrix or medium, i.e., the ink is cell-laden, then the term bioink is used for the ink. In addition to

cells, biomaterial ink or bioink can contain other biological molecules such as peptides, growth factors, proteins and DNA and those can be printed with hydrogels. [1], [4]

2.2 Applications of 3D printable hydrogels

The advance of hydrogels as a biomaterial in 3D printing is their tunable material, physical, chemical and biological properties like mechanical strength, crosslinking and degradation. This enables to modification of the material properties to suit desired applications or manufacturing methods. Other advantages of hydrogels are good biocompatibility and high water content, which makes the material properties similar to natural tissue. Hydrogels can also provide extracellular microenvironments for various cell functions, such as attachment, migration, reproduction and differentiation in 3D environment. [1]

Due the properties mentioned above, 3D printed hydrogels can be utilized in several biomedical applications. These are, for example tissue engineering and regenerative medicine, in vitro tissue modeling, drug delivery and novel devices. Tissue engineering and regenerative medicine is replacing, repairing or regeneration of missing, damaged or diseased tissue or organ. The purpose of tissue modeling is model native human tissue. It can be used for in vitro disease modeling, drug development and cancer research. 3D printed hydrogels can also be used to deliver drugs locally and with controlled discharge, in soft robotics, for example as a stimuli responsive materials and printing electrons such as wearable flexible biosensors, implants and other devices. With these applications can be done faster, cheaper, effective and safe drug screening and testing, and be used more effective drug treatment with fewer adverse effects. Utilizing these applications can also reduce the need for animal testing, the need and limitations for tissue and organ donors, and the risk of transplant rejections. [1], [11]

2.3 3D printing methods

3D printing is an additive manufacturing technique where 3D structures is made by adding materials layer-by-layer. It’s one part of rapid prototyping process, which also includes other steps such as model preparation, 3D modelling and post processing. [13] 3D printing allows fabrication of complex structures with complicated geometries and enables printing many materials and components at the same time. The technique

improves adaptability, efficiency and printing quality compared to traditional methods. [11] Printing quality is described by the term shape fidelity, which refers to how well the printed structure and the original computer model matches. The printing quality is also affected by the resolution, i.e. how small details can be produced by printing. [5]

In 3D printing of biomaterials there is possibility to categorize the methods into three main groups (figure 1). The classification is based on their working principles. The techniques are extrusion-based printing, droplet-based (also inkjet) printing and laser-based printing. [13] This chapter presents a few methods and their basic working mechanisms that are suitable for 3D printing of hydrogels.

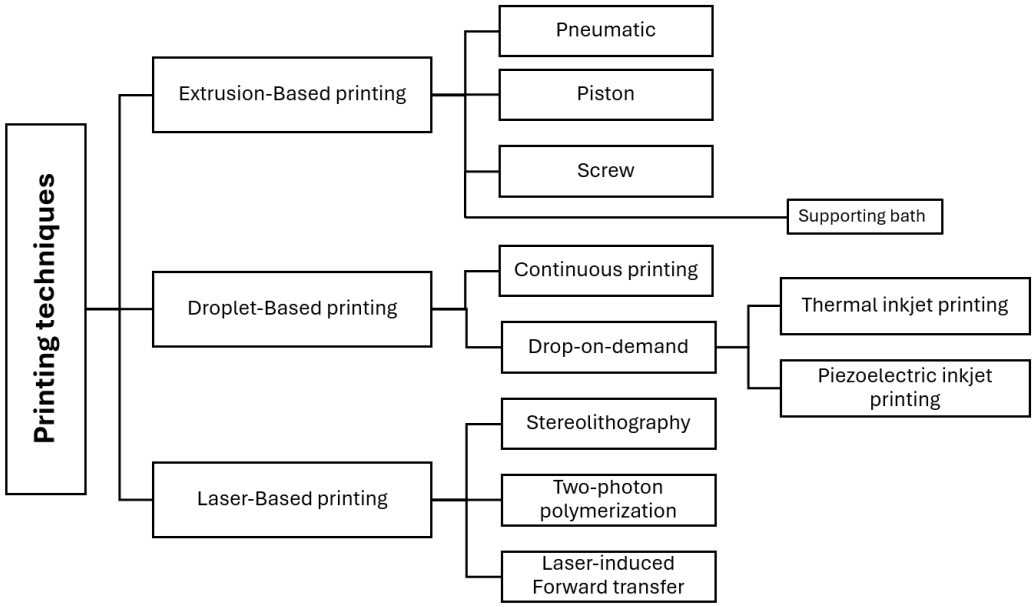

Figure 1: Classification of 3D printing methods. Adapted from [3].

2.3.1 Extrusion-based printing

Extrusion-based printing is one of the most common 3D printing technique [14]. In extrusion-based printing the ink is extruded thru a nozzle by pressure. The pressure can be achieved by pneumatic, piston or screw (figure 2). Created pressure by pneumatic is not as high as it is created by a piston or screw. The highest pressure is achieved with screw extrusion, which makes possible to print higher viscosity materials with it. Steady pressure creates an even flow of biomaterial. [1], [13] Depending on the material that is printed in extrusion, it can be already crosslinked or the crosslinking can occur during or

after the printing. [1], [10]. With this method, it is possible to achieve resolution to 100 µm. [15]

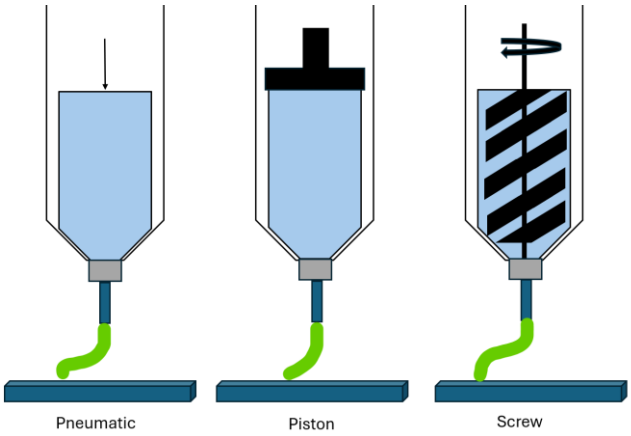

Figure 2: Extrusion based printing methods. Adapted from [1].

2.3.2 Printing into gel or suspension bath

Printing into supporting (also support or gel) bath enables printing complex well-defined structures from low viscosity and mechanically weak materials without settling and collapsing and also makes possible to manufacture hollow structures. There are two different methos depending on whether it is wanted to create a structure from ink or support bath material (figure 3). It is done by crosslinking the remaining material and removing the unwanted material. If the support bath material is removed after the ink is crosslinked, the method is called support-bath enabled 3D printing. If the ink is removed after the support bath material is cured, it is called embedded 3D printing and the term sacrificial ink is used for ink material. [16], [17] The properties of support bath has also an impact on final product [18].

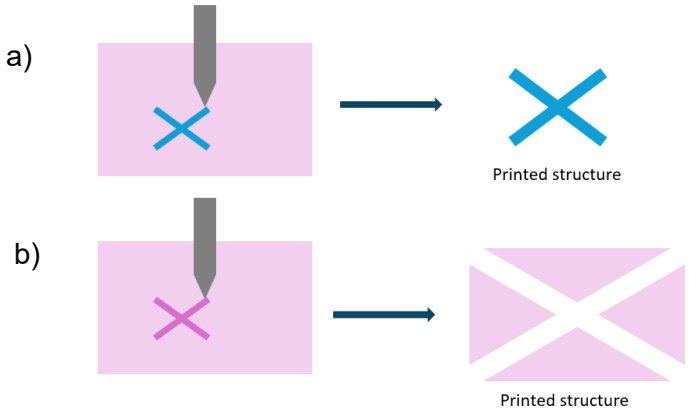

Figure 3: Printing into supporting bath. a) Crosslinking of ink and bath removal. b) crosslinking of bath and ink removal.

2.3.3 Droplet-based printing

Droplet-based printing (or inkjet printing) is drop-by-drop manufacturing technique, where the 3D scaffold is formed by adding a material one drop at a time. Printers can be classified into two groups: the drop-on-demand (DOD) jetting system and continuous inject (CIJ) system. Continuous inkjet system is a technique where ink is forced out of a print head continuously generating a jet. The jet breaks into droplets, which movement are controlled by electrical signals. The method is similar to traditional inkjet printer. The drop-on-demand jetting systems the main operative principle is eject individual droplets due the effect of pulses. There are different mechanisms for the droplet formation (figure 4). One is called thermal inkjet system, where a voltage pulse heats the ink and forms a vapor bubble. When the bubble expands rapidly and collapses, a pressure pulse is formed and the surface tension is exceeded in the nozzle, which leads to the formation of a droplet. Another mechanism is called piezoelectric inkjet system, where a voltage pulse causes a change in the volume and the droplet is formed, when the surface tensions overcome in the nozzle. The method makes it possible to achieve a high resolution of 50-300 µm but it is not favorable method for printing larger structures. Generally, the droplet printing is used to make smaller structures and extrusion printing to make larger structures. [1], [15]

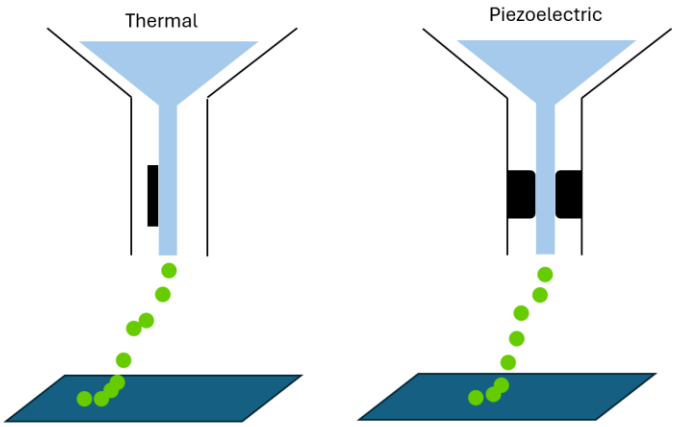

Figure 4: Drop-on-demand printing systems. Adapted from [1].

2.3.4 Laser-based printing

There are several laser printing techniques, but all the techniques are not suitable for printing hydrogels. Techniques that printing of hydrogels is possible are, for example stereolithography (SLA), two-photon polymerization (TPP) and laser-induced forward transfer (LIFT). The basic principle in all method is use light energy to create 3D structures. SLA and TPP method are only usable for photo-crosslinkable precursors. In stereolithography (Figure 5) a laser source, which is usually UV-light, is pointed to photocurable liquid resin. The 3D structure is formed by curing the resin layer by layer in 2D pattern. A new layer of resin is added on top of the hardened layer by moving the fabrication substrate up or down. Usually post treatments like cleaning the item and post-hardening is necessary. [1]

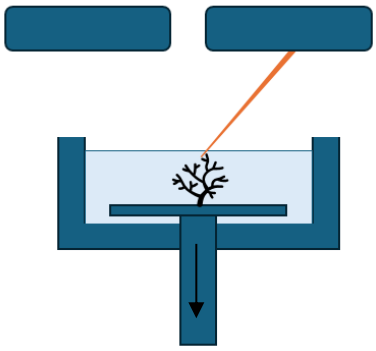

Figure 5: Working principle of SLA printing. Adapted from [1].

Two-photon polymerization (figure 6) is a technique to cure photosensitive materials using radiation. TPP is based on the principle of two-photon absorption (TPA), where the

photoinitiator absorbs two photons to be excited the similar energy state as, when excited by one photon of higher energy. After absorbing photons, the photoinitiator generates reactive radicals that initiates the polymerization. The process is utilizing femtosecond laser pulses, for example light of near-infrared range. The absorption happens in focal point of the pulse and thus the polymerization can also occur elsewhere than the surface of the ink. The method enables the mild processing conditions, which prevents photochemical damage and produces high-resolution scaffolds. The sizes of the structures is relatively small. [1],[19] Resolution as 100 nm is possible to achieve with this method. [15]

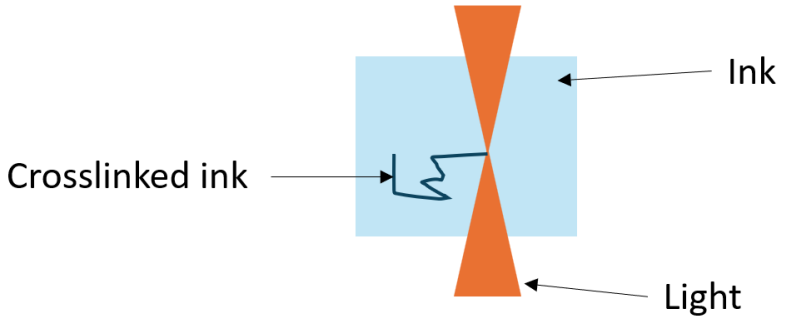

Figure 6: Working principle of TPP. Adapted from [1].

Laser-induced forward transfer technique differs from previous ones in that the laser beam is not focused directly on the ink (figure 7). The laser pulse is focused onto a thin layer of laser-absorbing material through laser-transparent donor substrate. Energy absorption causes the ink detach in the form of a droplet from the coating to toward the acceptor substrate. This method is good for sensitive materials that can be damaged by intense UV rays. [1] The resolution can even be 20-80 µm [15].

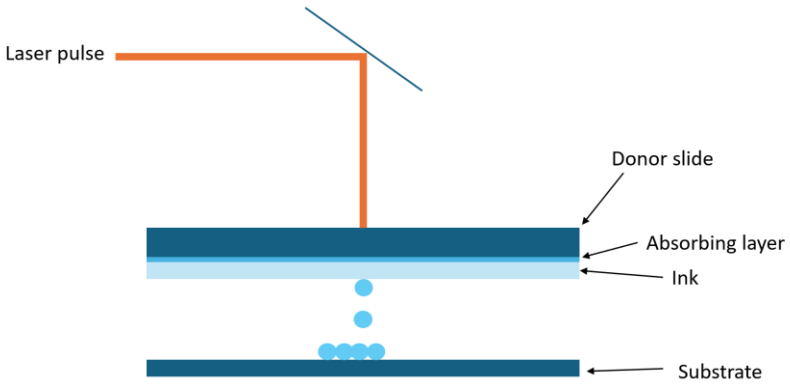

Figure 7: Working principle of LIFT. Adapted from [1].

3. RHEOLOGY AND VISCOSITY

Rheology is the study of material's flow and deformation under the influenced forces. [6] Rheology gives more information about flow behavior of liquids and deformation behavior of solids [7]. It is used in many fields, for example in material science and food industry and it can be applied in many applications in technologies and engineering of materials. The rheological properties of materials affect resolution, shape fidelity, shape retention (i.e. the ability of printing filament to retain its shape after printing) and processability, and that is the reason, why flow and deformation behavior are studied. Many other properties have also an effect on the rheology, for example type, degree and duration of loading, temperature, and other parameters such as concentration, pressure, and pH value have an influence on rheological behavior. [6], [7], [14]

Viscosity is one term that describe rheological behavior. Viscosity describes the ability of material to resist flow. For example, low viscosity materials like water flows easily but high viscosity materials like honey flows slower. Types of viscosity is more than one but often just the term viscosity refers to shear viscosity. Shear viscosity (η) is the ratio between shear stress (τ) and shear rate ($\dot{\gamma}$) as it is described in equation 1. [7]

$$\eta = \frac{\tau}{\dot{\gamma}} \tag{1}$$

The shear rate and shear stress such as other rheological parameters are defined often by using the two-plates-model (figure 8). In this model the matter is placed between two plates where other plate stays stationary and other is in motion by shear force.

Figure 8: Illustration of two-plates-model. Adapted from [7].

Shear stress is described in equation 2. It is a relationship between shear force and area of the plate.

$$\tau = \frac{F}{A} \quad (2)$$

where τ is shear stress*, F* shear force and *A* is area of the plate.

Shear rate can be calculated in two different ways that are presented in equations 3 and 4.

$$\dot{\gamma} = \frac{v}{h} \quad (3)$$

$$\dot{\gamma} = \frac{\gamma}{t} \quad (4)$$

where $\dot{\gamma}$ is shear rate, v velocity and *h* is the distance between two plates, γ shear strain and *t* is time. [6], [7]

3.1 Rheological behavior

Materials can be classified into different groups based on the behavior of matter. The classification can be made by different perspectives. [6], [14] One determining factor is change in viscosity when stressed. The viscosity may remain constant or change as the shear rate changes while other conditions remain unchanged. Newtonian fluid refers to a substance whose viscosity is independent with changes in shear rate or shear stress and in these cases also shear stress and shear rate are linearly related (figure 9) [6]. For example, water is a Newtonian fluid. Non-Newtonian fluid's viscosity is not constant in certain temperatures and pressures like Newtonian fluids. Viscosity changes as shear stress or shear rate changes and the ratio between shear stress and shear rate is not linear (figure 10). [7]

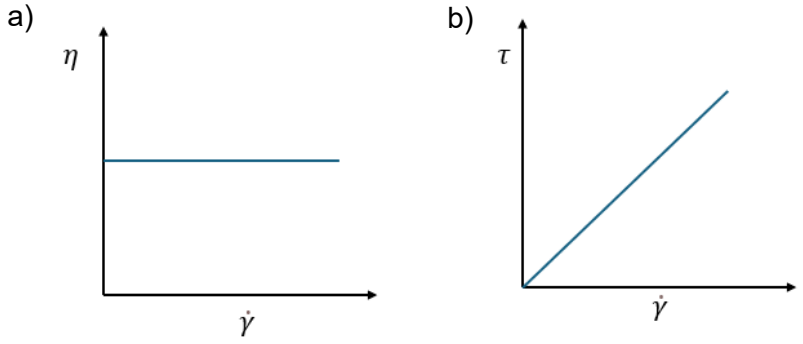

Figure 9: Newtonian flow behavior: a) viscosity as a function of shear rate and b) shear stress as a function of shear rate.

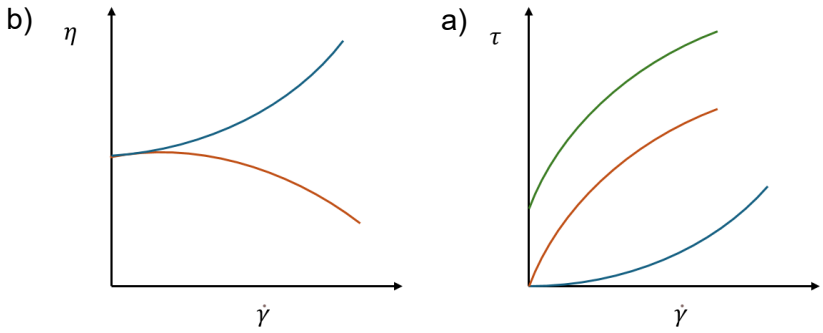

Figure 10: Non-Newtonian flow behavior: a) viscosity as a function of shear rate and b) shear stress as a function of shear rate. Red is shear thinning, blue shear thickening and green viscoplastic fluid.

Non-Newtonian fluid behavior can be divided into three categories: time-independent, time-dependent and viscoelastic behavior (figure 12). Time-independent behavior means that material behavior does not depend on the duration of the load. The behavior can be shear-thinning, shear-thickening or viscoplastic. The viscosity of shear-thinning material decreases when shear rate increases. Shear-thickening behavior is opposite of shear-thinning. Viscosity of shear-thickening materials increases when shear rate increase. Viscoplastic materials are materials that start to deform after a large enough force is applied. This force required for material to begin deform or flow is called yield stress or yield point (τ_0). The material that has a yield point but the relationship between

shear stress and shear rate is linear, is classified as Bingham plastic (figure 11a). If the relationship is not linear it is classified as a Bingham pseudoplastic (figure 11b). [14]

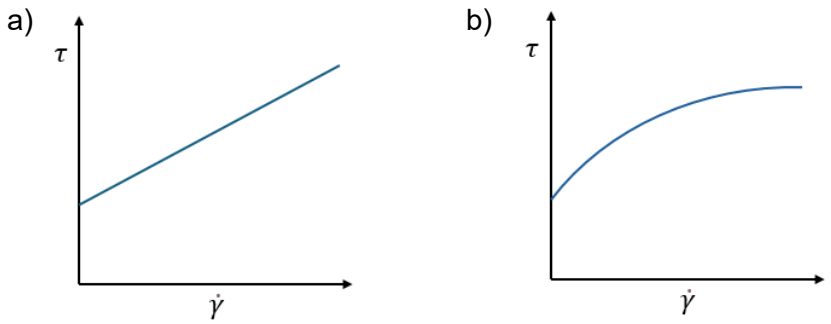

Figure 11: The shear stress as a function of shear rate. Difference between a) Bingham plastic and b) Bingham pseudoplastic.

Time-dependent fluid's behavior depends on duration of applied stress. There are two types of time-dependent fluids: thixotropic and rheopectic fluids. The viscosity of thixotropic fluids decreases over time when the shear rate and temperature remain constant. On the other hand, at the same conditions the viscosity of rheopectic fluids increases over time. To thixotropic and rheopectic fluid behavior it is not possible to depict simple mathematical equations. The recovery of viscosity after the load or stress is removed is an important characteristic of time-dependent fluids. This means the return of the viscosity to the pre-load level. The term recovery time in turn refers to the time it takes for viscosity to recover. [14]

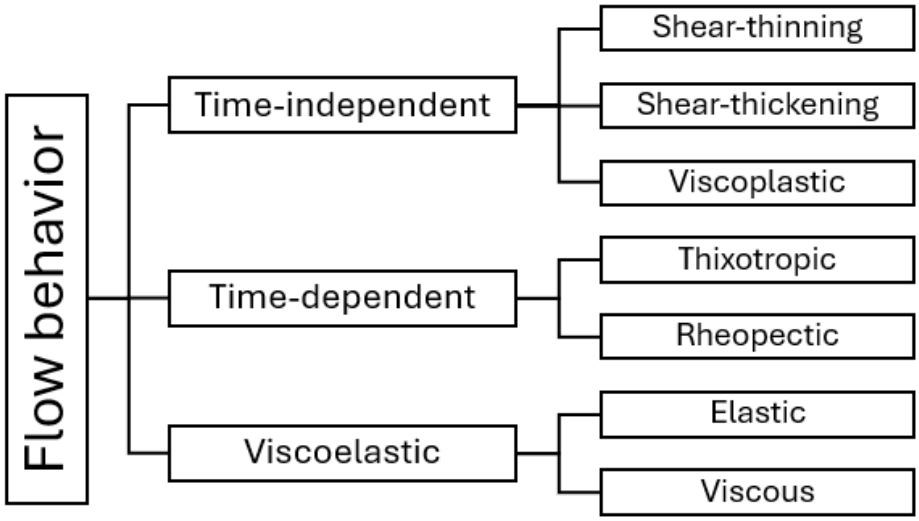

Figure 12: Classification of non-Newtonian fluid flow behavior. Adapted from [14].

The picture shows different classifications on flow behavior of non-Newtonian fluids based on response to shear stress. Often materials show not just one feature as most materials exhibit more than one non-Newtonian behavior [14].

The behavior of materials can also be divided into elastic, viscous and viscoelastic behavior. [6] Ideal elastic material starts deform immediately after its loaded or stressed and after the load or stress is removed the matter returns completely and immediately to its original shape. Elastic behavior can be modelled with a spring (figure 13a). The deformation of ideal viscous material happens at a constant deformation rate when force is applied. When the force is removed, there is not recovery and material do not return to its original shape. The change of shape is therefore irreversible. Viscous behavior is modelled by a dashpot (figure 13b). Viscous and elastic behavior can be also presented as Newton' and Hooke's models that are presented in the chapter 3.3 in equations 5 and 6. If the materials shows both elastic and viscous behavior, it is called viscoelastic material. The change of viscoelastic materials is partially reversible and occurs with a delay. Viscoelastic material behavior is so something between ideal elastic and ideal viscous and it can be modelled by combining viscous dashpot and elastic spring. [7] Two simple viscoelastic models are Maxwell-element (figure 13c) and Voigt-Kelvin model (figure 13d). In Maxwell- element the dashpot and the spring is in series and in Voigt-Kelvin model the dashpot and spring are in parallel. Polymers behaves usually viscoelastically. [6]

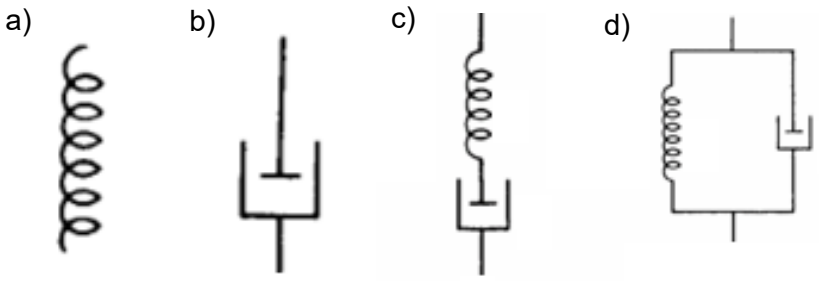

Figure 13: Models for a) elastic, b) viscous and c-d) viscoelastic behaviors. Adapted from [6].

3.2 Rheology and viscosity measurement methods

Technology that is used to measure and analyze the rheological properties of materials, is called rheometer. There are different types of rheometers, which can be used

measuring different parameters of different materials. Rotational and capillary rheometers can be used to measure rheological properties of hydrogel precursors and hydrogels. [7]

3.2.1 Rotational rheometer

The rotational rheometer is one technique to determine rheological characteristic of hydrogels. The measurement method is often modelled by two-plates model, where a sample is placed between two plates. One of the plates moves, when the other one usually the bottom plate stays stationary. The movement of the plate causes deformation of the material. There are many different geometries, which the most common are parallel-plate, cone-and-plate, and cup-and-bob (figure 14). The measurement is done by moving the upper part (i.e. bob) of the device. The movement can be unidirectional or back and forth. Both are used for hydrogels but they measure different parameters. [7]

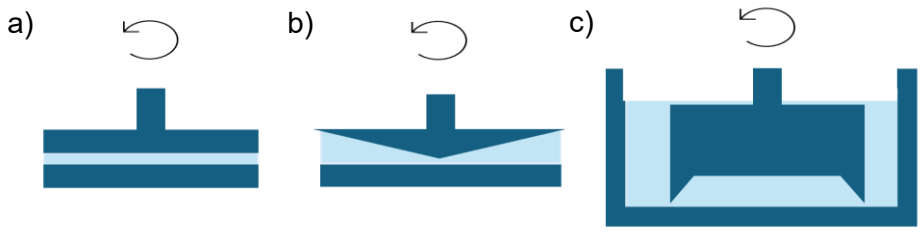

Figure 14: Geometries for rotational testing a) parallel-plate, b) cone-and-plate and c) cup-and-bob. Adapted from [20].

With a rotational test, where the movement is unidirectional, can be determined the viscosity of material. The test can be done in two different ways: controlling the shear rate (CSR) or controlling the shear stress (CSS). In CSR test the shear stress is measured in different shear rates and in CSS test the shear rate is measured in different shear stresses. The result is a flow curve showing the viscosity or as a function of shear stress or function of shear rate. In addition, test can show the stress required for deformation as a function of shear rate or shear stress. [7]

In test method where the bob is moving back and forth, the term oscillatory test is used. This test method is used for determining the viscoelastic properties of materials. The test is done by controlling the shear deformation (CSD) or the shear stress (CSS). In CSD the results are the torque and the phase shift angle and in CSS the result is the deflection angle and the phase shift angle. The results are used to determine the deformation and shear stress. Oscillatory test can also be performed changing amplitude while keeping

the frequency as a constant and vice versa. With these methods is possible to determine viscoelastic parameters for example storage modulus (*G')* and loss modulus (*G")*. *G'* represent the elastic behavior and *G"* the viscous behavior of material. It is also possible to measure loss factor (tan*δ)*, what is the relationship between *G'* and *G".* When *tanδ* < 1, *G'* > *G"*, and materials behave more solid like than viscous like. The where *G'* = *G"* (i.e. *tanδ* =1) is called gel-point and the behavior chances from liquid-like to solid-like. This kind of transition is caused due to gelation, which means the crosslinking and the forming of 3D structure from polymer chains. Determining the viscosity directly is not possible with oscillatory test. Instead, determination of complex viscosity (η^*) is possible with using *G'*, *G"* and angular frequency (ω). Complex viscosity means resistance of viscoelastic flow and is depending on angular frequency. Also determining other parameters like yield stress or testing material characteristics like creep or recovery is possible with oscillatory tests. [6], [7], [14]

However, rotational rheometers have limitations. With rotational rheometers, measurements can only be made at low shear rates and all the materials are not suitable for all geometries. Cup-and-bob geometry is better for low viscosity materials than parallel-plate or cone-and-plate, because the material cannot flow out of the cup just as easily. Cone-and-plate is not suitable when the sample contains large particles and the size of the particles affects the size of the gap in parallel-plate. [7]

3.2.2 Capillary rheometer

Capillary rheometer (figure 15) is used for measuring the viscosity of material by pushing the material with a piston through a die and measuring the required pressure to cause it to flow. Usually, the test is done by controlling the speed of the piston and measuring the pressure drop across the capillary with pressure transmitter on the barrel near the die or force transmitter what is on the top of the piston. With the speed of the piston and its geometry, the flow rate is determined. It is also possible to control the pressure and measure the flow rate. The advantage of the capillary rheometer is possibility to use higher shear rates than with rotational rheometers. [21] The movement of the piston is unidirectional so oscillatory measurements are not possible with capillary rheometer. However, with capillary rheometer it is possible to better study the material behavior in processing conditions or processes where shear rates can be high. For example, this metho is suitable for testing and illustrating how materials behave in extrusion based 3D printing, where the shear rates can become high. [20]

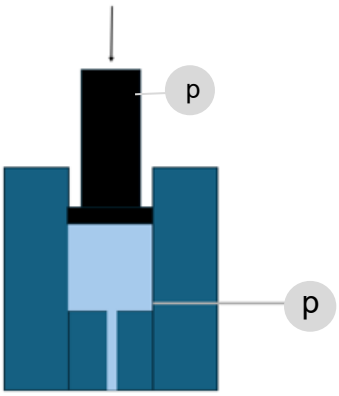

Figure 15: Capillary rheometer device. Adapted from [21].

3.3 Equations for modelling the flow behavior

There are a lot of models to model the behavior of materials [7]. The simples models to model rheological behavior are Newton's law that is described in equation 5 for idealviscous fluids and Hooke's law is described in equation 6 for idealelastic materials [22].

$$\frac{F}{A} = \tau = \eta\dot{\gamma} \tag{5}$$

where *F* is force, *A* is area, τ is shear stress, η is viscosity and $\dot{\gamma}$ is shear rate.

$$\varepsilon = \frac{\sigma_E}{E} \tag{6}$$

where ε is deformation, σ_E tensile stress and *E* elastic modulus. [14]

Most polymers have viscoelastic behavior, so other models are needed [22]. A few of them will be reviewed below.

3.3.1 Shear-thinning and shear-thickening materials

Neither Hooke's or Newton's law are not suitable for materials that viscosity changes as a function of shear rate or materials that behavior is viscoelastic, so other models are needed to describing the behavior of them. These models are for example Power law (or Ostwald-de Waele) and Sisko model.

Power law model, that described in equation 7, is utilized to model shear-thinning and shear-thickening flow behavior. The model is often used in the polymer industry. Power law is one of the most used mathematical models for shear thinning fluids.

$$\tau = K\dot{\gamma}^n \tag{7}$$

where τ is shear stress, *K* is consistency index, *n* is the flow behavior index and $\dot{\gamma}$ is shear rate. [14] Power law can be also presented:

$$\eta = K\dot{\gamma}^{n-1} \tag{8}$$

where η is viscosity. [14]

The value of *n* describes the shear behavior of material. Material behaves as a Newtonian or idealviscous fluid when *n* = 1. If *n* > 1 material behavior is shear thickening and if n<1 material is shear thinning. [14] A disadvantage of this model is that fitting in the low-shear and high-shear range is difficult i.e. it is suitable for medium shear rates. [7].

Sisko model is described in equation 9. It is a modification of Power law. The model considers the viscosity values when the shear rate is high.

$$\tau = \eta_\infty \cdot \dot{\gamma} + K\dot{\gamma}^n \tag{9}$$

where η_∞ is viscosity at an infinite shear rate. [14] Sisko model can be expressed also:

$$\eta = \eta_\infty + K\dot{\gamma}^{n-1} \tag{10}$$

3.3.2 Viscoplastic fluids

When modelling the behavior of viscoplastic materials, yield point must be taken into account, because viscoplastic materials start to flow after the yield stress is reached. Bingham model and the Herschel-Bulkley model are the most common models used to describe viscoplastic fluids. The third model that is often used for viscoplastic fluids is Casson model.

Bingham model is represented in equation 11. It used when the relationship between shear stress and shear rate is linear after materials start to deform. [14]

$$\begin{aligned} \tau &= \tau_0 + \eta_B(\dot{\gamma}) \quad if\ |\tau| > |\tau_0| \\ \dot{\gamma} &= 0 \qquad\qquad\quad if\ |\tau| < |\tau_0| \end{aligned} \tag{11}$$

where τ_0 is yield point and η_B is plastic viscosity.

Bingham model is not well suitable for materials which relation of shear stress and shear rate is not linear, so for this kind of materials Herschel-Bulkley model is better. The equation of Herchel-Bulkley is described in equation 12. Herschel-Bulkley is an adaptation of Bingham model. [20]

$$\begin{aligned} \tau &= \tau_0 + K\dot{\gamma}^n \quad if\ |\tau| > |\tau_0| \\ \dot{\gamma} &= 0 \qquad\qquad\quad if\ |\tau| < |\tau_0| \end{aligned} \tag{12}$$

Casson model is also used to model viscoplastic fluids and is suitable also for materials that relation of shear stress and shear rate is not linear. Casson model can be more precis than Bingham model at the low shear rates [14].

$$\begin{aligned} (\tau)^{\frac{1}{2}} &= (\tau_0)^{\frac{1}{2}} + (\eta_c\dot{\gamma})^{\frac{1}{2}} \quad if\ |\tau| > |\tau_0| \\ \dot{\gamma} &= 0 \qquad\qquad\qquad\qquad\quad if\ |\tau| < |\tau_0| \end{aligned} \tag{13}$$

where τ_0 is yield point, η_c= plastic viscosity and $\dot{\gamma}$ is shear rate. Plastic viscosity in Casson and Bingham model is the viscosity of material at very high shear rates. [7], [22]

4. UTILIZATION OF PARAMETERS

In this chapter is discussed the material properties of hydrogels required by different 3D printing methods to achieve successful printing results and how viscosity parameters can be utilized in 3D printing. Different printing methods have ink behavior requirements, that may also vary depending on the printing stage.

Extrusion printing is one of the most common additive manufacturing techniques and there is a lot of information about the rheological properties of the inks. However, other parameters such as printing speed, nozzle size and the force used can also affect printing and ink behavior. On the other hand, the ink behavior reciprocally effect on printing window, which tells which printing speeds or pressures can or should be used. In laser based and droplet-based printing, viscosity parameters are not always the most critical factor. The result can be controlled by, for example the properties of the laser beam (e.g. light energy, wavelength) and used amplitude. In droplet printing and LIFT the density and surface energy has also an impact on the droplet formation. [14], [15], [23]

In extrusion-based printing there is three critical stages, where the rheological properties of material have an effect on the printing of hydrogels. These stages are (1) the material behavior in the nozzle before extruding, (2) the behavior after extruding, and (3) the behavior, when the material lands on the substrate. The behavior in these stages affects the final result of the of the printing process. [14] In inkjet printing there are two critical stages: the formation and detachment of an ink droplet, and the impact and reaction of the droplet with the substrate. In laser-based printing the ink properties have to be suitable for photocuring. [15], [24]

4.1 Rheological behavior during and after printing

There are recommended viscosities for the printing methods. In extrusion it is 30-6·10^7 Pa·s, in SLA it is below 5 Pa·s, in LIFT 1-300 mPa·s and in inkjet printing it is 3.5-12 mPa·s. In TPP there aro no limitations. [15] In extrusion printing where the mechanical force can achieve high values, the viscosity range is wide. The viscosity should however be high enough that the printed structure does not collapse after printing. With the higher viscosities the inks are mechanical stronger after printing, but the printing is harder. The

shear thinning and thixotropic nature are suitable characteristics, because it reduces the flow resistance and make the ink flow steadier. After the extrusion the shear stress is removed and the ink should recover rapidly. Rapid shear thinning and thixotropic recovery is relevant to the formation of the printable structure and its mechanical stability. The shape fidelity and resolution increases, if the flow is steady, and the structure do not collapse due the weight of new printing layers. Fast recovery also improves the printing speed, when the printed structure can withstand its own weight and the weight of the new layers. [14], [25], [26] The extrusion printing is also possible with lower viscosities, but the then should use printing into supporting bath. The bath material supports the ink before it becomes strong enough. The recovery time should be short ($< 1\ s$) to ensure sufficient shape fidelity. Faster recovery time improves printing quality and shape retention, because the ink cannot spread in crevasses of the bath. [17], [27]

In laser-based printing the inks do not have to be shear thinning because there are no significant shear forces. However, in SLA low viscosity inks are beneficial, because those spread quickly and evenly on the new printing layer. That increases the printing speed and quality. Lower viscosity also allows the ink removal of the uncured ink from the porous scaffold more easily. Additionally, viscosity cannot be too low because it can cause polymer shrinkage and too high viscosity can cause agglomeration especially if the ink contains cells. In TPP there are no limitations for ink viscosity, but higher viscosities are recommended and even semi solid-like inks can be processed. [28], [29] LIFT utilized a little bit different technique than SLA and TPP. The rheological properties effects on the shape and the speed of the jet formed. At higher viscosities the jet forming is harder and the jet becomes smaller and slower, and the needed energy for laser pulse is higher. If the viscosity is too high, the jet formation might be prevented completely or the high laser energy can be harmful for bioinks. However, higher viscosity inks decreases the jet size and produce less jet scattering, and thus increases printing resolution. [23], [30], [31]

In droplet-based printing, the viscosity affects also like in LIFT. If the viscosity is too high, the droplet formation is difficult and it can even be prevented. The nozzle clogging can occur, if the viscosity or density is too high. Too low viscosity, on the other hand can lead formation of satellite drops (i.e., the disintegration of the main droplet into smaller drops), which reduces printing resolution and quality. Shear thinning can be either an advantage or disadvantage. It can make easier to print inks with higher viscosities or decrease viscosity too much and thus cause formation of satellite droplets. In turn, in some cases it has been found that the shear thinning behavior can also suppress the formation of

satellite drops. The printability of Newtonian fluid in DOD is described by the Ohnesorge number (*Oh*), which is affected by density, surface tension, nozzle size and viscosity. The value decreases when ink viscosity decreases. The ink is printable when $0.1 < Oh < 1$. If *Oh* is below 0.1, the viscosity is too low and if $Oh > 1$, the viscosity is too high. For viscoelastic materials additional parameters, for example Weissenberg (*Wi*) and Deborah (*De*) numbers are needed. In addition to density, viscosity and surface tension, these parameters are affected by other characteristics, such as deformation rate, relaxation time or velocity of the jet. [15], [24] The rheopectic nature is recommended, because increased viscosity helps the droplet to keep the shape of the ink drop by reducing spreading, splashing and deformation when it lands on the substrate. It improves the shape fidelity, when the droplets can keep their shape. Although, too pronounced rheopectic characteristic can cause nozzle clogging. [32]

Parameters *n* and *K*, that appear in many viscosity models, can be calculated and used in the first (printing) stage [14], [25]. In extrusion printing, parameters *n* and *K* can be utilized to improve the printing quality, predict the process, and they can be used to estimate ink velocity, shear stress and viscosity in nozzle during printing. The values can also be used to determine the suitable printing window for the material, i.e. appropriate printing parameters such as pressure and velocity to achieve the most shape fidelity product as possible. The value of *n* describe the behavior of material viscosity and value *K* illustrates the viscosity of the ink, as the value of *K* increase, the viscosity of the ink increases. The values of *n* and *K* are affected by, for example concertation and temperature. A decrease in temperature and increase in concentration increase the value of parameter *K*. Usually as the *K* increases, *n* decreases. The value of *n* only begins to change due the effect of temperature when the temperature change is large enough. However, the dependence on temperature and concentration is not as strong for *n* as it is for index *K*. [25], [26], [34] In order to print easily and with the necessary resolution, the material should be shear thinning, i.e. the value of *n* is below 1. The lower of the value, the stronger is the shear-thinning behavior. Lowering the viscosity reduces the flow resistance in the nozzle. [14] Shear thinning behavior is essential when *n* is less than 0.6. [34] Studies state that a higher *K* value causes higher pressure during printing, which makes it more difficult to extrude the ink and increases extrusion resistance. Larger *K* value reduces the printing resolution. However, the increase of shear-thinning behavior decreases the pressure during extrusion. [26], [33] Paxton et al. state in their study that with high *K* and low *n* value the printing window is narrower than with other materials [25]. The *K* and *n* should therefore be low enough so that the material flow from

the nozzle is even. Lower values also indicate easier extrusion and better printing resolution.

The yield stress can be determined, for example by using the Herschel-Bulkley model and can be utilized in many stages in printing. It affects the flow and self-supporting capacity in extrusion and droplet printing. In support bath printing it effects to the nozzle movement and shape fidelity.

Exhibiting the yield point is very important in supporting bath printing. The bath material behaves as a solid-like at rest, but starts to yield after the shear stress is higher than the yield stress. Material should yield and be shear thinning when shear stress is applied, so that the nozzle can move effectively and printing is successful, and the ink can keep its shape in support material. A low yield stress improves the nozzle movement, but higher yield stress improves the shape fidelity. A too high yield stress, on the other hand can cause crevasses. This can be mitigated, for example by using inks that have higher viscosity and shear thinning. A suitable yield stress in support bath printing is from Pascals to hundreds of Pascals and in extrusion printing it could be up to hundreds of Pascals, depending on the applied shear stress and used inks. [17], [26], [27]

Yielding after the high enough stress can be useful also in extrusion and droplet printing, because it can prevent accidental leaks. In addition, in extrusion printing modifying the yield point it is possible to influence to flow and shape fidelity. With lower yield stresses the ink flow is steadier during extrusion and the printed filament has better quality. However higher yielding points increases the mechanical strength of the printed structure after the extrusion and thus improves the shape fidelity. The inks withstand its own weight and the weights of the new layers better without collapsing or deforming. [14], [32], [33] In droplet-based printing and LIFT the high yield point has a same effect as a high viscosity, i.e. it makes the droplets smaller. The yield stress can be useful after printing, when the ink is important to keep its shape before the structure is stabilized with crosslinking. In SLA and TPP the yielding is not necessary. [17], [23], [27]

After the printing, the most important material properties are the ability to keep its shape and structure and have good enough mechanical properties that it withstands the weight of the new layers (i.e. self-supporting capacity) that is possible to print on top of it and its own weight without collapsing. These properties enable good printing quality, in which the resolution and shape fidelity is high. Viscosity, yield stress, thixotropic nature and recovery time have an effect but so does the crosslinking and viscoelastic properties.

The crosslinking effects highly on the mechanical strenght and elasticity of hydrogels. [14]

Rapid gelation should occur after printing in extrusion, droplet and LIFT printing. In extrusion the crosslinking can occur before, during or after the printing, so the shape or structure does not collapse. If the ink is already crosslinked, the ink should recover rapidly and have self-healing nature. When printing into supporting bath the ink gelation can be slower, because the bath material gives the necessary support to the ink before crosslinking and stabilization. In droplet and LIFT printing the gelation occurs after the droplet is landed. [15], [23], [24]

Other influencing rheological property is viscoelasticity. When the *tanδ* is lower than one, the elastic portion of material is dominating. The higher the elastic portion the stronger the structure. In supporting bath printing the support bath should have solid-like behavior at rest in order to hold the material in place. The storage modulus of material should be sufficiently high and $G' > G''$. Although, it must to be remembered that the bath should behave liquid-like when the stress is applied, so the nozzle can move in it. The G' value of support bath is higher than G' of ink, otherwise it may cause pressure to the ink and induce deformation on the printed filament and decrease the printing quality. [17], [27]

The high elastic behavior can be advantageous or disadvantageous in extrusion printing. The high elastic modulus enables good printing quality if the elastic behavior is not too strong to hamper printing. Low *tanδ* value means that the ink has self-supporting capacity. Strong gels have *tanδ* value below 0.1 and if it is above that, the ink might need further crosslinking, because the structure is prone to collapse. The G' value can be increased with pre-crosslinking of the ink. The disadvantage of low *tanδ* value is that the fluidity of ink decreases as the value decreases, which can cause problems with printing. [25], [33]

Balancing between the rheological characteristics is essential, to achieve a sufficiently good printing quality, resolution and shape fidelity. For example, in extrusion the flow has to be steady and the mechanical properties has to be good enough that the structure do not collapse. The greater elastic modulus gives better mechanical strength but can make the ink flow badly. In droplet printing higher viscosities enables the smaller droplets but too high viscosity can cause high cell-damaging stresses and even restrain the printing due clogging the nozzle or preventing the droplet formation.

5. CONCLUSIONS

3D printing methods require certain material behaviors. Not all hydrogel materials are suitable for all printing techniques. Wrong rheological properties may cause weaker printing quality or the printing process to be interrupted. The results from rheological tests can be fitted into mathematical equations, which can be utilized to choosing the suitable materials or produce materials, that behavior is suitable for printing. The figure 16 below summarizes the rheological characteristic requirements for different printing methods.

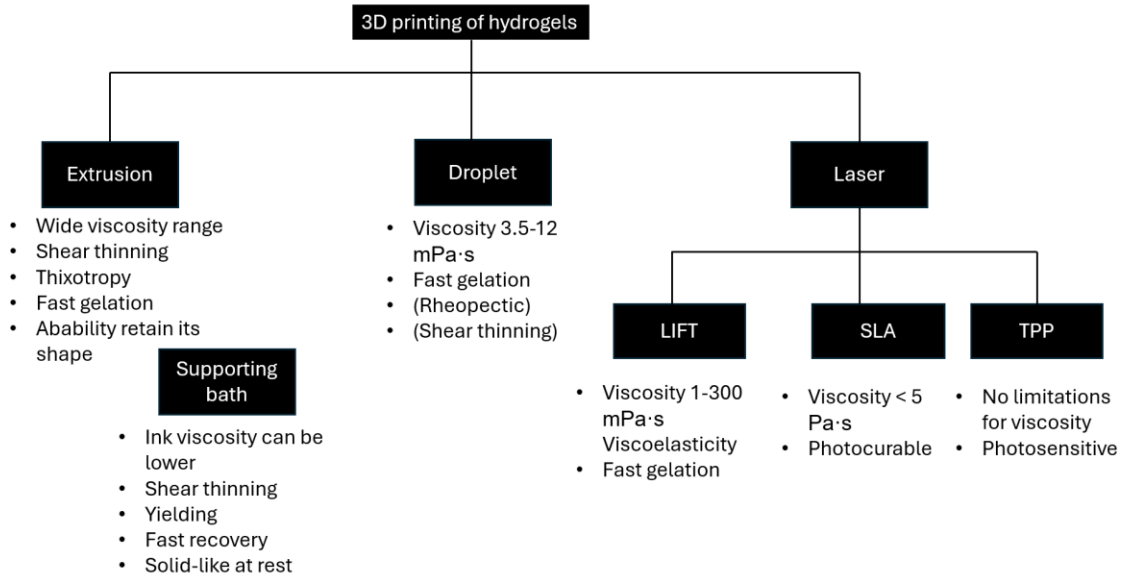

Figure 16: The recommended and required rheological behavior of inks in 3D printing. Features in the parentheses are not required, but recommended behaviors.

In extrusion printing the ink materials with a wide range of viscosities are suitable, but the material should have shear thinning and thixotropic nature and be self-supporting. However, printing into a supporting bath can be uses, in which case the support bath material should support the printed ink. Lower viscosity inks are suitable in droplet, LIFT and SLA printing. Ink that are used in droplet and LIFT should have fast gelation speed. TPP does not have specific limitations for viscosity. It is not always possible to get all the desired properties at the same time, so it is necessary to find a balance between the characteristics of the inks in order to achieve the highest possible printing quality.

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