

CREATING THREE-DIMENSIONAL HYDROGEL ARCHITECTURES FOR BIOMEDICAL APPLICATIONS

BY
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A thesis paper submitted in partial fulfillment of the requirements for the award of
Master of Science in
Materials and Metallurgical Engineering



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CANDIDATE’S DECLARATION

I, Raihan Atahar, declare that this thesis is my own work and any part of it has not been submitted elsewhere for the award of any degree or diploma. Where use has been made of the work of other people, it has been fully acknowledged and referenced accordingly.

.....

Raihan Atahar

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CERTIFICATE OF RESEARCH

This is to certify that the work presented in this thesis was carried out by the author under the supervision of Dr. Fahmida Gulshan, professor of Department of Materials and Metallurgical Engineering, Bangladesh University of Engineering and Technology, Dhaka-1000, Bangladesh.

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Dedication

*To my parents,
Who sacrificd their whole life for me.*

Abstract

Hydrogels are water-soluble polymers which can contain a huge amount of water inside them. This property of hydrogels has attracted specific interests in biomedical fields such as 3D organ/tissue printing, wound dressing, drug delivery, trans-dermal systems, dental materials, implants, injectable polymeric systems, ophthalmic applications, hybrid-type organs, contact lenses etc. Three-dimensional (3D) printing of hydrogels can be considered as a significant advancement in this regard because this may fascilate the use of hydrogels. In this research work, a hydrogel of poly(acrylic acid)-cysteine (PAA-cysteine) conjugates was 3D printed. The hydrogel synthesized was chosen after in-depth literature study. PAA-cysteine conjugates were synthesized by the covalent attachment of L-cysteine to poly(acrylic acid) (PAA). Synthesis followed by purification of the hydrogel. The reaction mixture was dialyzed using Spectra/Por[®] 2 membrane (MWCO: 12-14kD) for 3 days in a fume hood under stirring at room temperature. After that, the dialyzed sample was transferred into petri dishes and freeze-dried for two days and stored in a closed desk until use. Swelling test was performed to get the swelling ratio of poly(acrylic acid)-cysteine (PAA-cysteine) conjugated hydrogel. The swelling ratio was found to be ~290% of the dry weight of the hydrogel. This high swelling ratio is very exciting for biomedical application. After that printing of hydrogel was tried. It was very challenging as hydrogel possesses a significant portion of porosity inside them. Inkjet printing and fused deposition modeling (FDM) printing technology were applied for the printing purpose. Inkjet printing was performed successfully with the synthesized hydrogel. A Standard operating procedure (SOP) was prepared for the use of FDM printer. But FDM printing was not possible due to the difficulty in finding the melting point of the hydrogel. Different biomolecules (proitens, amino acids etc.) can be delivered in vivo through the printed hydrogels. This work can be considered as a precursor to facilitate the use of hydrogels in biomedical applications.

Keywords: hydrogel, swelling ratio, 3D printing, inkjet printing, fused deposition modeling (FDM).

Table of contents

CANDIDATE’S DECLARATION	i
CERTIFICATE OF RESEARCH	ii
Acknowledgements	iv
Dedication	vi
Abstract	vii
Table of contents	viii
List of figures	xii
List of tables	xiv
Abbreviations	xv
Nomenclature	xvii
Chapter 1: Introduction	1
1.1 Motivation	1
1.2 Objectives	2
1.3 Research questions	2
1.4 Research hypothesis	3
1.5 Scope of the thesis	3
Chapter 2: Literature Review	4
2.1 Introduction	4
2.2 Hydrogel	4
2.2.1 Definition	4
2.2.2 Properties	6
2.2.2.1 Swelling properties	6
2.2.2.2 Porosity	8
2.2.2.3 Biocompatibility	8
2.2.2.4 Other properties	9
2.2.3 Classifications	10
2.2.3.1 According to origin	10

2.2.3.2	According to crosslinking.....	11
2.2.3.3	According to the basis of preparation.....	11
2.2.3.4	Classification based on physical appearance.....	12
2.2.4	Synthesis	13
2.2.4.1	Three-dimensional polymerization.....	14
2.2.4.2	Direct crosslinking of water-soluble polymers.....	15
2.2.5	Hydrogel technical features	16
2.2.6	Problems associated with hydrogels	17
2.2.6.1	Low mechanical strength.....	17
2.2.6.2	Inhomogeneity of Hydrogels.....	17
2.2.6.3	Environmental aspects.....	17
2.2.7	Applications	17
2.3	3D Printing.....	18
2.3.1	Definition	18
2.3.2	Classification.....	19
2.3.2.1	Fused deposition modeling (FDM).....	19
2.3.2.2	Inkjet printing	21
2.3.3	Advantages.....	24
2.3.4	Disadvantages	25
2.3.5	Applications	27
2.4	Challenges in printing hydrogels.....	29
Chapter 3: Standard Operating Procedure (SOP) for ABS and PLA Filament on Cartesio FDM 3D Printer Using Cura 3D Modeling Software		31
3.1	Objective	31
3.2	Introduction	31
3.3	Definitions.....	32
3.4	Safety Precautions.....	32
3.5	Materials & Equipment	32
3.6	Procedure.....	33
3.7	Preparing the printer.....	36

3.8	Support Generation	36
3.9	Change of Filament	36
3.10	Troubleshooting	38
3.10.1	Not Extruding at Start of Print	38
3.10.2	Print Not Sticking to the BED	39
3.10.3	Warping.....	42
3.10.4	Layer shifting or Misalignment	43
3.10.5	Stops Extruding in the Middle of a Print	43
3.10.6	Weak Infill.....	44
3.11	Summary	45
Chapter 4: Hydrogel Synthesis		47
4.1	Materials.....	47
4.2	Synthesis of hydrogel	47
4.2.1	synthesis.....	47
4.2.2	Purification.....	48
4.3	Swelling behavior of hydrogel	50
4.4	Results and discussion.....	50
4.4.1	Hydrogel synthesis.....	50
4.4.2	Swelling behavior of hydrogel.....	52
Chapter 5: Printing hydrogels		55
5.1	Inkjet printing.....	55
5.1.1	Inkjet printer.....	55
5.1.2	Ink formulation	55
5.1.3	Inkjet printing.....	56
5.2	Fused deposition modeling (FDM) printing.....	57
Chapter 6: Conclusion.....		59
6.1	Summary of the findings	59
6.2	Answers to the Research Questions	59
6.3	Testing research hypothesis	59

6.4	Limitations and Future Directions.....	60
Chapter 7:	References	61

List of figures

Figure 2.1: Schematic diagram of hydrogel showing their affection for water	5
Figure 2.2: Schematic of (a) the classic covalent single-network hydrogel, (b) dual-crosslinked hydrogel, (c) polymer-intercalated nanocomposite hydrogel, (d) hydrogel with elastomer-like segments and non-covalent bonds, and (e) double-network (DN) hydrogel. Blue and red long lines: polymer backbones; short green lines: covalent crosslinking points; yellow short lines: non-covalent crosslinking points [18].	5
Figure 2.3: Stimuli response of unswollen hydrogel to swollen hydrogel [34].	7
Figure 2.4: The transmission path models of water molecule in porous structure of hydrogels: (a) PEG-modified hydrogels, (b) CNF/PEG-modified hydrogels [33].	8
Figure 2.5: Schematic of methods for formation of hydrogels by chemical modification of hydrophobic polymers [41].	11
Figure 2.6: Schematic diagram of Semi-Interpenetrating network polymeric (Semi-IPN) hydrogels and Interpenetrating network polymeric (IPN) hydrogels	12
Figure 2.7: Synthesis of hydrogels by three-dimensional polymerization [50].	14
Figure 2.8: Synthesis of hydrogels by cross-linking of ready-made water-soluble polymers [50].	16
Figure 2.9: Different biomedical applications of hydrogels	18
Figure 2.10: 3D printing technique.	19
Figure 2.11: A schematic illustration of FDM –	20
Figure 2.12: Thermoplastic materials pyramid available in FDM.	21
Figure 2.13: Types of inkjet printer, (a) continuous, (b) drop-on-demand.	22
Figure 2.14: Types of drop-on-demand inkjet printer, (a) thermal, (b) piezoelectric.	23
Figure 2.15: Common 3D printable materials with their advantages and disadvantages.	26
Figure 2.16: The pros and cons of 3D printing.	27
Figure 2.17: Different applications of 3D printing.	28
Figure 2.18: Biomedical applications of 3D printing.	29
Figure 3.1: Schematic of a typical FDM printer.	31
Figure 3.2: Cartesio FDM 3D Printer.	33
Figure 3.3: Adhesive spray.	33
Figure 3.4: Cura software interface.	34

Figure 3.5: Cura software interface showing ‘open file’ icon.	34
Figure 3.6: Cura software interface showing ‘layer view’.	35
Figure 3.7: Cura software interface showing ‘generate support’.....	36
Figure 3.8: Cartesio FDM 3D printer’s power switch.	37
Figure 3.9: Cartesio FDM 3D printer’s working area.	37
Figure 3.10: Cartesio FDM 3D printer’s extruder head.....	38
Figure 3.11: Skirt.	39
Figure 3.12: Print not sticking to the BED.	40
Figure 3.13: Cartesio FDM 3D printer’s settings for ‘printing speed’.	41
Figure 3.14: Cartesio FDM 3D printer’s settings for temperature control.	41
Figure 3.15: Warping.	42
Figure 3.16: Layer shifting or Misalignment.	43
Figure 3.17: Stoppage of extrusion in the middle of a print.	44
Figure 3.18: Infill pattern settings.....	45
Figure 4.1: Flow diagram of PAA-Cysteine conjugate synthesis.....	47
Figure 4.2: Dialysis of PAA-Cysteine conjugates.	48
Figure 4.3: Purification of PAA-Cysteine conjugates.	49
Figure 4.4: The dialyzed sample was freeze-dried in Severn Freeze-Dryer.....	49
Figure 4.5: Chemical substructure of PAA-Cysteine conjugates.	51
Figure 4.6: Synthesized PAA-Cysteine conjugates after purification.	51
Figure 4.7: A tablet form of PAA-cysteine conjugates	52
Figure 4.8: Swelling behavior of PAA-Cysteine conjugates.....	53
Figure 5.1: A customized piezoelectric printer was used for inkjet printing.....	55
Figure 5.2: Hydrogel structure of a 10*10 mm ² square- (a) as printed, (b) swollen printed structure after 5 minutes of water addition, (c) disintegrated printed structure after 45 minutes of water addition.	57
Figure 5.3: Burnt hydrogel samples during the attempt to find out the melting point.	58

List of tables

Table 2.1: Factors affecting the properties of hydrogels.	9
Table 2.2: Hydrogels classification according to origin.	10
Table 2.3: Different types of hydrogels.	13
Table 2.4: Commonly used synthetic monomers for hydrogel production.	15
Table 2.5: Types of inkjet printer.	23
Table 2.6: Summary of fused deposition modeling (FDM) and inkjet printing.	24
Table 3.1: Comparison between ABS and PLA filament.	45
Table 4.1: Process conditions for freeze-drying.	50
Table 4.2: Swelling behavior data of PAA-Cysteine conjugates.....	53
Table 5.1: Solubility data of PAA-Cysteine conjugates.	56
Table 5.2: Parameters for inkjet printing of hydrogels.	56

Abbreviations

2D	Two-dimensional
3D	Three-dimensional
AM	Additive manufacturing
CAD	Computer aided design
CD	Cyclodextrin
CT	Computed tomography
ECM	Extracellular matrix
EG	Ethylene glycol
HA	Hyaluronic acid
HA	Hydroxyapatite
HEMA	Hydroxyethyl methacrylate
HIPS	High impact polystyrene
MMA	Methyl methacrylate
PAAm	Polyacrylamide
PBO	Poly(butylene oxide)
PCL	Polycaprolactone (PCL)
PEEK	Polyether ether ketone
PEG	Poly(ethylene glycol)
PEO	Poly(ethylene oxide)
PF	Propylene fumarate
PGEMA	Poly(glucosylethyl methacrylate)
PHB	Poly(hydroxy butyrate)
PHPMA	Poly(hydroxypropyl methacrylamide)
PLGA	Poly(lactic-co-glycolic acid)

PLGA	Poly(lactic-co-glycolic acid)
PMMA	Poly(methyl methacrylate)
PNIPAAm	Poly(N-isopropyl acrylamide)
PNVP	Poly(N-vinylpyrrolidone)
PPE	Polyphenylene ether
PPO	Poly(propylene oxide)
PS	Polystyrene
PVA	Poly(vinyl alcohol)
PVAc	Poly(vinyl acetate)
RP	Rapid prototyping
SOP	Standard Operating Procedure

Nomenclature

gm	Gram
mm	millimeter
pl	picoliter
T_g	Glass transition temperature
$^{\circ}\text{C}$	Degrees Celsius
γ	Gamma

Chapter 1: Introduction

1.1 Motivation

Researchers have been interested in polymeric materials over the past few decades specially for biomedical applications. In some cases, there needs to achieve a certain amount of cell growth inside body. Water-soluble polymers, i.e., hydrogels have attracted specific interest in this regard. Hydrogels are water-swollen three-dimensional (3D) networks consisting of cross-linked polymer chains. These can be done by either physical interactions or chemical bond formation between polymer chains. The chemical and mechanical similarities between hydrogels and the mammalian extra-cellular matrix can impart hydrogels systems with the ability to remain undetected following placement within the body [1], as the body's host defenses may not deem the implanted hydrogel as being different from the body's native tissues.

Hydrogels may provide an environment for directed adhesion, spreading, growth and/or differentiation of cells into the desired tissue construct. They may potentially offer selectivity over nutrient uptake or even influence the fate of cellular growth based on the physical properties of the surrounding hydrogel environment that has been pre-seeded with precursor cells [2].

Although several advantageous properties of hydrogels in biomedical applications have motivated significant research into such materials, the practical introduction of hydrogels into the body is a significant challenge limiting their application. Materials that are difficult to introduce into the body are generally associated with high levels of patient discomfort and may therefore result in a lower degree of patient compliance. The high elasticity of conventional hydrogels has particularly limited the administration of these materials through non-invasive techniques, such as through injection. As a result, gels pre-formed outside of the body must often be introduced surgically to their target site.

In previous work, this limitation has been addressed by using gels that form spontaneously *in vivo* through physical interactions between polymer chains following injection, taking advantage of unique properties of the biological environment such as ionic strength, pH, or temperature to trigger self-associations [3,4]. However, physically cross-linked hydrogels face two major challenges that limit their overall efficacy in application-

First, a reliance on physical interactions between polymer chains for gelation limits the degree of control that can be exerted over which the timescale of gel degradation within the body, with most gels susceptible to rapid dissolution by the body and subsequent dissolution (or at least rapid loss of mechanical integrity). This can be a major challenge in the biomedical context, as pre-loaded cargoes may not be secured within the context of a protective matrix throughout a pre-determined therapeutic window. For example, poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) has been shown to degrade and be reabsorbed by the body in a matter of hours subsequent to administration [5].

Second, the inherently dynamic nature of inter-chain physical interactions often limits the mechanical strength of these physically cross-linked gels, particularly under shear. Although this shortcoming can in part be addressed by increasing the number of polymer chains composing the physical gels, an

increase in the local concentration of polymer can increase the osmotic gradient into the matrix and potentially dehydrate surrounding tissues [6].

These limitations have encouraged the growth of a class of hydrogels formed via covalent bond formation between polymer chains. By controlling the number of complimentary reactive groups (typically electrophiles and nucleophiles) present on component precursor polymer chains, it is possible to pre-program the relative density and types of cross-links present within the hydrogel matrix [7]. In this way, the rate of hydrogel formation and degradation and gel mechanical properties can be controlled to a degree not offered by physical gels.

Unfortunately, hydrogels pre-formed in this way outside of the body generally do not possess the properties of physically cross-linked hydrogels, making them difficult to introduce into the body through minimally invasive means. For this reason, there is a growing interest in developing a procedure to prepare such hydrogels that can be inserted into human body and show required properties. Three-dimensional (3D) printing can be a breakthrough in this regard. In extrusion based 3D printing, there may be some difficulties in extruding the hydrogels into filaments for printing. But in case of inject printing, there is no such difficulties.

1.2 Objectives

From the biomedical point of view, hydrogels have gained interest. If the hydrogels can be printed, it will open a new horizon in biomedical field. But because of lack of strength 3D printing of hydrogel is quite challenging. This thesis project has been aiming to face that challenge. To do so, the objectives of the thesis has been differentiated into several parts.

- (i) The first objective is to develop a standard operating procedure (SOP) for 3D printable polymers that can be used to inform the printing of different hydrogels.
- (ii) In the meantime, an in-depth literature study has been conducted to get necessary information and properties required for hydrogels and 3D printing.
- (iii) After that, a number of polymers has been listed to check the feasibility of 3D printing.
- (iv) From the list, a polymer has been chosen for the synthesis of hydrogel under required conditions.
- (v) When the hydrogel is synthesized, a standard purification method has been applied to eliminate unbound reacting species.
- (vi) After that, printing of the hydrogel is tried.

1.3 Research questions

The study aims to answer the following research questions (RQ)-

RQ1: Does hydrogel show required properties that can be used to inform the feasibility of biomedical applications?

RQ2: Should hydrogel be 3D printable?

1.4 Research hypothesis

The research questions were investigated based on the following research hypothesis (RH)-

RH1: The most important property that is required for hydrogels to be used in biomedical applications like cell growth is swelling properties. Hydrogels are hydrophilic polymer networks which may absorb from 10–20% (an arbitrary lower limit) up to thousands of times their dry weight in water [8]. The water uptake by hydrogels is sensitive to external environment, including the temperature, the pH, the ionic strength, and the electric field [9]. So, hydrogels are strong candidates to be used to inform the feasibility of cell growth inside the body.

RH2: Hydrogels are mostly thermoplastic polymers and they possess amorphous structure. Amorphous polymers have a disordered structure and a viscosity high enough to facilitate the adhesion of the layers and also maintain the shape of the printed layers [10]. So, hydrogels should be printable.

1.5 Scope of the thesis

There are several scopes of this thesis work. They are listed chapterwise in the following section:

Chapter 1 of this thesis provides the motivation behind the thesis, objectives of the work, research questions, research hypothesis and scope of the thesis.

Chapter 2 of this thesis provides the background literature study of hydrogels and 3D printing. The chapter includes their definition, classification, advantages, disadvantages, applications and so on.

Chapter 3 includes a standard operating procedure (SOP) of printing ABS and PLA filament using Ultimaker Cura 3D modeling software on a Cartesio FDM 3D Printer.

Chapter 4 covers the synthesis of PAA-cysteine conjugated hydrogel and its printing. Before printing swelling behavior of the hydrogel was studied.

Chapter 5 summarizes the whole thesis and points out the limitations and future scope of the thesis.

Chapter 2: Literature Review

2.1 Introduction

The thesis is based on two major keywords- ‘hydrogel’ and ‘printing’. The literature review will be mainly focused on these two topics.

2.2 Hydrogel

2.2.1 Definition

Loosely crosslinked polymeric materials that swell in the presence of water or other solvents are often used to make gel-like absorbent materials. These gels are typically made via a free radical polymerization mechanism, where monomer units are connected into long chains through their double bonds [11]. These gel-like polymers are called hydrogels.

‘Hydrogels’ is a combination of two words- ‘hydro’ and ‘gels’. ‘Hydro’ is a Greek word that means water. And ‘gels’ means jelly like substance in simple words. Gels are mainly materials composed of a three-dimensional crosslinked polymer or colloidal network immersed in a fluid. They are usually soft and weak, but can be made hard and tough. So, in general hydrogels are gels that have water as their main constituent.

Hydrogels are three-dimensional, cross-linked hydrophilic polymers with the ability to absorb large quantities of water even up to 1000 times of their dry weight without being dissolved [12-16]. This happens due to the absorption of water by capillary pressure through interconnected pores (i.e. open channels) in hydrogels [17].

Hydrogels resemble the extracellular matrix (ECM) in biological tissues, which is mainly composed of macromolecular components, such as fibrous collagens and proteoglycans. However, unlike naturally-occurring ECM that can withstand large deformations and loads, classic covalent single network hydrogels (Fig. 2.2) are mechanically weak [18], which makes them unsuitable for most physiological load-bearing situations. Many approaches have been used to improve the toughness of hydrogels, for example, non-covalent bonds (e.g., hydrogen bonds and ionic interactions) toughened hydrogels, hydrogels with highly stretchable networks, and double-network (DN) hydrogels [19-23].

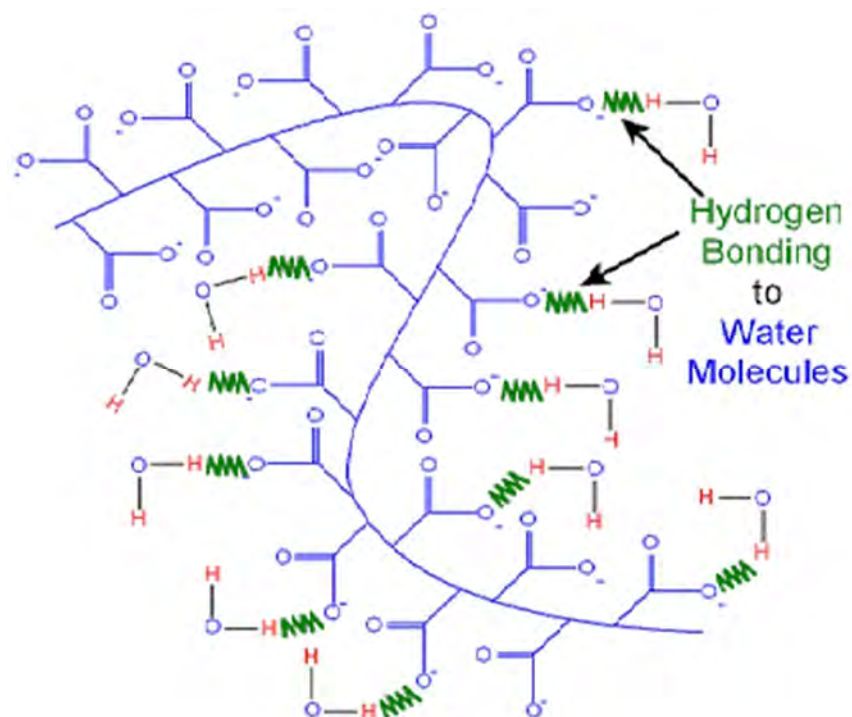


Figure 2.1: Schematic diagram of hydrogel showing their affection for water
(Ref: <https://bcachemistry.wordpress.com/tag/sodium-polyacrylate/>).

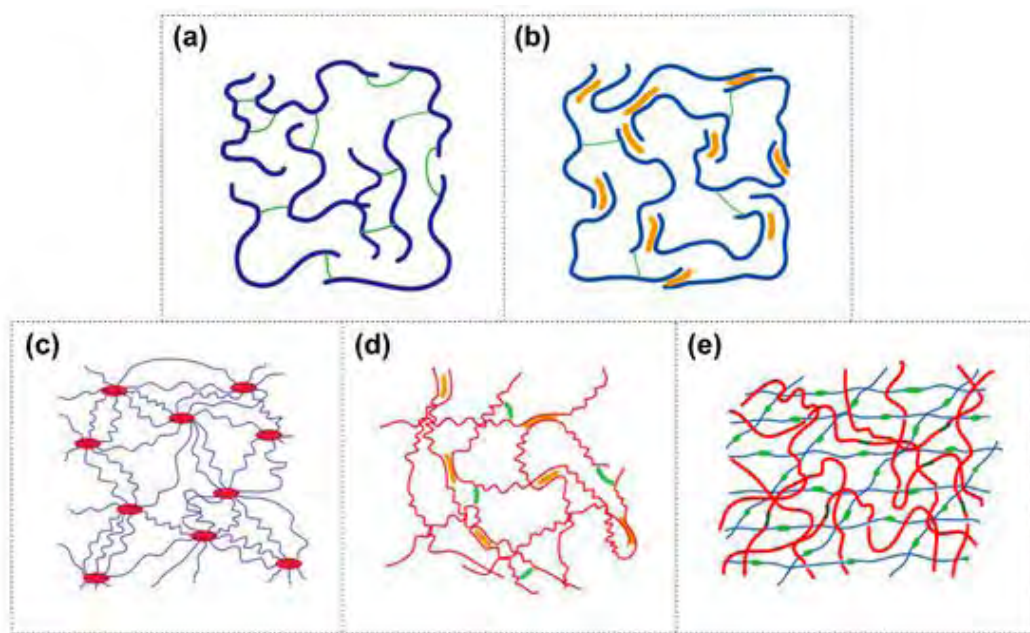


Figure 2.2: Schematic of (a) the classic covalent single-network hydrogel, (b) dual-crosslinked hydrogel, (c) polymer-intercalated nanocomposite hydrogel, (d) hydrogel with elastomer-like segments and non-covalent bonds, and (e) double-network (DN) hydrogel. Blue and red long lines: polymer backbones; short green lines: covalent crosslinking points; yellow short lines: non-covalent crosslinking points [18].

2.2.2 Properties

Hydrogels have been an important class of biomaterial in last few decades. Properties of these materials are easily controlled by their preparation method and composition, so that hydrogels can be created with desired mechanical properties, biocompatibility, biodegradability, permeability, and responsivity to environmental cues [24,25].

2.2.2.1 Swelling properties

The most important property of hydrogels is to swell in water. The nature of water is important in understanding the swelling behavior of hydrogels. Andrade et al. classified three types of water in hydrogels- bound water, interfacial water and free water [26].

Bound water

Bound water is strongly associated with the macromolecular chains by hydrogen bonding or by polar interactions.

Interfacial water

Interfacial water is characterized by ice-like configurations around the hydrophobic groups. This type of water is not as strongly held by the polymer chains as bound water.

Free water

Free water, also known as bulk water, has the same physical properties as normal water. It is not attached to the polymer chains.

Hydrogels swell in water due to the presence of water-stabilizing groups in them, such as -OH, -COOH, -CONH₂ etc. Swelling happens in response to physical and chemical stimuli, where physical stimuli include external environment like temperature [27], electrical stimuli [28], magnetic field, light, pressure, sound etc. and chemical stimuli include pH [29], solvent composition, ionic strength, molecular species etc. (Fig. 2.3).

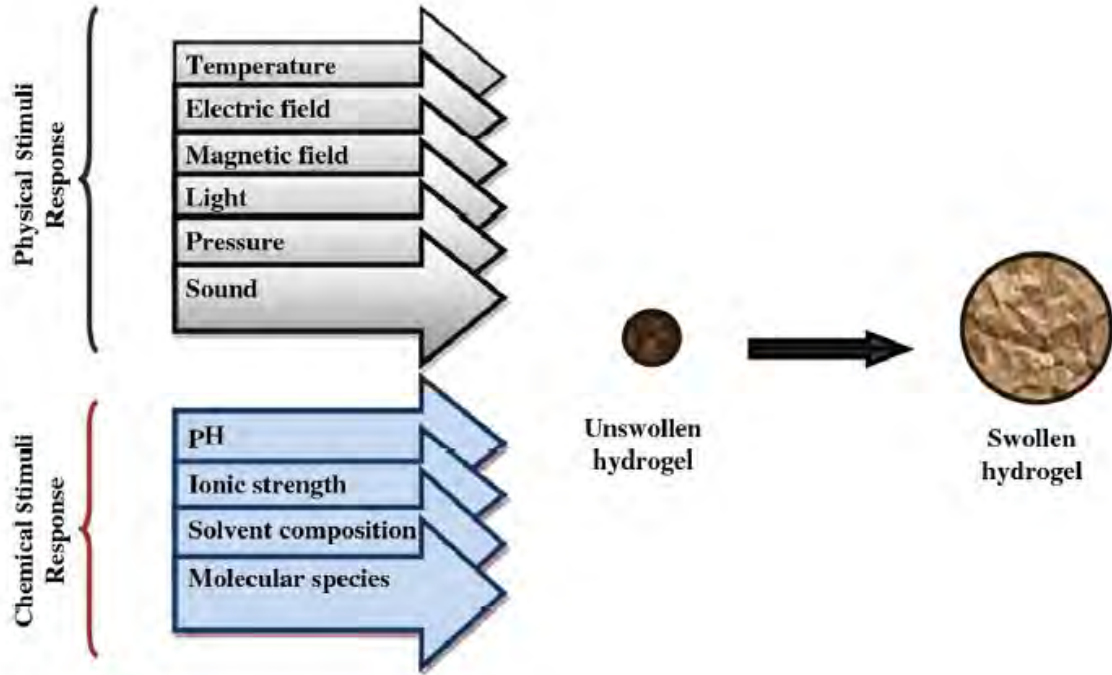


Figure 2.3: Stimuli response of unswollen hydrogel to swollen hydrogel [34].

The porosity and fine internal pores play a significant role in enhancing the total swelling capability and the response rate by reducing the transport resistance to fabricate functional hydrogel [30-32]. Fig. 2.4 is the transmission path models of water molecule in porous structure of hydrogels [33]. These models express the effect of interpenetrating porous structure. As the models shown, the water molecules have different transmission path to go through the porous structure when hydrogels are swelling in the water. Due to the porous structure of PEG-modified (Fig. 2.4a), some water molecules can go straight into the hydrogels, but the distribution and direction of pores still limit the transmission of water molecules. However, the structure with high connectivity of CNF/PEG-modified (Fig. 2.4b) provides much more space and diffusion channels for water molecules. These spaces and channels greatly reduces the transport resistance of water molecules, shorten the time for water molecules motion, and greatly improves the swelling properties and mass transfer of hydrogels.

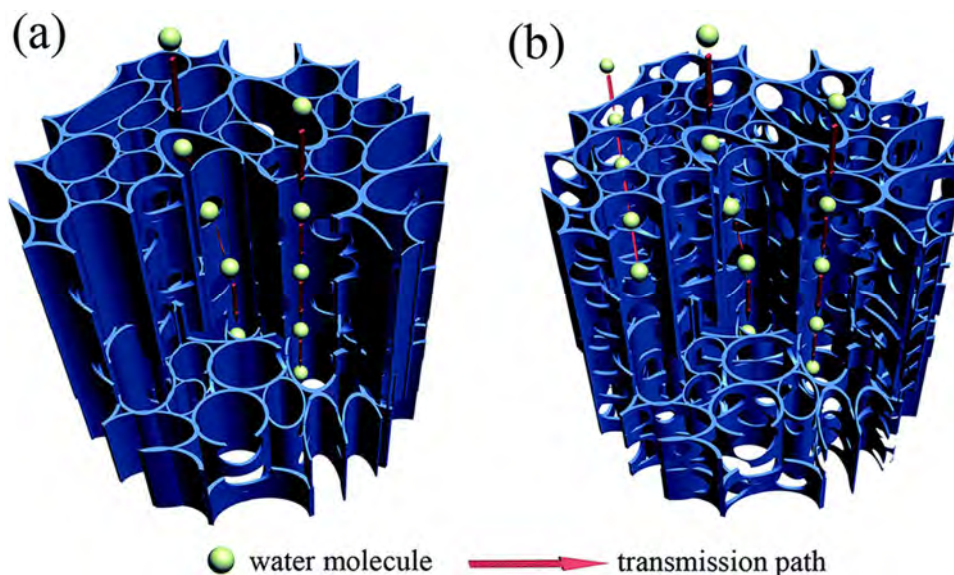


Figure 2.1: The transmission path models of water molecule in porous structure of hydrogels: (a) PEG-modified hydrogels, (b) CNF/PEG-modified hydrogels [33].

2.2.2.2 Porosity

Porosity is a morphological characteristic of a material that can be illustrated as the presence of void cavity inside the bulk. Pores may be created in hydrogels by the process of phase separation during synthesis or they may present as smaller pores within the network. The average pore size, the pore size distribution and the pore interconnections are essential factors of a hydrogel matrix that are often challenging to compute. Pore-size distributions of hydrogels are effectively influenced by three factors [34]-

- a) Concentration of the chemical cross-links of the polymer strands. That concentration is calculated by the initial ratio of cross-linker to monomer.
- b) Concentration of the physical entanglements of the polymer strands. That concentration is ascertained by the initial concentration of all polymerizable monomers in the aqueous solution.
- c) Net charge of the polyelectrolyte hydrogel. That charge is determined by the initial concentration of the cationic and/or anionic monomer.

2.2.2.3 Biocompatibility

It is important for the hydrogels to be biocompatible and nontoxic to make it pertinent in biomedical field. Most polymers used for this purpose must pass cytotoxicity and *in vivo* toxicity tests. Biocompatibility is the capability of a material to function with an appropriate host response in a specific application. Biocompatibility analysis consists of two parameters namely biosafety and bio-functionality:

- a) Biosafety i.e. adequate host response not only systemic but also local as well (i.e. surrounding tissue), the absence of cytotoxicity, mutagenesis, and/or carcinogenesis.

- b) Bio-functionality i.e. the capacity of material to perform the specific task for which it is intended.

This explanation is exceptionally applicable in tissue engineering since the nature of tissue construct is to constantly interact with the body through the healing and cellular regeneration process as well as during scaffold degradation. Moreover, initiators, organic solvents, stabilizers, emulsifiers, unreacted monomers and crosslinkers utilized in polymerization and hydrogel synthesis may be toxic to host cells if they ooze out to tissues or encapsulated cells. To eradicate harmful chemicals from preformed gels, certain purification processes should be implemented such as solvent washing or dialysis [35].

2.2.2.4 Other properties

Elasticity, permeability, solute loading capacity are other important properties of hydrogels. Hydrogels also have lubricating properties, low friction coefficients and high mechanical strength, like cartilage [36]. These properties can be determined by four structural parameters such as water structure in the hydrogel, the heterogeneity of the gel, the chemical composition of structure, and the mesh size of the network. The application of hydrogel can be determined by altering these parameters. There are differences for each type of material with respect to synthesis techniques and properties. The nature of the monomer, the chemical nature and extent of crosslinking agents, different experimental conditions of polymerization and the method of polymerization can be varied to prepare different types of hydrogels with different properties like swelling behavior, crosslinking density, pore size etc. Table 2.1 shows factors affecting the properties of hydrogels-

Table 2.1: Factors affecting the properties of hydrogels.

Factors	Hydrogels properties
Monomer strength ↑	Stimulus- sensitivity ↓
Free water ↑	Permeability ↑
Crosslinking density ↑	Permeability ↓
	Elastic modulus and strength ↑
	Swelling ↓
Hydrophobicity ↑	Swelling ↓
Hydrophilicity ↑	Swelling ↑
Ionization ↑	swelling ↑
Charge ↑	Swelling ↑
	Charge-solute binding capacity ↑

2.2.3 Classifications

Hydrogels can be classified on different bases as detailed below:

2.2.3.1 According to origin

According to origin, hydrogels can be classified into the following types-

- a) **Natural hydrogels:** This type of hydrogels is commonly found in nature. Human body is the best example of natural hydrogels. They can be found in tissues, tendons, membranes for kidney and blood vessels, surface of the stomach, intestines, lungs etc. [37,38].
- b) **Synthetic hydrogels:** Synthetic hydrogels are mixtures that contains substantial amount of water and highly swollen, hydrophilic polymer networks [39]. They have been developed using various polymerization techniques. In the past decades, many synthetic hydrogels have been developed for a large variety of applications [40].
- c) **Combination of natural and synthetic hydrogels:** Sometimes natural hydrogels are combined with synthetic hydrogels to get desired properties. They can be said to be a sub-class of synthetic hydrogels.

Some common hydrogels are listed in table 2.2 according to their origin [8].

Table 2.2: Hydrogels classification according to origin.

Natural polymers and their derivatives ($\pm$crosslinkers): Anionic polymers: HA, alginic acid, pectin, carrageenan, chondroitin sulfate, dextran sulfate Cationic polymers: chitosan, polylysine Neutral polymers: dextran, agarose, pullulan
Synthetic polymers ($\pm$crosslinkers): Polyesters: PEG-PLA-PEG, PEG-PLGA-PEG, PEG-PCL-PEG, PLA-PEG-PLA, PHB, P(PF-co-EG) $\pm$ acrylate end groups, P(PEG/PBO terephthalate) Other polymers: PEG-bis-(PLA-acrylate), PEG $\pm$ CDs, PAAm, P(NIPAAm-co-AAc), PVAc/PVA, PNVP, P(MMA-co-HEMA), P(AN-co-allyl sulfonate), P(biscarboxy-phenoxy-phosphazene), P(GEMA-sulfate)
Combinations of natural and synthetic polymers: P(PEG-co-peptides), alginate-g-(PEO-PPO-PEO), P(PLGA-co-serine), collagen-acrylate, alginate-acrylate, P(HPMA-g-peptide), P(HEMA/Matrigel), HA-g-NIPAAm

2.2.3.2 According to crosslinking

According to the type of crosslinking, hydrogels are classified into two types as shown in Fig. 2.5 [41]-

- a) **Physically crosslinked hydrogels:** In physically crosslinked hydrogels, hydrogels have transient junctions that arise from either polymer chain entanglements or physical interactions such as ionic interactions, hydrogen bonds, or hydrophobic interactions [42].
- b) **Chemically crosslinked hydrogels:** Chemically crosslinked hydrogels have permanent junctions. The crosslinking can take place by ultraviolet irradiation, heating or chemical crosslinking via crosslinker with a huge collaborative reaction, such as Michael's reaction, Michaelis-Arbuzov reaction, nucleophile addition and so on [42,43].

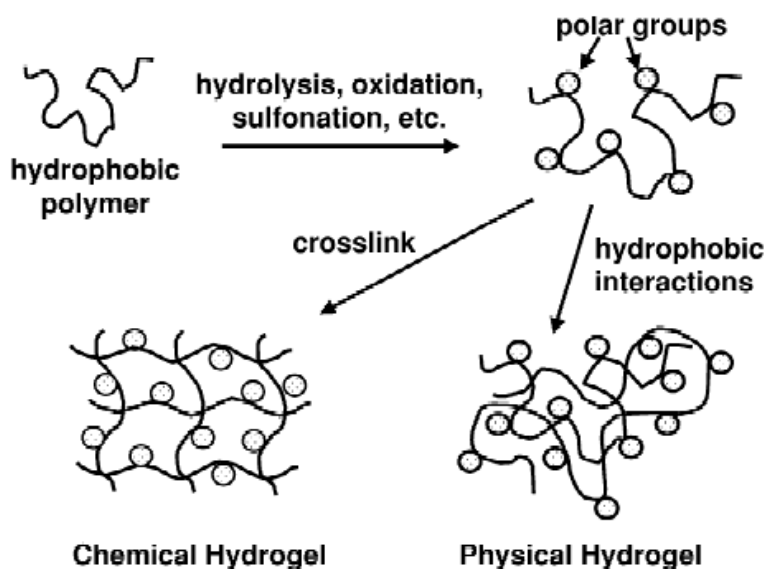


Figure 2.2: Schematic of methods for formation of hydrogels by chemical modification of hydrophobic polymers [41].

2.2.3.3 According to the basis of preparation

The method of preparation leads to formations of some important classes of hydrogels. These can be exemplified by the following-

- a) **Homopolymeric hydrogels:** Homopolymeric hydrogels are referred to polymer network derived from a single species of monomer, which is a basic structural unit comprising of any polymer network [44]. They may have cross-linked skeletal structure depending on the nature of the monomer and polymerization technique.
- b) **Copolymeric hydrogels:** Copolymeric hydrogels are comprised of two or more different monomer species with at least one hydrophilic component, arranged in a random, block or alternating configuration along the chain of the polymer network [45].

- c) **Semi-Interpenetrating network polymeric (Semi-IPN) hydrogels:** In semi-IPN hydrogel, one component is a cross-linked polymer and other component is a non-cross-linked polymer [46].
- d) **Interpenetrating network polymeric (IPN) hydrogels:** Interpenetrating network polymeric (IPN) hydrogels are intimate combination of two polymers, at least one of which is synthesized or cross-linked in the immediate presence of the other [47]. The main advantages of IPN hydrogels are relatively dense hydrogel matrices can be produced which feature stiffer and tougher mechanical properties, controllable physical properties and more efficient drug loading compared to conventional hydrogels [35].

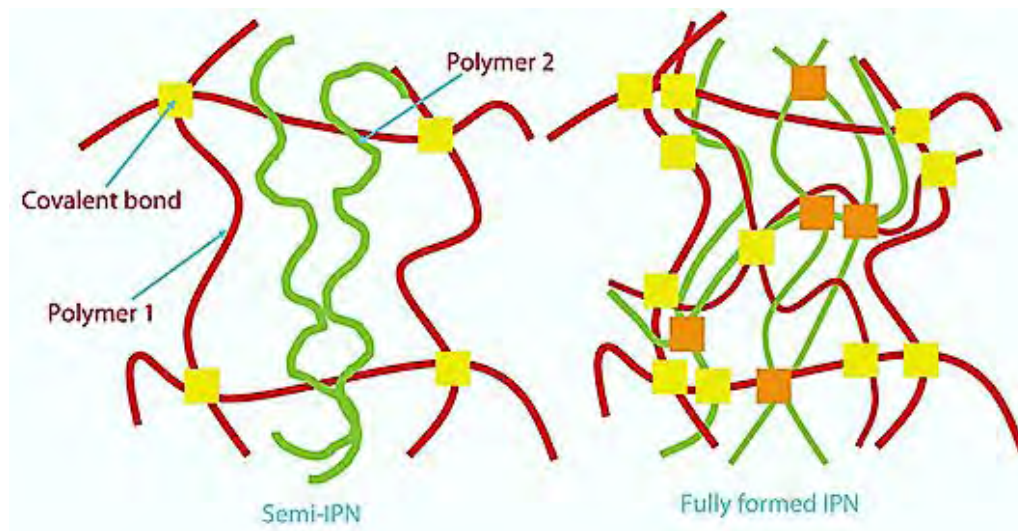


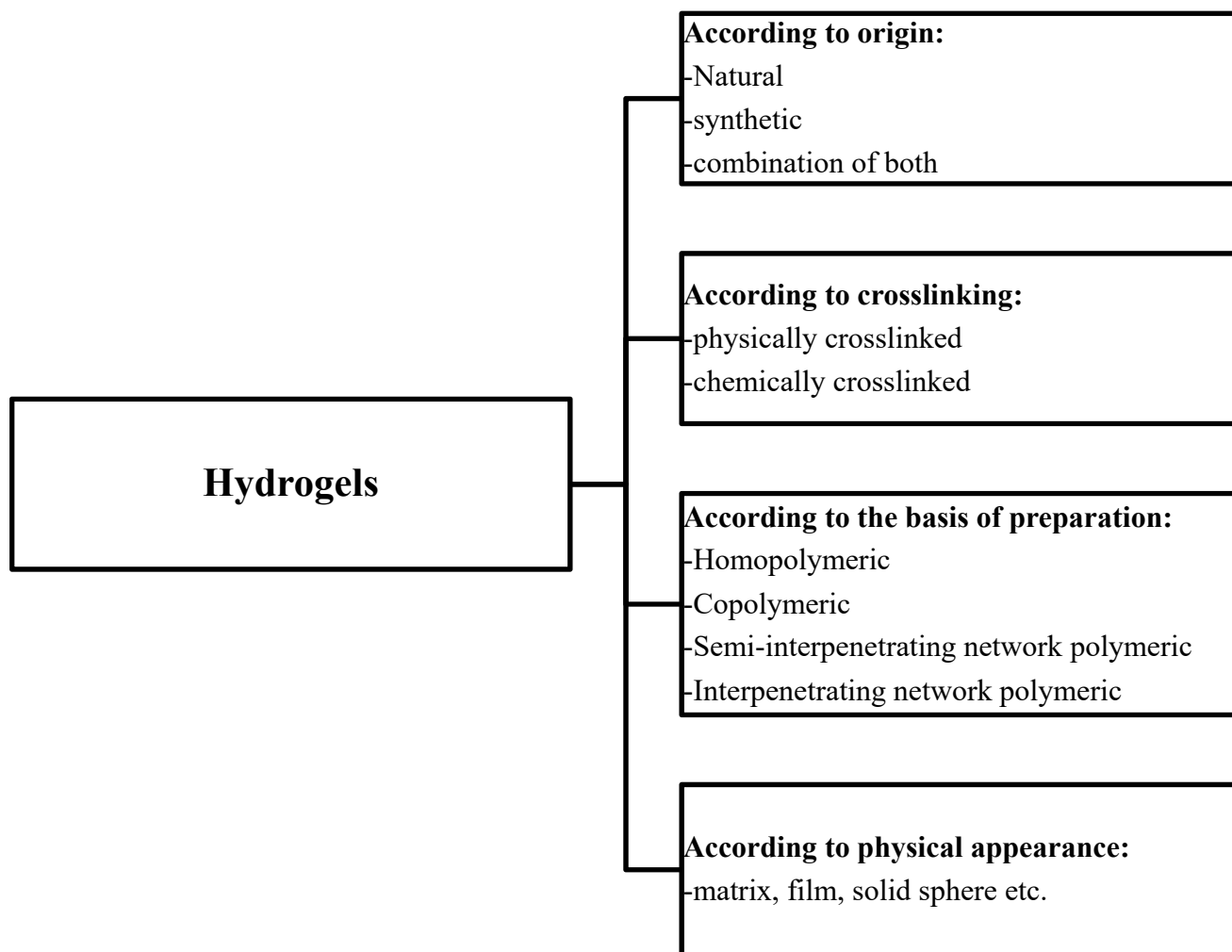
Figure 2.3: Schematic diagram of Semi-Interpenetrating network polymeric (Semi-IPN) hydrogels and Interpenetrating network polymeric (IPN) hydrogels

(Ref: <https://www.intechopen.com/books/aspects-of-polyurethanes/polyurethane-epoxy-interpenetrating-polymer-network>).

2.2.3.4 Classification based on physical appearance

Hydrogels appearance as matrix, film, or microsphere depends on the technique of polymerization involved in the preparation process.

Table 2.3: Different types of hydrogels.



2.2.4 Synthesis

In general, hydrogels can be prepared from either synthetic polymers or natural polymers. The synthetic polymers are hydrophobic in nature and chemically stronger compared to natural polymers. Their mechanical strength results in slow degradation rate, but on the other hand, mechanical strength provides the durability as well. These two opposite properties should be balanced through optimal design [48]. Also, it can be applied to preparation of hydrogels based on natural polymers provided that these polymers have suitable functional groups or have been functionalized with radically polymerizable groups [49].

Chemical hydrogels are commonly prepared in two different ways: ‘three-dimensional polymerization’, shown in fig. 2.7, in which a hydrophilic monomer is polymerized in the presence of a polyfunctional cross-linking agent, or by direct cross-linking of water-soluble polymers shown in Fig. 2.7 and 2.8 [50].

2.2.4.1 Three-dimensional polymerization

Hydrogels are most commonly synthesized by free-radical chain polymerizations of hydrophilic monomers containing a carbon double bond. Polymerization begins when an initiating species produces active radical centers, typically by homolytic dissociation of a weak bond or by a redox reaction. Once formed, the active radical centers rapidly propagate through the carbon double bonds of many monomer units to form polymer chains. When two active radical centers meet, they may react with each other by combination or disproportionation to terminate the polymerization process.

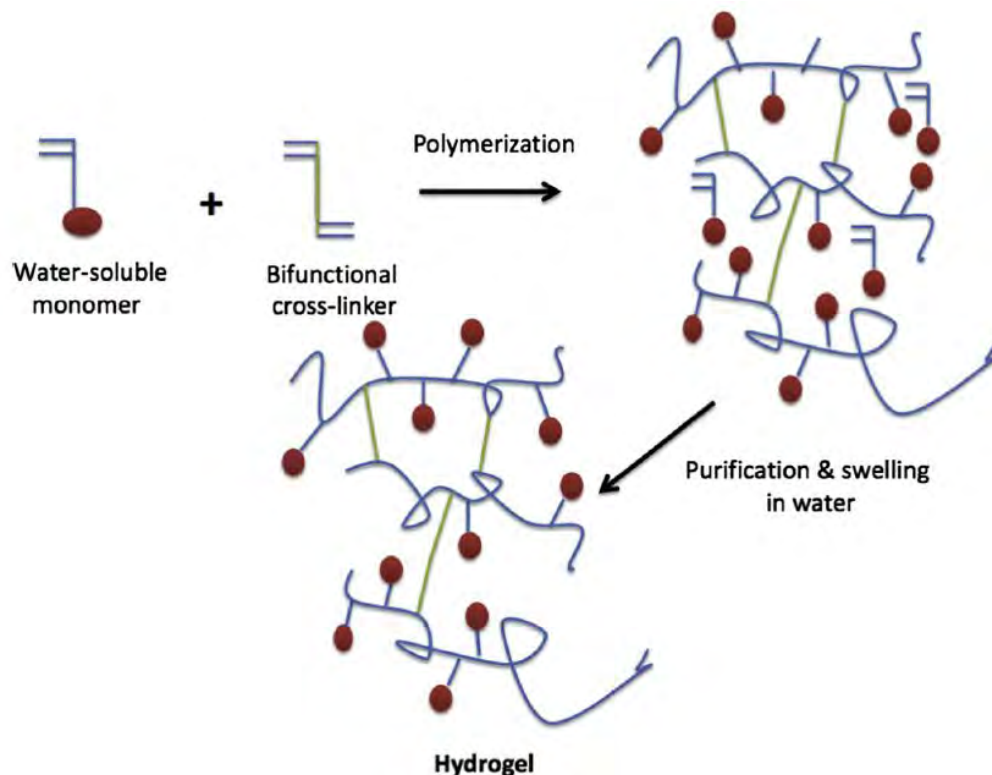


Figure 2.4: Synthesis of hydrogels by three-dimensional polymerization [50].

Table 2.2 shows a variety of monomers that may undergo free-radical copolymerizations with crosslinking agents to form hydrogels. Each of these monomers contains a carbon double bond through which an active center may propagate to produce polymer chains. The method for generating active centers depends on the particular monomers, solvents, and the reaction conditions to be employed, but may be based on heat (thermal initiators), light (photo initiators), γ -radiation, or electron beams.

Table 2.4: Commonly used synthetic monomers for hydrogel production.

Monomer	Abbreviation	Chemical structure
Hydroxyethyl methacrylate	HEMA	$\text{CH}_2=\text{C}(\text{CH}_3)\text{COOCH}_2\text{CH}_2\text{OH}$
Methoxyethyl methacrylate	MEMA	$\text{CH}_2=\text{C}(\text{CH}_3)\text{COOCH}_2\text{CH}_2\text{OCH}_3$
Ethylene glycol dimethacrylate	EGDMA	$\text{CH}_2=\text{C}(\text{CH}_3)\text{COOCH}_2\text{CH}_2\text{OCC}(\text{CH}_3)=\text{CH}_2$
Acrylic acid	AA	$\text{CH}_2=\text{CHCOOH}$
Methacrylic acid	MA	$\text{CH}_2=\text{C}(\text{CH}_3)\text{COOH}$
Methyl methacrylate	MMA	$\text{CH}_2=\text{C}(\text{CH}_3)\text{COOCH}_3$
N-Vinyl-2-pyrrolidone	NVP	$\text{CH}_2=\text{CHNCOCH}_2\text{CH}_2\text{CH}_2$
Vinyl acetate	VAc	$\text{CH}_2=\text{CHCOOCH}_3$
N-Isopropylacrylamide	NIPAAm	$\text{CH}_2=\text{CHCONHCH}(\text{CH}_3)_2$

However, three-dimensional polymerization often results in materials containing significant levels of residual monomers and therefore purification of these materials must be performed thoroughly because the unreacted monomers are often toxic and could leach out from the hydrogels continuously. The purification of hydrogels containing residual monomers is typically performed by extraction into excess water, and can take up to several weeks to be completed [51,52].

2.2.4.2 Direct crosslinking of water-soluble polymers

Recently, Khutoryanskiy et al. [53] reported an alternative method to synthesize hydrogels from ready-made water-soluble polymers in aqueous solutions using thermal treatment or microwave irradiation. In this method the aqueous solutions of specific water-soluble polymers such as poly(methyl vinyl ether-alt-maleic anhydride) and poly(vinyl alcohol) are mixed together at room temperature and the crosslinking process is achieved by thermal treatment under high pressure via autoclaving or microwave radiation. Both radiation and thermal crosslinking methods are inexpensive, safe, do not require a purification step and result in sterile hydrogels if a suitable combination of hydrophilic polymers is used [50].

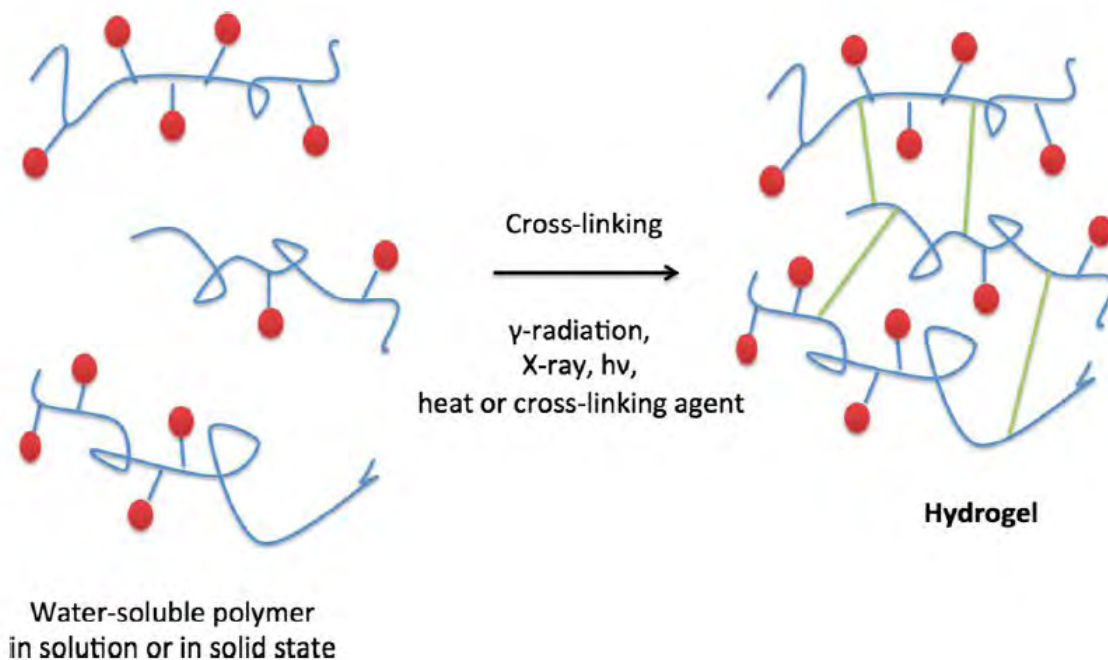


Figure 2.5: Synthesis of hydrogels by cross-linking of ready-made water-soluble polymers [50].

2.2.5 Hydrogel technical features

The functional features of an ideal hydrogel material can be listed as follows [54]:

- The highest absorption capacity (maximum equilibrium swelling) in saline.
- Desired rate of absorption (preferred particle size and porosity) depending on the application requirement.
- The highest absorbency under load (AUL).
- The lowest soluble content and residual monomer.
- The lowest price.
- The highest durability and stability in the swelling environment and during the storage.
- The highest biodegradability without formation of toxic species following the degradation.
- pH-neutrality after swelling in water.
- Colorlessness, odorlessness, and absolute non-toxic.
- Photo stability.
- Re-wetting capability (if required) the hydrogel has to be able to give back the imbibed solution or to maintain it; depending on the application requirement (e.g., in agricultural or hygienic applications).

Obviously, it is impossible that a hydrogel sample would simultaneously fulfill all the above mentioned required features. In fact, the synthetic components for achieving the maximum level of some of these features will lead to inefficiency of the rest. Therefore, in practice, the production

reaction variables must be optimized such that an appropriate balance between the properties is achieved. For example, a hygienic product of hydrogels must possess the highest absorption rate, the lowest re-wetting, and the lowest residual monomer, and the hydrogels used in drug delivery must be porous and response to either pH or temperature.

2.2.6 Problems associated with hydrogels

There is no unmixed blessing in this earth. Hydrogels having so many technical features, also have some problems associated with them.

2.2.6.1 *Low mechanical strength*

The scope of hydrogel applications is often severely limited by their mechanical behavior [55]. Hydrogels contain a major portion of water of its own weight. That makes hydrogels soft and incapable of load bearing. Most hydrogels do not exhibit high stretchability; for example, an alginate hydrogel ruptures when stretched to about 1.2 times its original length. Some synthetic elastic hydrogels have achieved stretches in the range 10–20, but these values are markedly reduced in samples containing notches [56]. Most hydrogels are brittle, with fracture energies of about 10 J m^{-2} [57], as compared with $\sim 1,000 \text{ J m}^{-2}$ for cartilage [58] and $\sim 10,000 \text{ J m}^{-2}$ for natural rubbers [59]. Intense efforts are devoted to synthesizing hydrogels with improved mechanical properties [60–62].

2.2.6.2 *Inhomogeneity of Hydrogels*

Another problem associated with hydrogels is spatial gel inhomogeneity [63]. In contrast to ideal gels with a homogeneous distribution of cross-links, hydrogels always exhibit an inhomogeneous cross-link density distribution, known as the spatial gel inhomogeneity. The spatial inhomogeneity is undesirable because it dramatically reduces the optical clarity and strength of hydrogels.

2.2.6.3 *Environmental aspects*

Some hydrogels that are currently being used are not completely degradable. However, more and more research is being done and possibilities are being sought to form hydrogels on an organic basis. These are not harmful to the environment. Nevertheless, many hydrogels from biomaterials are currently being used in, for example, wound dressings and contact lenses. These types of products are not harmful to humans.

2.2.7 Applications

Hydrogel of many synthetic and natural polymers have been produced with their end use mainly in tissue engineering [64], pharmaceutical, and biomedical fields [50]. In every case, it is necessary to fulfill the primary requirements of material stability and good biocompatibility [65].

Due to their high-water absorption capacity and biocompatibility they have been used in 3D organ/tissue printing [66], wound dressing [67], drug delivery [68], trans-dermal systems, dental materials, implants, injectable polymeric systems, ophthalmic applications, hybrid-type organs (encapsulated living cells) [69], soft and hard contact lenses [70,71], polymers are used as intraocular

lenses [72], implants (in otorhinolaryngology [73], neurology [74], urology [75] and gynecology [76]). Polymers are also used in ointments [77], blood extenders [78], sensors and actuators in biochips [79,80].



Figure 2.6: Different biomedical applications of hydrogels
(Ref: <http://stickykaist.ac.kr.menu2/menu3.php>).

2.3 3D Printing

2.3.1 Definition

Printing is a unique technology with a wide range of applications. Printers are being used in fields from mass communication to electronics, and recently in medicine [81-86]. The invention of 3D printing has opened a new avenue for research and industry to make complicated objects not possible otherwise. [87]

Three-dimensional (3D) printing, otherwise known as additive manufacturing (AM), layered manufacturing, rapid prototyping (RP), or solid freeform fabrication is a process in which a 3D object is created by laying down successive deposits of material (liquid, powder, or sheet material) in two-dimensional (2D) layers as the printing machine reads data from the computer aided design (CAD) [88-91]. This process was first described in 1981 by Hideo Kodama of Nagoya Municipal Industrial Research Institute when he invented manufacturing methods to create a 3D plastic model [92-93]. Since then the process has matured and many different printers currently exist that are much more sophisticated than the original machines.



Figure 2.7: 3D printing technique.

Numerous sophisticated 3D printed instruments and tools have been created and are being employed in academic laboratories and industry. Until recently, 3D printing was mostly used for prototyping devices for design and manufacture. It allowed for application in a range of fields including aerospace, engineering, consumer products, arts, food industry, education, manufacturing, and medicine. More recently, 3D bioprinting has been expanded to applications such as cell and tissue printing [94,95].

2.3.2 Classification

3D Bioprinting aims to fabricate biologically relevant structures using tissue-like materials, such as hydrogels, for regenerative medicine applications including pharmaceutical drug screening and organ repair and replacement [96]. Several bioprinting approaches have been successfully used to fabricate 3D structures layer-by-layer [97]:

- (i) Selective layer/laser sintering (SLS);
- (ii) Fused deposition modeling (FDM) also known as fused filament fabrication (FFF) or molten polymer deposition;
- (iii) Stereolithography;
- (iv) Digital light processing;
- (v) Inkjet 3D printing and;
- (vi) Electronic beam melting.

Only FDM and inkjet printing are related to this thesis work and they are discussed below.

2.3.2.1 Fused deposition modeling (FDM)

Fused deposition modeling (FDM) printers include a stage and one or a few temperature-controlled cartridges (i.e., syringes or pens) that can be loaded with cells, cell-laden hydrogels, and biomaterials to print [82,97,98]. The materials inside the cartridge may be dispensed using a pneumatic or mechanical dispensing system [98-100]. The operator can control the extrusion procedure, speed, and the displacement of the cartridge, and/or the stage in x, y, and z directions, by means of a computer software program [87].

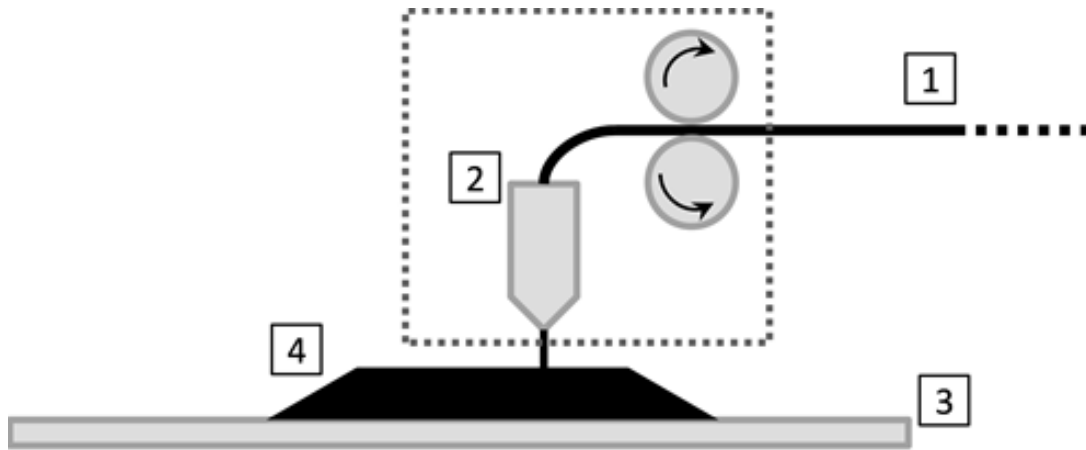


Figure 2.8: A schematic illustration of FDM–

Material (1) is forwarded into the heated extruder (2). The plastic melts and is extruded onto the printer bed (3), where it cools down. Due to the relative motion of the extruder and the printer bed to one another, the printed part (4) is formed [101].

Through this technique, filaments made of thermoplastic materials are extruded in thin threads and deposited layer by layer in the desired 3D structure and adhere to each other by physical interactions. The layer underneath hardens as it cools and binds with the new layer that is added on the top, remaining a fully solidified structure throughout the process (fig. 2.11) [101]. FDM is already used to produce commercial plastics and, in general, is the most used among all the techniques; this is partially because of the low price of the printer and the facile manipulation, which makes it possible even for home use. Thermoplastic polymers currently processed with FDM are acrylonitrile butadiene styrene (ABS) and poly(lactic acid) (PLA). Other polymers were also found suitable for this technique: acrylonitrile-styrene-acrylate, polycarbonate, polyethylene terephthalate, Thermoplastic polyurethane (TPU), thermoplastic elastomers etc. [102]. Thermoplastic materials pyramid for different common polymers available in FDM is shown in fig.2.12. As a rule of thumb, the higher a material is the better its mechanical properties.

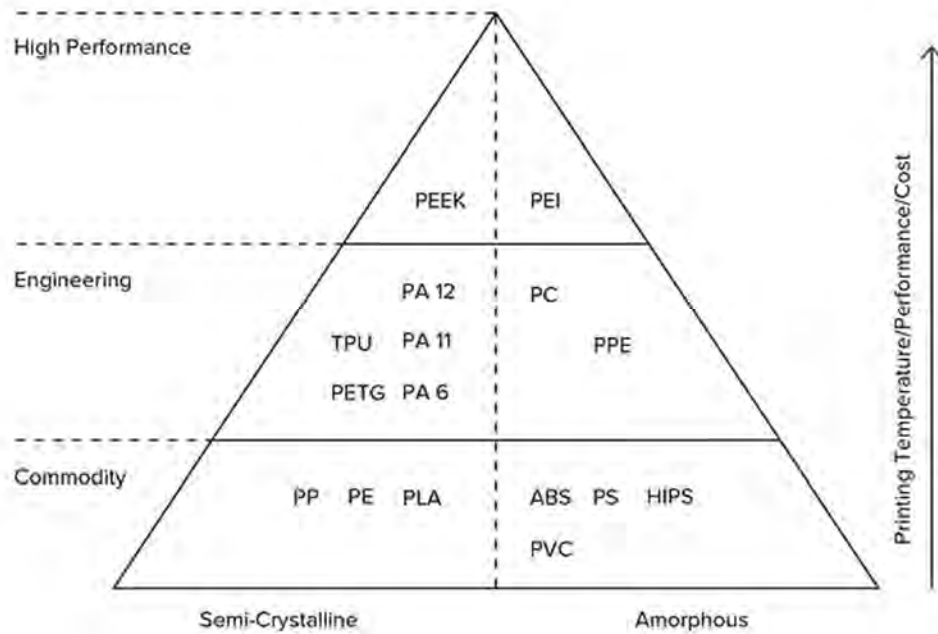


Figure 2.9: Thermoplastic materials pyramid available in FDM.

The roughness of the 3D-printed structures is an important issue in the case of FDM, since it affects not only the appearance but also the mechanical resistance of the products. A polishing device connected to the 3D printer [103], the use of the vaporized acetone to melt uniformly the surface of 3D-printed prostheses made of ABS [104] and filling the grooves with the material dissolved by the solvent stored in a pen-style device [105] were among the solutions proposed to remove the layer grooves.

2.3.2.2 Inkjet printing

Inkjet printing is a well-established tool for commercial and consumer image production, and has been incorporated into 3D printing methods for prototyping [106] and manufacture [107]. It has been highlighted as a promising additive method due to its precision, accuracy, low cost, ability to deposit multiple materials contemporaneously, and simple scale up/out, with material throughput being dependent on the size of the printer and number of jets [108-110].

Inkjet printing is a non-contact approach that enables processing of 1–100 pl droplets of liquid into 2D and 3D structures. This approach involves dissolving or dispersing the material of interest in a liquid to form an ink. Drops of ejected from a micrometer-scale nozzle are commonly created by either (a) heating of the liquid to a temperature greater than the boiling temperature or (b) application of a voltage to a piezoelectric transducer, which leads to vibration of the material [111,112]. Inkjet printing is commonly referred to as a ‘maskless’ or ‘tool-less’ approach since either movement of the inkjet nozzle or movement of the substrate enables accurate and reproducible formation of the desired structure [113].

The exact printing sequence procedure differs by printer type and material. In general, inkjet printers create the printed model from the “bottom up” by depositing material in a layer-by-layer fashion [114]. Both photopolymers and bioink require special modifications to their printing process that bear mention. Photopolymers are jetted in ultrathin sequential layers onto a build tray and are cured (each layer at a time) by ultraviolet light [115,116]. Bioink is a water-based liquid that may contain proteins, enzymes, and/or cells suspended in media or saline to form a dense cellular slurry [117,118]. After printing, the cells are initially supported by inert gel. The pre-specified geometry of the bioink is maintained as the cellular model matures [117], lending support to the exciting prospect of 3D bioprinted organs in the future.

Inkjet printers are traditionally categorized into two classes, continuous and drop-on-demand [119] as shown in fig. 2.13. Continuous inkjet printers have high speed capabilities and can print on large-scale areas; hence, they are normally used in printing industries. When precision and cost matter, the drop-on-demand inkjet printers are preferred.

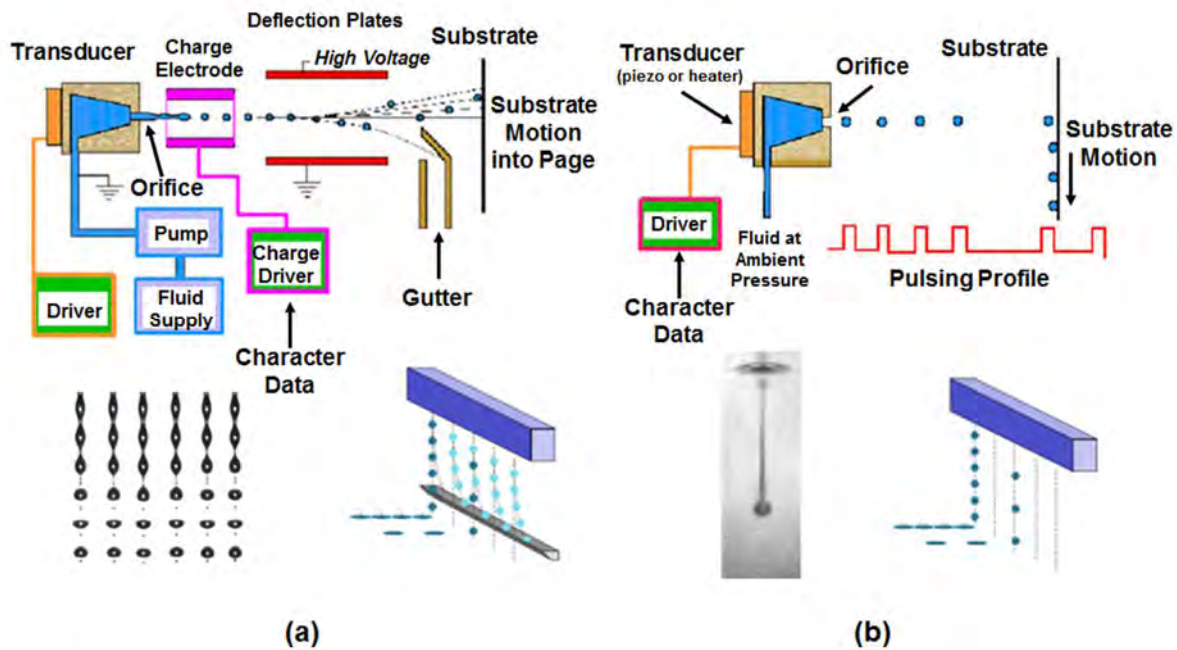


Figure 2.10: Types of inkjet printer, (a) continuous, (b) drop-on-demand

(Ref: <https://urlzs.com/CQTXo>).

Thermal and piezoelectric inkjet printers are the most popular drop-on-demand printers as shown in fig. 2.13 and 2.14 [120]. In thermal printers, a heater makes a bubble of ink out of a reservoir and ejects it from the nozzle [119,121]. In piezoelectric drop-on-demand printers, the application of a voltage pulse changes the shape and size of the piezoelectric material and, consequently, a droplet of ink is produced by pressure on the ink reservoir caused by the piezoelectric material [119]. Droplet shape and size can be adjusted by tuning the applied voltage to the piezoelectric material [83].

Table 2.5: Types of inkjet printer.

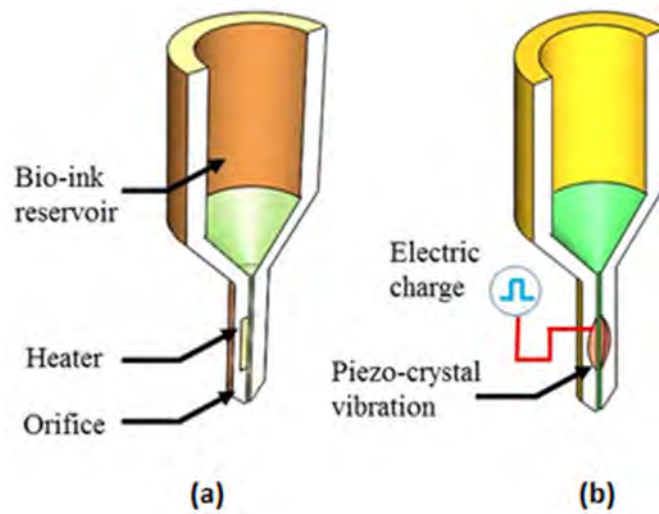
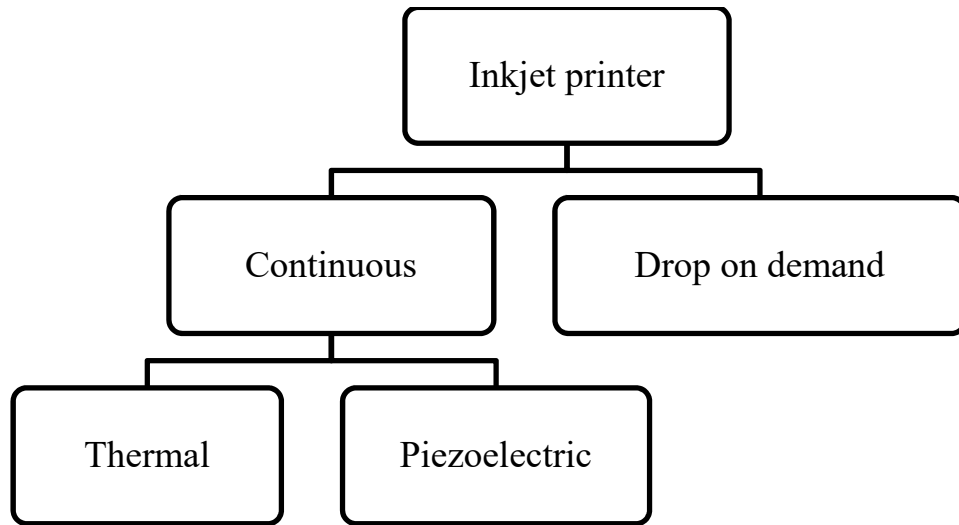


Figure 2.11: Types of drop-on-demand inkjet printer, (a) thermal, (b) piezoelectric.

Table 2.6: Summary of fused deposition modeling (FDM) and inkjet printing.

Printer type	Printing process	Materials used	Advantages and disadvantages	References
Fused deposition modeling (FDM)	Strands of thermoplastic polymers or polymer/ceramic composites extruded through a tip and deposited layer by layer	Structural and biopolymers, PLA, ABS, ceramic-polymer, or metal polymer composites	Advantage: – Easy to use and can be used with a large variety of materials Disadvantage: – Material restriction related to thermoplastic polymers	122-124
Inkjet printer	Extrusion-based layer-by-layer deposition	Polycaprolactone (PCL), hydroxyapatite (HA), bioactive glasses, polylactic acid (PLA)/polyethylene glycol (PEG), poly(hydroxymethylglycolideco- ϵ -caprolactone).	Advantage: – Easy to incorporate both drug and biomolecules (proteins and living cells) Disadvantages: – Post-processing may be needed for some materials. – Process works well within a certain range of viscosity.	125-128

2.3.3 Advantages

The interest in 3D printing technology is highlighted by the vibrant growth of the sales reported by 3D printer producers, who claim an increase of 17.4% in worldwide revenues, in 2016, as compared with previous years. A substantial amount of research predicts the proliferation of this industry and a

potential increase of the products and services from \$6 billion in 2016 to \$21 billion worldwide by 2021 [129].

3D printing offers many advantages over traditional manufacturing, which include the ability to create objects with complex internal structures, improved versatility and customization, and less space requirements [130]. Advantages of 3D printing also include onsite production, less waste, and ability to create one-off (single use) objects and small series of personalized products [101,131]. Another important advantage of 3D printing is the “zero lead time” between the design stage and final construction which allows for rapid prototyping. This makes 3D printing an important adjunctive tool for research and innovation purposes [90]. When combined with medical imaging, 3D printing opens new opportunities in the advancement of medicine. Its application within the medical field is facilitated by the ease of converting modern medical images into CAD designs [132].

2.3.4 Disadvantages

3D printing technology has been a major breakthrough in past few decades. But it has some drawbacks like cost, limited size, limited raw materials, questionable accuracy, violation of copyrights etc. Cost is perhaps the biggest factor in determining the usefulness of 3D printers in clinical practice. Despite the drop-in prices of printers in the past few years, high-resolution commercial machine costs a huge sum of money. Many institutions do not produce the volume of 3D models needed to justify the cost of an in-house printer. Instead, third-party companies that specialize in the manufacturing of 3D printed objects for medical use offer an alternative. Potential disadvantages without sourcing include a higher cost per model after discounting the cost of hardware, patient privacy issues due to off-site transfer of files, and longer turnaround times due to shipping [89].

While 3D printing is a significant manufacturing breakthrough, materials that can be used are still limited, and some are still under development. For example, the 3D printing material of choice is plastic. Plastic is preferred as it can quickly and easily be deposited down in melted layers to form the final product. However, plastic may vary in strength capacity and may not be the best for some components. Some companies offer metal as a material, but final product parts are often not fully dense. Other specialized materials including glass and gold are being used but are yet to be commercialized.

PRIMARY 3D PRINTING PLASTICS		
PLA	ABS	POLYMAX PLA
Reliable	High impact strength	Extremely strong
Large prints	Deep colours, matte finish	Reliable
Lots of colours, glossy finish	High UV resistance	Large prints
Fastest print times	Easily post-processed	Sometimes needs sanding down
Weak to high temperatures	Not ideal for large parts	More expensive
Recommended for: Prototypes, props and models	Recommended for: End-use parts, environment resistance	Recommended for: Functional parts not used in high temperatures
SECONDARY 3D PRINTING PLASTICS		
WOOD	T-GLASE / NYLON	FLEXIBLE
Wooden texture	Extremely strong	Highly flexible
Easy to grind/sand/smooth	Transparent	Very similar to rubber
Small to medium prints only	Small to medium prints only	Small-medium prints only
More Expensive	More Expensive	More expensive
Recommended for: Wooden structures in architecture models	Recommended for: Maximum strength polycarbonate-like prints	Recommended for: Rubber-like components

Figure 2.12: Common 3D printable materials with their advantages and disadvantages.

The size of objects created with 3d printers is currently limited and the accuracy isn't good enough yet specially for smaller products. However, in the near future; large items such as architectural structures with better accuracy can be created using 3d printing.

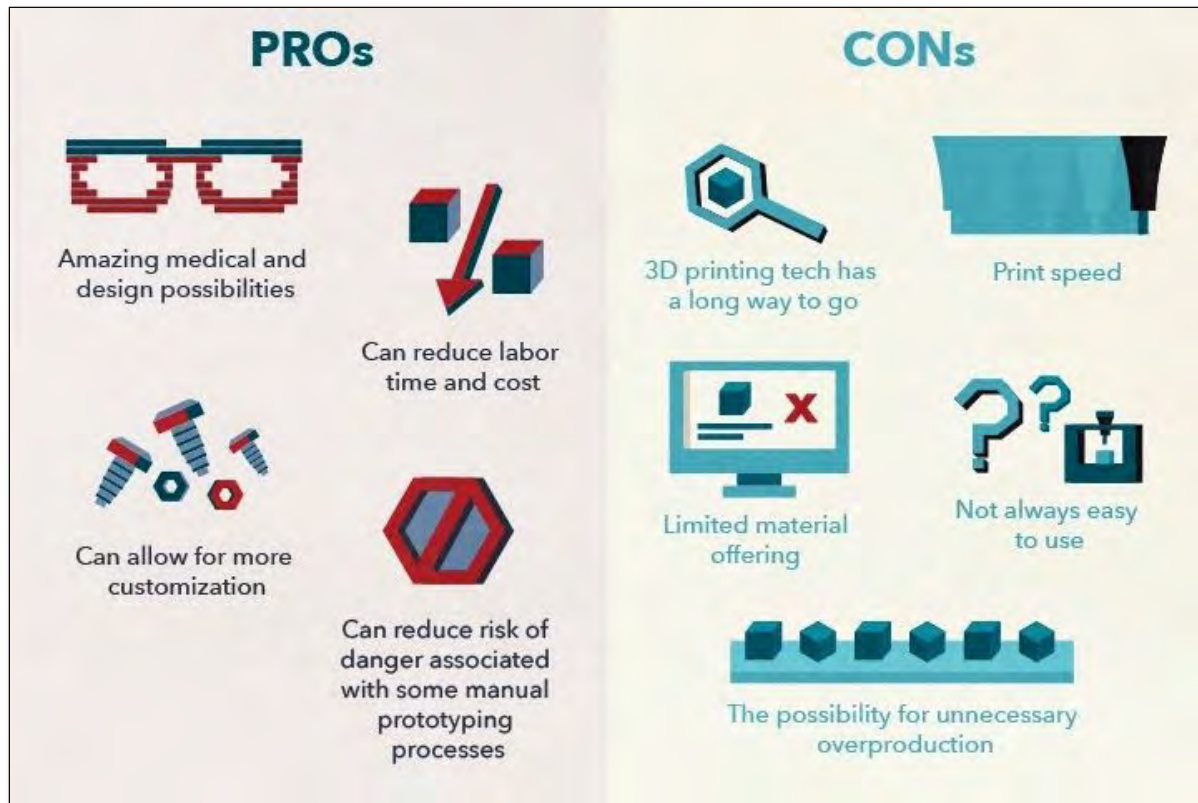


Figure 2.13: The pros and cons of 3D printing.

2.3.5 Applications

3D-printing technology is attractive for many applications: (i) in the research field for prototyping or for a limited production of prototypes; (ii) in medicine to create 3D biomedical structures using digital models obtained with different medical imaging techniques (computer tomography, magnetic resonance imaging, ultrasound); (iii) in industry for prototyping and manufacture of spare parts for automotive, airplanes, etc. 3D-printing development is speeding up annually due to the reduction of the production cycles, waste, limited use of cutting fluids, and it becomes more accessible for small companies etc. [133]. Thus, 3D printing is often used to develop medical devices [134], flexible electronics [135,136], various pieces for automotive or robotics [137], art objects [138], precise replica of archeological objects etc.

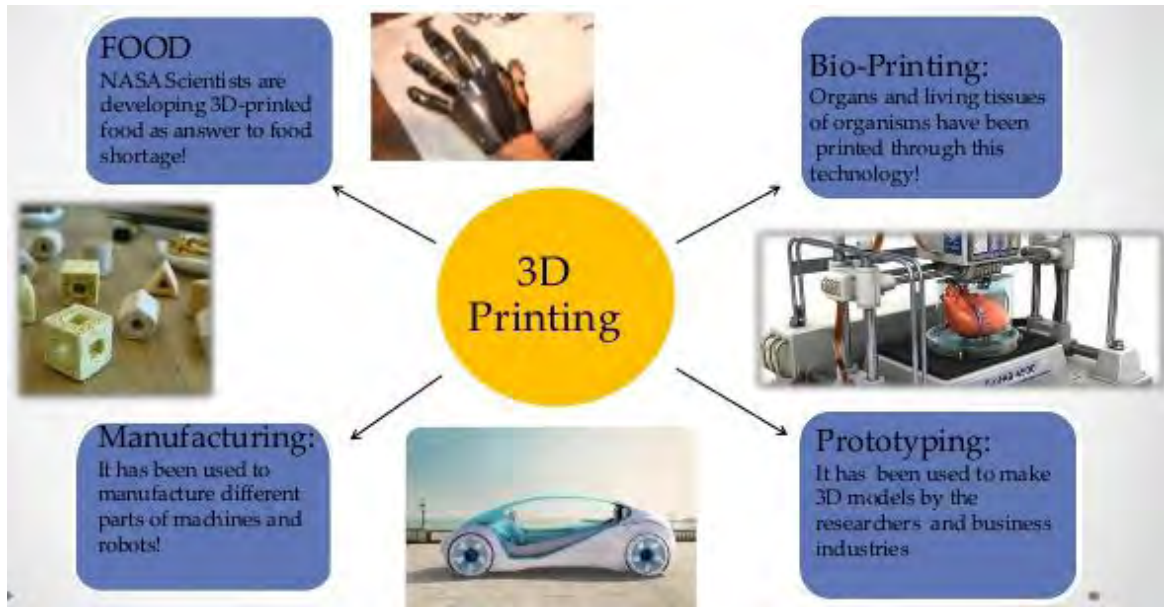


Figure 2.14: Different applications of 3D printing.

3D printing has been recognized for its potential in surgical modeling. Surgeons can fabricate a 3D printed model of the surgical task and this model can be used to educate the patient, plan the surgical approach, and act as an intraoperative surgical guide. 3D printed medical models are being used for cardiac, orthopedic, dental, and craniofacial applications [139]. Surgical models may decrease surgical time and decrease complications. For orthopedic and craniofacial applications, 3D printed surgical guides can be used intra-operatively to determine the optimal location for internal plates and screws. 3D printed surgical reconstructive plates can be manufactured which are customized for the patients and specific to their surgical requirements. Drill guides can be printed in advance to assist the surgeon with regards to optimal orientation, location and depth for reconstructive screws and these guides are becoming increasingly accurate and more useful as innovations in transparency and flexibility become available for 3D printing [132].

3D anatomical models are proving their practicality in the training and education of physicians. 3D printed models are safe, reusable, and can accurately represent actual pathology [89,140]. In recent years the development of bioprinting has enabled researchers to produce living tissues using the principle of 3D printing. A variety of tissues, including bone [141], skin [142], and cartilage [143], have already been manufactured for transplantation. Medical imaging is considered to be an essential tool in the organ replication process by providing engineers with information on the 3D structure and function of tissues and organs [98]. A customized, bioresorbable tracheal splint was created through 3D printing for a 2-month old infant with tracheobronchomalacia [144]. The splint was manufactured from polycaprolactone (PCL) based on computed tomography (CT) images of the patient's airway.

Current research is under way to develop functional kidney, liver, and other solid organs for transplantations [98]. Medical imaging likely plays an important role in the development of

regenerative medicine by providing a noninvasive means of identifying damaged tissues, providing valuable information used in the creation and implantation of organs, and subsequent monitoring following surgery [89].



Figure 2.15: Biomedical applications of 3D printing.

2.4 Challenges in printing hydrogels

The use of hydrogels and 3D printing in biomedical application is projected to grow exponentially in the coming years. Although the theoretical applications for their use in medicine are endless, there are barriers that would need to be addressed before their potential can be fully used. Some of the key challenges associated with 3D printing of hydrogels lie in achieving target mechanical properties, durability, and designed architectures to satisfy both mechanical and functional requirements. This is especially important in load-bearing implants, where mechanical performance and fatigue are critical under diverse conditions both *in vitro* and *in vivo* [88].

Hydrogels have porous structure which is their premier property. But challenges lie in the ability of 3D printing technologies to accurately produce desired porous architectures meeting design intent. Often, the length scales associated with the designed porous architectures (ranging from tens to hundreds of microns) are beyond the resolution capability of the 3D printing technology in use. The effective mechanical properties and failure modes of such porous architectures need to be well understood and integrated into implant design procedures to avoid undesirable implant failure outcomes. Computational design techniques validated with experimentally measured property data need to be integrated into implant design procedures [88].

With the materials currently used for 3D models, the texture does not accurately imitate that of human tissue. Surface finish or surface roughness is not up to the marks in many cases. Thus, careful attention has to be paid to understand the surface finish and surface texture requirements as well as choosing appropriate post-processing procedures to attain final desired surfaces in biomaterials fabricated by

3D printing. The amount of time and effort spent in postprocessing affects the cost-effectiveness of using 3D printable hydrogels. Many companies are developing software to speed up these steps that allow users to easily perform segmentation and 3D reconstruction [88,89,140].

Last but not least, there are some limitations regarding the hydrogels to become fully environment friendly. 3D printing is a layer by layer process. However, every layer and every interface between adjacent layers provides the opportunity for the introduction of defects during 3D printing that can lead to failure of the part under the intended application conditions. Because of their porous structure hydrogels are prone to this problem. Cytotoxicity test is recommended before using hydrogels in biomedical applications.

Chapter 3: Standard Operating Procedure (SOP) for ABS and PLA Filament on Cartesio FDM 3D Printer Using Cura 3D Modeling Software

3.1 Objective

The objective of this standard operating procedure (SOP) is to describe the method of operation of ABS and PLA filament using Ultimaker Cura 3D modeling software on a Cartesio FDM 3D Printer.

3.2 Introduction

3D printing or additive manufacturing is a process of making three dimensional solid objects from a digital file. The creation of a 3D printed object is achieved using additive processes. In an additive process an object is created by laying down successive layers of material until the object is created.

It all starts with the creation of a 3D model in the computer. This digital design is for instance a CAD (Computer Aided Design) file. A 3D model is either created from the ground up with 3D modeling software or based on data generated with a 3D scanner. With a 3D scanner you're able to create a digital copy of an object.

There are many types of 3D printing processes as described in the previous chapter. In this SOP, we are interested in FDM (Fused Deposition Modeling) i.e. material extrusion printing process. In FDM, an object is built by selectively depositing melted material in a pre-determined path layer-by-layer. The materials used are thermoplastic polymers and come in a filament form.

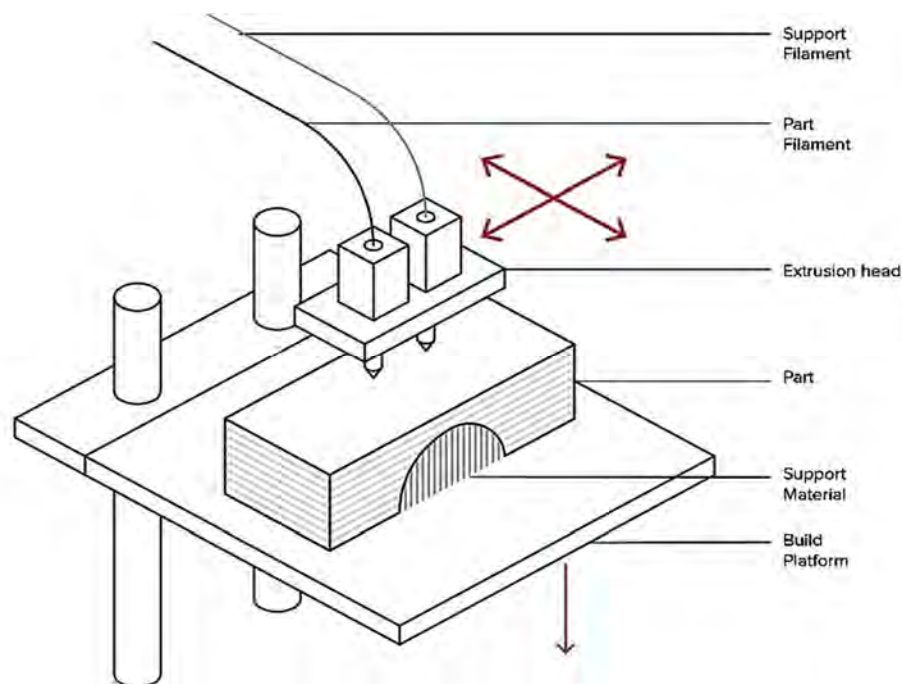


Figure 3.1: Schematic of a typical FDM printer.

3.3 Definitions

°C	The Celsius scale used as a temperature scale, degrees Celsius
3D	Three-dimensional
ABS	Acrylonitrile butadiene styrene, a thermoplastic polymer
Adhesion	A spray used on the BED to help filament cling to it.
BED	Also known as bed platform. It is the flat-area within the 3D printer on which the filament extruder prints onto.
Cura	A 3D modeling software to examine print before printing
Extruder	A device within the 3D printer which the plastic filament flows into.
Filament	Thermoplastics which are used in 3D printing (ABS and PLA)
PC	Personal Computer
PLA	Polylactic Acid, a thermoplastic polyester
SOP	Standard Operating Procedure
T _g	Glass transition temperature

3.4 Safety Precautions

Some safety precautions are needed before going for FDM printing. They are listed below:

- a) Safety training must be carried out by a person who is familiar with the printer for all equipment and materials used.
- b) Lab coat and safety glass should be worn during lab work.
- c) One must be careful while 3D printer is in use, many hot parts can be exposed while printing (Example - extruder can reach temperature of >180°C).
- d) All waste generated must be disposed of in appropriate containers

3.5 Materials & Equipment

- a) ABS and PLA Filament
- b) 3D Printer
- c) PC from which to operate 3D printer and Cura software
- d) Adhesive Spray (if required)
- e) SD card



Figure 3.2: Cartesio FDM 3D Printer.



Figure 3.3: Adhesive spray.

3.6 Procedure

1. Turn on the PC system.
2. Open the Cura software from the desktop icon or through the PC control panel.

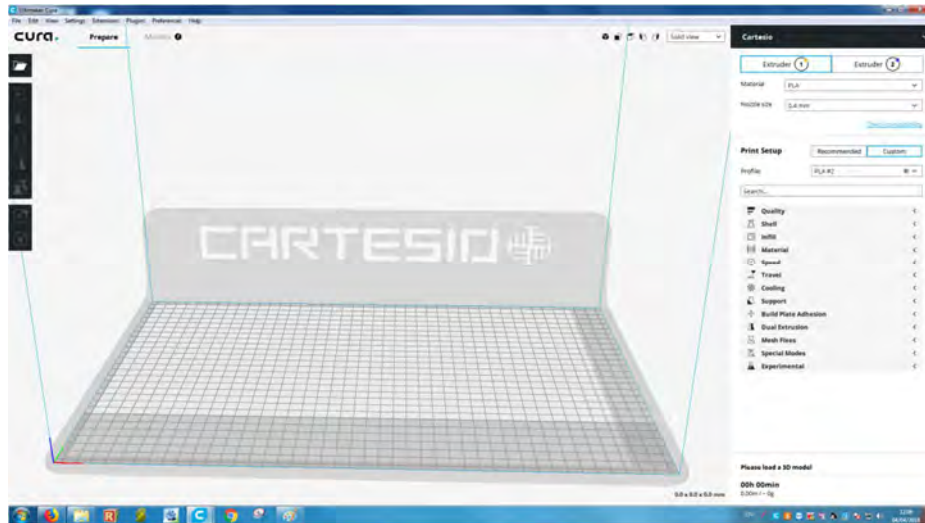


Figure 3.2: Cura software interface.

3. Upload digital file onto the Cura interface, e.g. STL file, which holds the requested 3D print. This can be accomplished by using the 'Open File' icon on the top left of Cura.



Figure 3.3: Cura software interface showing 'open file' icon.

4. Ensure that the 3D model is completely issue free by viewing it layer by layer i.e., layer view (fig. 3.6).

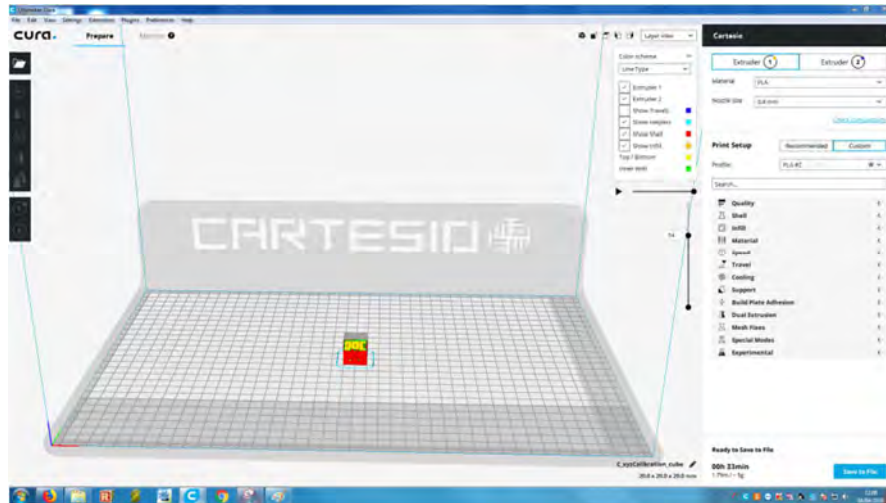


Figure 3.4: Cura software interface showing ‘layer view’.

5. If support is required, provide support. (see 3.8)
6. Name 3D model.
7. Insert SD card and save the 3D model.
8. Remove the SD card, and insert it into the 3D printer.
9. Prepare the printer (see 3.7)
10. On the 3D printers control panel select ‘Saved Files’.
11. Choose the recently saved and named file.
12. Once file is selected, close the lid of the printer and let the printer prepare for printing.
13. Once the selected temperature is reached with the extruder and BED, printing will begin.
14. As printing begins, oozing will occur. This oozing of filament should be clean off by a wire brush automatically. But if doesn’t, do it manually.
15. Allow the system to print.
16. Ensure first few layers print problem free. It should be okay if there is no problem in the initial layers. But occasional checkup is recommended.
17. If a problem is encountered during printing, see the troubleshoot section (see 3.10) to find out a possible solution.
18. After a successful print, allow sufficient time for the piece to cool down.
19. Once cool, open the lid and remove the print.
20. Ensure that any extra printed material such as rafts, skirts, brims is removed from the printer.
21. After removal from the bed, inspect the work piece for any faults, impurities or poor-quality printing.
22. If faults/impurities/problems/poor quality printing are found on the sample, see the troubleshoot section (see 3.10) to find out a possible solution.
23. Once finished, make sure to clean the working environment used.
24. Close down Cura and all running programs and then finally the PC.

3.7 Preparing the printer

1. Check if there are any printed object/rafts/skirts left onto the printer. If the printer is occupied with them, remove them and clean the printer.
2. Make sure glass is firmly attached to bed.
3. Ensure to apply adhesion to the printing Bed, by lightly drizzling one or two quick sprays of adhesion onto the BED. Typically, you need to spray after 5/6 printing.
4. Ensure sufficient filament for print, if not change filament (see 3.9).
5. Turn on the printer and insert SD card in it. Now, the printer is ready to print.

3.8 Support Generation

Sometimes you may need to provide support parts to the model which have overhangs, without these, the model would collapse during printing. Here are rules of providing support to a model-

1. If there is an overhang of $>45^\circ$ in a model, support is needed.
2. Enable 'Generate Support'. This can be found in settings on the right hand-side of Cura (fig. 3.7).

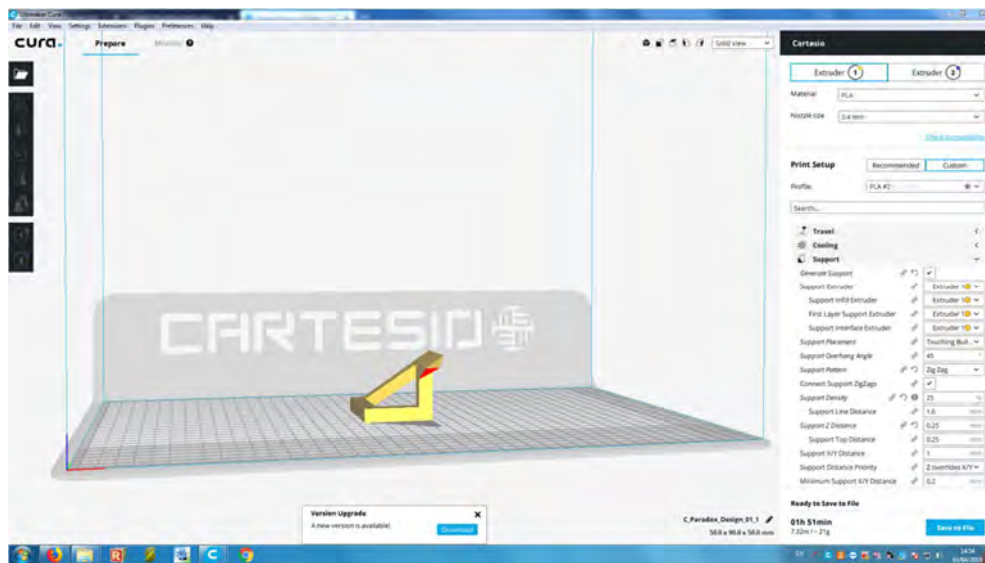


Figure 3.5: Cura software interface showing 'generate support'.

3. Once Support is generated, inspect the piece again in layer view.
4. If any problem is observed, see the troubleshoot section (see 3.10) to find out a possible solution.

3.9 Change of Filament

1. Once a change of filament is needed or desired, Switch off the 3D printer (fig. 3.8).



Figure 3.6: Cartesio FDM 3D printer's power switch.

2. Open lid on 3D printer and pull extruder head to the middle of the bed.

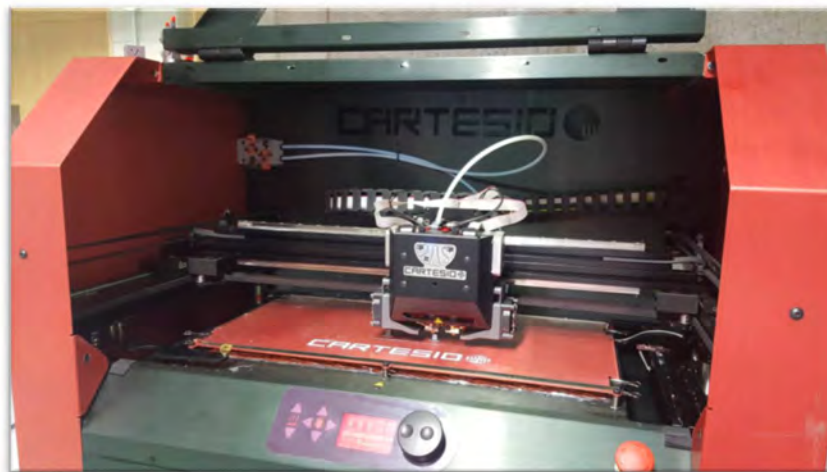


Figure 3.7: Cartesio FDM 3D printer's working area.

3. On top of the extruder head, notice the two pipes which lead the filament into the extruder.
4. On extruder head, notice the two spring-loaded switches where the pipes lead (fig. 3.10).



Figure 3.8: Cartesian FDM 3D printer's extruder head.

5. Push down on the spring-loaded switch which leads the desired filament into the extruder.
6. With the spring-loaded switch pushed down, grab the filament and pull out the filament from extruder head.
7. Now from the roll of filament, slowly roll-back the filament to draw out filament from the 3D printer.
8. Once filament is safely removed, ensure no knotting occurs as it will create problem in printing (see 3.10).
9. Store roll of filament back in its box and place the box in a dry area. Silica gel beads package is highly recommended to protect filament from moisture in the air.
10. Select a filament to go back into the extruder.
11. Place filament on roll and again push filament through pipe which lead to extruder.
12. Once a significant amount of filament is pushed through the end of the pipe, switch on 3D printer.
13. Now heat the extruder up to a certain amount of temperature ($>200^{\circ}\text{C}$ for ABS and $>180^{\circ}\text{C}$ for PLA).
14. Use manual extrusion to remove the previous stuck filament in the extruder.
15. When there starts to extrude the desired filament, change of filament is completed.

3.10 Troubleshooting

Here is an extensive list of the most common 3D printing issues along with their possible causes and solutions.

3.10.1 Not Extruding at Start of Print

This issue is a very common issue in 3D printing. This may cause because of-

a) Extruder was not primed before beginning the part

Most extruders have a bad habit of leaking plastic when they are sitting idle at a high temperature. The hot plastic inside the nozzle tends to ooze out of the tip, which creates a void inside the nozzle where the plastic has drained out.

A common way to do this in Simplify3D is by including something called a skirt, an outline that surrounds your part but does not touch the part (fig. 3.11) The skirt will draw a circle around your part, and in the process, it will prime the extruder with plastic.

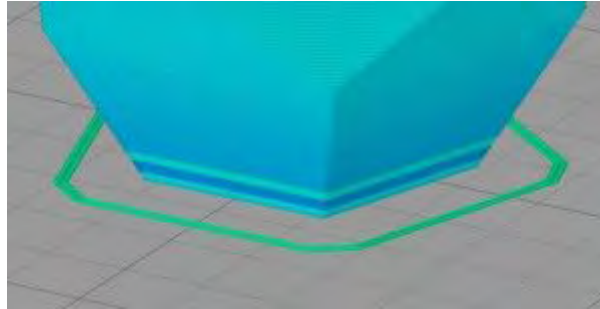


Figure 3.9: Skirt.

b) Nozzle starts too close to the BED

If the nozzle is too close to the build table surface, there will not be enough room for plastic to come out of the extruder. The hole in the top of the nozzle is essentially blocked so that no plastic can escape. An easy way to recognize this issue is if the print does not extrude plastic for the first layer or two, but begins to extrude normally around the 3rd or 4th layers as the bed continues to lower along the Z-axis.

To solve this problem, you can use the very handy G-Code offsets which can be found on the G-Code tab. This allows you to make very fine adjustments to the Z-axis position.

c) The extruder is clogged

If none of the above suggestions are able to resolve the issue, then it is likely that your extruder is clogged. This can happen if foreign debris is trapped inside the nozzle, when hot plastic sits inside the extruder too long, or if the thermal cooling for the extruder is not sufficient and the filament begins to soften outside of the desired melt zone.

Fixing a clogged extruder may require disassembling the extruder, so please contact your printer manufacturer before you proceed.

3.10.2 Print Not Sticking to the BED

It is very important that the first layer of your print is strongly connected to the printer's build platform so that the remainder of your part can be built on this foundation. If the first layer is not sticking to the

build platform (fig. 3.12), it will create problems later. There are many reasons behind these first layer adhesion problems-

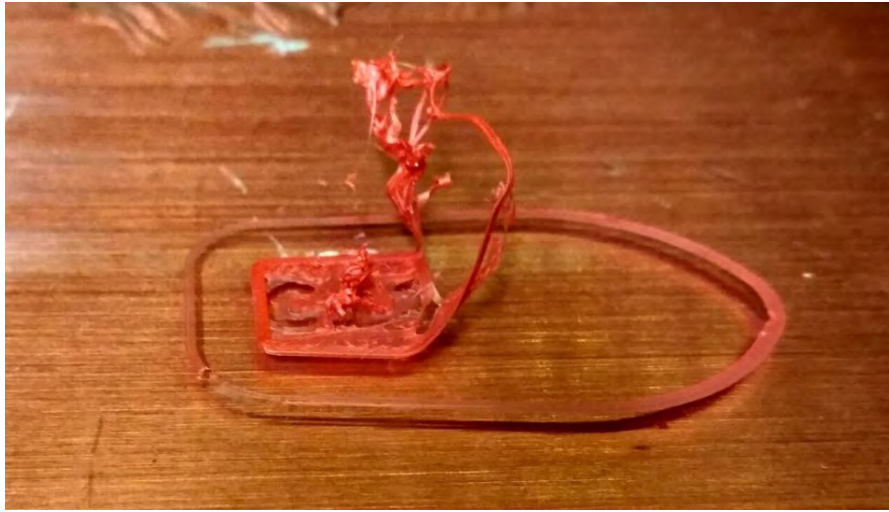


Figure 3.10: Print not sticking to the BED.

a) Lack of adhesive spray

An adhesive spray is required for Cartesian FDM 3D Printer so that the model sticks to the BED. It is done by lightly drizzling one or two quick sprays of adhesion onto the BED. Typically, you need to spray after 5/6 printing. Note that, too much adhesive spray may damage the model.

b) First layer printing is too fast

As you extrude the first layer of plastic on top of the build platform, you want to make sure that plastic can properly bond to the surface before starting the next layer. If you print the first layer too fast, the plastic may not have time to bond to the build platform.

If you feel that your printer is moving too fast on the first layer, try reducing this from settings (fig. 3.13)



Figure 3.11: Cartesio FDM 3D printer's settings for 'printing speed'.

c) Temperature or cooling settings

Plastic tends to shrink as it cools from a warm temperature to a cool temperature. Because of this fact, the plastic will tend to separate from the build platform as it cools. If you notice that the layer seems to stick initially, but later separates from the print bed as it cools, it is possible that your temperature and cooling settings are to blame.

As a general starting point, ABS and PLA tends to adhere well to the BED that is heated to 100-100°C and 50-70°C respectively. You can adjust these settings by clicking on "Materials" tab and then adjusting the "Build Plate Temperature" (fig. 3.14)



Figure 3.12: Cartesio FDM 3D printer's settings for temperature control.

d) Nozzle starts too far away from the BED

If the nozzle is too far away from the BED, then the model won't stick properly to the BED.

To solve this problem, you can use the very handy G-Code offsets which can be found on the G-Code tab. This allows you to make very fine adjustments to the Z-axis position.

3.10.3 Warping

As you start printing larger models, you may start to notice that even though the first few layers of your part successfully adhered to the bed, later the part begins to curl and deform (fig. 3.15). This behavior is common when printing very large or very long parts with high temperature. The main reason for this problem is the fact that plastic tends to shrink as it cools. This can be solved by the following procedures-



Figure 3.13: Warping.

a) Use a heated BED

Many machines come equipped with a heated bed that can help keep the bottom layers of your part warm throughout the print. As a general starting point, ABS and PLA tends to adhere well to the BED that is heated to 100-100°C and 50-70°C respectively. You can adjust these settings by clicking on “Materials” tab and then adjusting the “Build Plate Temperature” (fig. 3.14).

b) Use a heated enclosure

While a heated BED can keep the bottom layers of your part warm, it may struggle to keep the upper layers of the part from contracting once you start printing taller objects. In this situation, you may find it useful to close the lid of the printer that can help regulate the temperature of the entire build volume.

3.10.4 Layer shifting or Misalignment

3D printer simply attempts to move the toolhead to a specific location, and hopes that it gets there. In most cases, this works fine because the stepper motors that drive the printer are quite powerful, and there are no significant loads to prevent the toolhead from moving. However, if something does go wrong, the printer would have no way to detect this. For example, if you happened to bump into your printer while it was printing, you might cause the toolhead to move to a new position. The machine has no feedback to detect this, so it would just keep printing as if nothing had happened. If you notice misaligned layers in your print (fig. 3.16), it is usually due to-

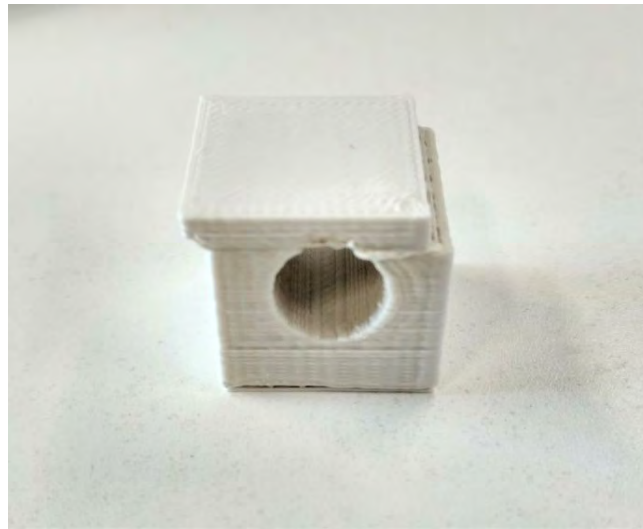


Figure 3.14: Layer shifting or Misalignment.

a) Toolhead is moving too fast

If you are printing at a very high speed, the motors for your 3D printer may struggle to keep up. If you feel that your printer may be moving too fast, try to reduce the printing speed. To do this, click “Speed” and adjust the “Printing Speed” (fig. 3.13).

3.10.5 Stops Extruding in the Middle of a Print

If your printer was extruding properly at the beginning of your print, but suddenly stopped extruding later (fig. 3.17), there are typically only a few things that could have caused this problem-

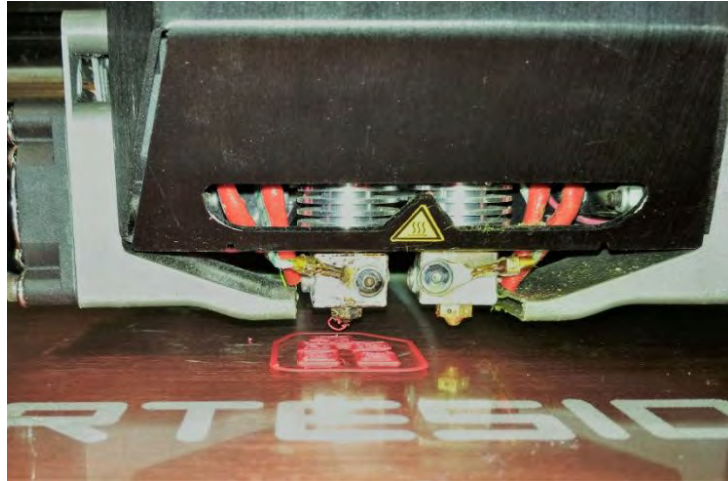


Figure 3.15: Stoppage of extrusion in the middle of a print.

a) Out of filament

This one is pretty obvious, but before checking the other issues, first verify that you still have filament leading into the nozzle. If the spool has run out, you will need to load a new spool before continuing the print (see 3.9).

b) The extruder is clogged

Sometimes your print may stop midway as a result of clogged extruder. If you find so, then you've to ask an expert to clean the extruder.

3.10.6 Weak Infill

The infill inside your 3D printed part plays a very important role in the overall strength of your model. The infill is responsible for connecting the outer shells of your 3D print, and must also support the upper surfaces that will be printed on top of the infill. If your infill appears to be weak or stringy, you may want to adjust a few settings-

a) Try alternate infill pattern

One of the first settings you should investigate is the infill pattern that is used for your print. You can find this setting by clicking "Infill" and selecting "Infill Pattern" (fig. 3.18). Some patterns tend to be more solid than others. For example, grid and triangles are all strong infill patterns.



Figure 3.16: Infill pattern settings.

b) Lower the printing speed

The infill is typically printed faster than any other portion of your 3D print. If you try to print the infill too fast, the extruder won't be able to keep up and you will start to notice under-extrusion on the inside of your part. This under-extrusion will tend to create weak, stringy infill since the nozzle is not able to extrude as much plastic as the software would like. If you have tried several infill patterns, but continue to have problems with weak infill, try reducing the print speed. To do this, click "Speed" and adjust the "Printing Speed" (fig. 3.13).

3.11 Summary

A table is listed below regarding the findings while preparing the SOP:

Table 3.1: Comparison between ABS and PLA filament.

Property	ABS	PLA
Glass transition temperature, T_g ($^{\circ}\text{C}$)	105	60
Melting temperature ($^{\circ}\text{C}$)	N/A (amorphous)	173
Printing temperature ($^{\circ}\text{C}$)	210-230	180-200
BED temperature ($^{\circ}\text{C}$)	80-110	50-70 (heated BED is not mandatory)
Tendency to warp	Tends to warp for larger parts	Almost warping-free
Health hazard during printing	High (generates toxic gas)	Minimal (generates non-toxic lactic acid)

ABS has superior mechanical properties but is harder to print with compared to PLA. PLA is ideal for 3D prints where aesthetics is important. Due to its lower printing temperature is easier to print with and therefore better suited for parts with fine details. ABS is best suited for applications where strength, ductility, machinability and thermal stability are required. ABS is more prone to warping.

Chapter 4: Hydrogel Synthesis

4.1 Materials

The following chemicals were used to synthesize poly(acrylic acid)-cysteine conjugates- L-cysteine hydrochloride from Alfa-Aesar; 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) from abcr GmbH, PAA (450 kDa), hydrochloric acid (ACS reagent, 37%), sodium hydroxide and sodium chloride salt powder from Sigma-Aldrich. All other reagents used were of analytical grade.

4.2 Synthesis of hydrogel

4.2.1 synthesis

Poly(acrylic acid)-cysteine (PAA-cysteine) conjugates were selected to synthesize as hydrogel after in-depth literature study because of its availability. PAA-cysteine conjugates were synthesized by the covalent attachment of L-cysteine to poly(acrylic acid) (PAA) according to a method described previously by Bernkop-Schnürch et al. [145]. The synthesis process was slightly modified. Briefly, one gram of PAA (450 kDa) was hydrated in deionized water and the pH value of the PAA solutions was adjusted to 4.5 by the addition of 5M NaOH. Then, EDAC of 1.7 mL in the final concentration of 0.2M was added to activate the carboxylic acid moieties of the hydrated polymers. After 20 min of incubation under stirring at room temperature, 1g of L-cysteine HCl was added to the hydrated PAA solution and the pH was adjusted to 6. Reaction mixtures were incubated for 3 hours at room temperature in a fume hood under stirring.

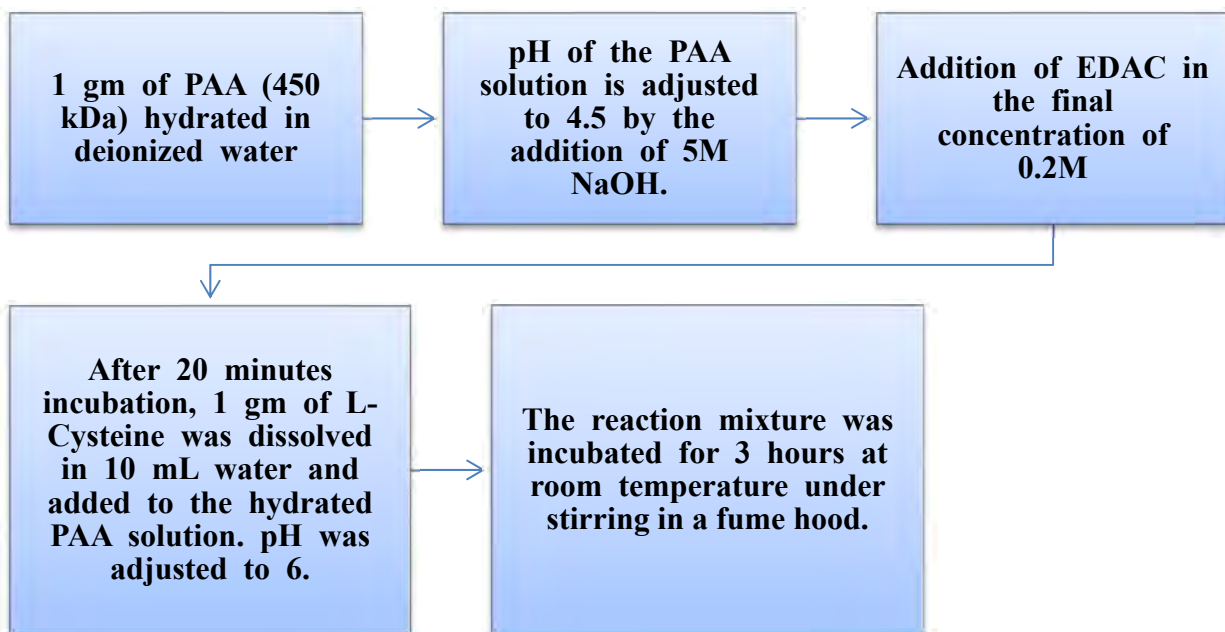


Figure 4.1: Flow diagram of PAA-Cysteine conjugate synthesis.

4.2.2 Purification

Hydrogels may contain unbound reacting species that can cause problem inside body. To eliminate the unbound reacting species from the polymers, the reaction mixture was dialyzed according to a method described previously [146]. The reaction mixture was dialyzed using Spectra/Por® 2 membrane (MWCO: 12-14 kD) for 3 days in a fume hood under stirring at room temperature as shown in fig. 4.2. In the first day, the reaction mixture was dialyzed two times against 5 mM HCl. In the second day, the reaction mixture was dialyzed two times against the same medium but containing 1% NaCl. Then the sample was dialyzed one time against 1 mM HCl. After that, the dialyzed sample was transferred into petri dishes. Then the sample was transferred in Severn Freeze-Dryer for two days as shown in fig. 4.4. Freeze-drying was performed to make sure that the structure of the sample is not affected by the external environment. After freeze-drying, the sample was stored in a closed desk until use. The conditions of the freeze-dryer are shown in table 4.1.



Figure 4.2: Dialysis of PAA-Cysteine conjugates.

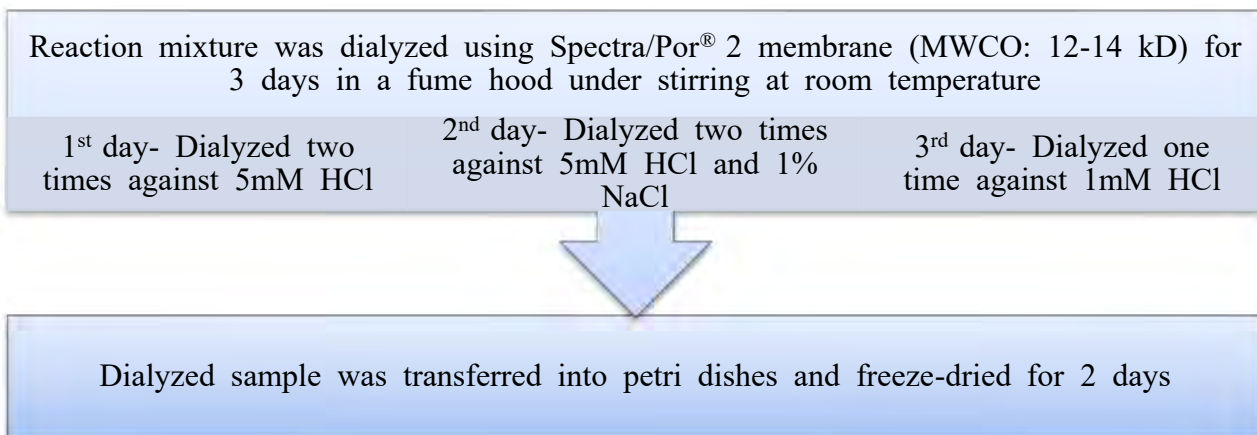


Figure 4.3: Purification of PAA-Cysteine conjugates.



Figure 4.4: The dialyzed sample was freeze-dried in Severn Freeze-Dryer.

Table 4.1: Process conditions for freeze-drying.

Steps	Time(hours)	Temperature(°C)	Pressure(mbar)
Step 1: Freezing	10	-30	1013.25
Step 2: Drying	24	-5	0.1
Step 3: Secondary drying	14	20	0.1

4.3 Swelling behavior of hydrogel

The swelling ratio was defined and determined as the ratio of the weight of water in the swollen hydrogel to the weight of the dry hydrogel. It was determined by a gravimetric method. Tablets were prepared (diameter- 6 mm, compaction load 350 kg, speed- 10 mm/min) from the samples and immersed in deionized water at room temperature for different period of times, wiped off carefully to remove excess water droplets on the surface, and weighed until no weight variation was observed. The swelling ratios were calculated from the given formula-

$$\text{Swelling ratio (SR, \%)} = \frac{W_t - W_o}{W_o} \times 100$$

Where,

W_o = Initial weight of the sample

W_t = Weight of the sample after swelling

4.4 Results and discussion

4.4.1 Hydrogel synthesis

During hydrogel (PAA-cysteine conjugates) synthesis, L-cysteine was covalently attached to PAA (450 kDa) by the formation of amide bonds between carboxylic acid groups of respective PAA and primary amino groups of L-cysteine. PAA of higher molecular mass (450 kDa) was taken as it showed better properties in previous study [146]. The carboxylic acid moieties of PAA were activated using EDAC prior to the coupling reaction.

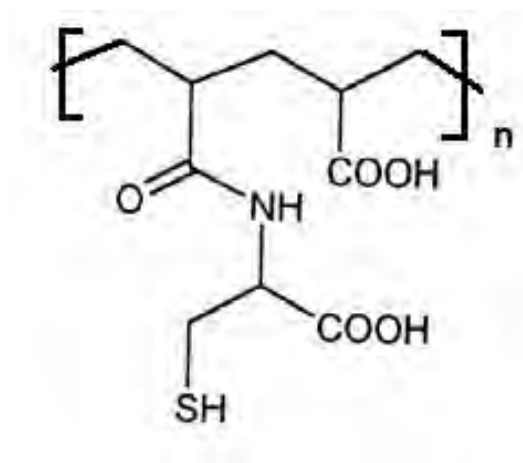


Figure 4.5: Chemical substructure of PAA-Cysteine conjugates.

The obtained hydrogels after freeze-drying appeared as white, odorless powder of fibrous structure as shown in fig. 4.6. Remember that the black particles in the fig. are nothing but the ink used for the leveling purpose of the sample.

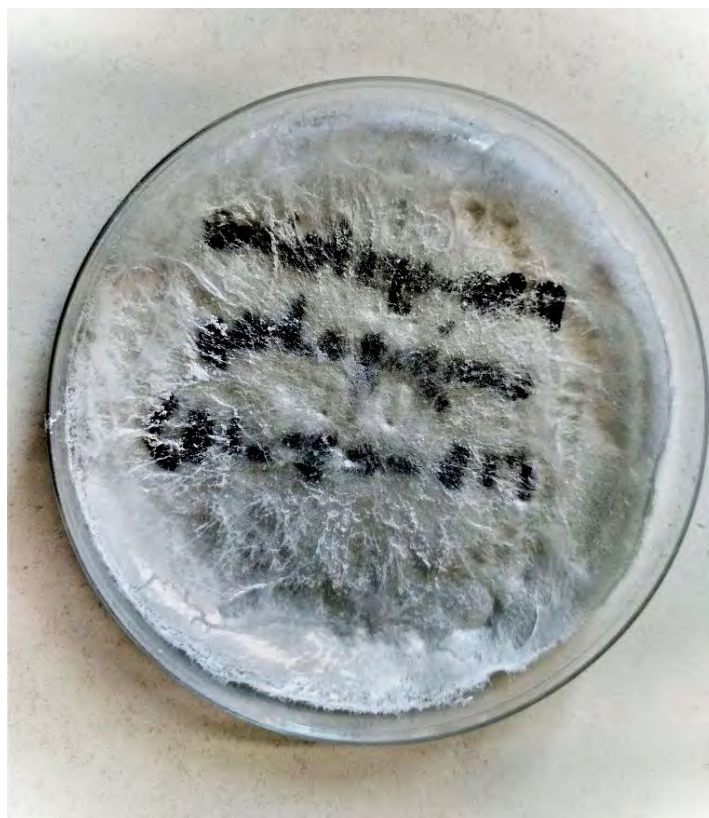


Figure 4.6: Synthesized PAA-Cysteine conjugates after purification.

4.4.2 Swelling behavior of hydrogel

Swelling study was undertaken after 5 minutes interval in this project. Several tablet samples were prepared for this study (fig. 4.7). Over swelling was avoided as it may result in the failure of the sample and give misleading data. The swelling data are listed in table 4.2. Three tablets were used to calculate the swelling ratio. The variation of the data for different tablets was the result of compaction pressure variance. The averaged swelling data are plotted against time (min.) in fig. 4.8. Percentage error bar has been used to express potential error amounts.



Figure 4.7: A tablet form of PAA-cysteine conjugates (shown above the petri dish).

Table 4.2: Swelling behavior data of PAA-Cysteine conjugates.

Time interval (min)	Swelling ratio (%)			Average swelling ratio (%)
	Tablet 1	Tablet 2	Tablet 3	
0	0	0	0	0
5	54.47	89.66	66	70.04
10	75.97	116.24	119	103.74
15	129.31	137.05	143.77	136.71
20	179.5	182.82	169.36	177.23
25	230.57	182.42	209.68	207.56
30	239.12	221.61	220.82	227.18
35	283.9	250.6	260.34	264.95
40	318.74	273.42	275.86	289.34

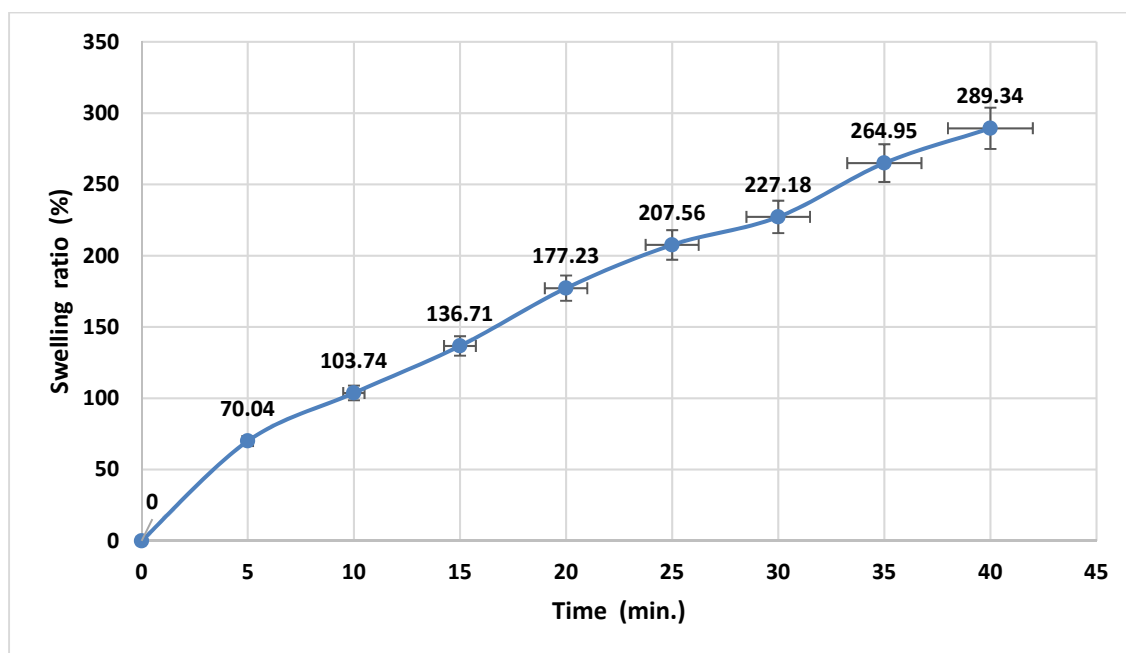


Figure 4.8: Swelling behavior of PAA-Cysteine conjugates. The values represent an average of three experiments. Percentage error bar has been used to express potential error amounts.

From the fig. and chart, it can be said that with increasing time the samples absorbed more water. The increase of water content in the sample continued up to 40 minutes. At that point the swelling ratio was found to be 289.34%. After that, the samples started to disintegrate, and the experiment was stopped.

Chapter 5: Printing hydrogels

Additive manufacturing, or 3D printing, is a promising approach for the fabrication of biological structures for regenerative medicine applications using tissue-like materials such as hydrogels. The main objective of this project is to print the hydrogels. An inject printer and a fused deposition modeling (FDM) printer were used in this regard.

5.1 Inkjet printing

5.1.1 Inkjet printer

There are different types of inkjet printers (fig. 2.5). Herein, a customized piezoelectric inkjet printer (fig. 5.1) was used for printing hydrogels. The printer uses a piezoelectric material in an ink-filled chamber behind each nozzle instead of a heating element. When a voltage is applied, the piezoelectric material changes shape, generating a pressure pulse in the fluid, which forces a droplet of ink from the nozzle. Liquid ink squirted at the paper set below the nozzle builds up the desired image.

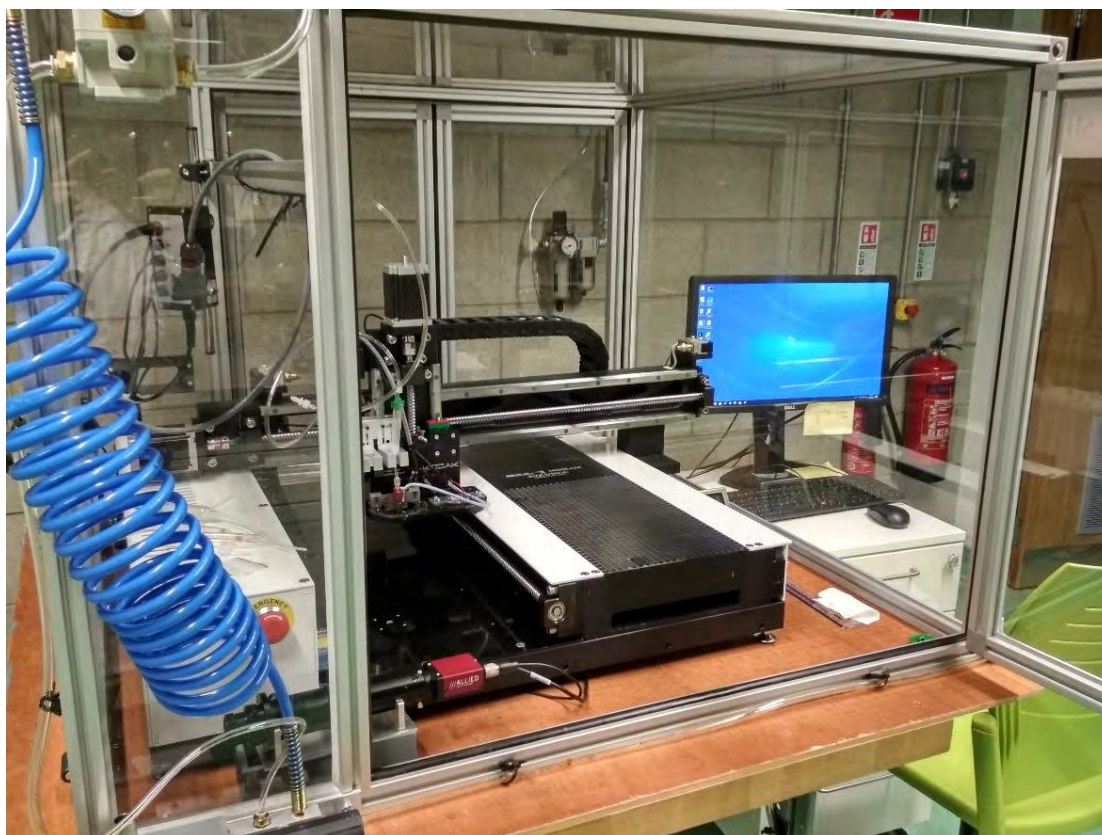


Figure 5.1: A customized piezoelectric printer was used for inkjet printing.

5.1.2 Ink formulation

Hydrogel of PAA-cysteine conjugates was used as the printing material. The dried hydrogel samples were kept in the closed desk. For inkjet printing, the dried samples in the concentration of 0.5% was

attempted to dissolve in various organic solvents like methanol, ethanol, dimethylformamide (DMF), chloroform and tetrahydrofuran (THF). The solution was stirred with a magnetic stirrer for 1 day in the fume hood. The results are shown in table 5.1.

Table 5.1: Solubility data of PAA-Cysteine conjugates.

Solvent	Solubility
Methanol	Soluble
Ethanol	Partially soluble
DMF	Partially soluble
Chloroform	Insoluble
THF	Insoluble

From naked eye observation, it was found that among the solvents methanol was the only one in which the dried hydrogel samples were soluble. So, methanol was selected as the solvent for ink formation. Prior to the use, the solution was filtered with 30mm syringe filter to ensure that there were no undissolved particles in the ink. Finally, the dried samples of hydrogel in the concentration of 0.5% in methanol was used as ink for inject printing.

5.1.3 Inkjet printing

Different shapes are printed with the customized piezoelectric inkjet printer. Different parameters are tried in this respect. The best prints were obtained from the following set-

Table 5.2: Parameters for inkjet printing of hydrogels.

Pressure	0.154 bar
Frequency	50 Hz
Line spacing	1 mm
Point spacing 1 mm	1 mm
Opening time for the nozzle	195 μ seconds
Drying time between each layer	40 seconds

In fig. 5.2 (a), a 5-layered $10 \times 10 \text{ mm}^2$ square of hydrogel structure is shown. After printing, some drops of water was transferred to the structure with the aid of a dropper. After 5 minutes, the structure swelled (fig. 5.2 (b)) even though the concentration is only 0.5% in methanol solution. After 45 minutes, the structure was found to contract (fig. 5.2 (c)) which means disintegration of the hydrogel.

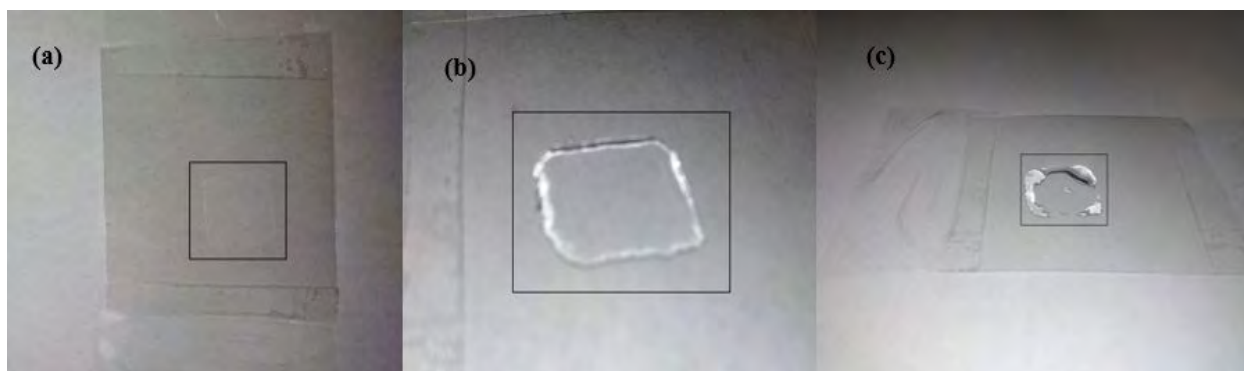


Figure 5.2: Hydrogel structure of a 10*10 mm² square- (a) as printed, (b) swollen printed structure after 5 minutes of water addition, (c) disintegrated printed structure after 45 minutes of water addition.

Because of swelling, different biomolecules like enzymes, proteins, amino acids etc. can be transported to the structure. This can open a new horizon in the *in vivo* application of hydrogels in biomedical applications such as cell growth, tissue engineering, drug delivery etc.

5.2 Fused deposition modeling (FDM) printing

The initial plan of the project involved the printing of hydrogels with both inkjet and FDM printer. SOP for the FDM printer was also prepared. But when the hydrogels were synthesized, the melting point of that particular hydrogels was necessary to be known. There was no previous study on the fact. So, finding out the melting point of the hydrogel was quite challenging. Without knowing the melting point, it wouldn't be possible to print the hydrogels with FDM printer. When attempt was made to figure out the melting point of the hydrogel, it was found that the hydrogels were burnt directly without giving a melting point after it passes 250⁰C (fig. 5.3). Several attempts were made but in vain. It may be as a result of the amorphous structure of the hydrogel or the purification process was incomplete. So, the project was then focused only in inkjet printing of the hydrogels which was giving exciting results. If the pressure could be reduced during the experiment, there was a possibility to find out the melting point. But the laboratory set up was not enough to do such experiment. So, FDM printing was left for future study.

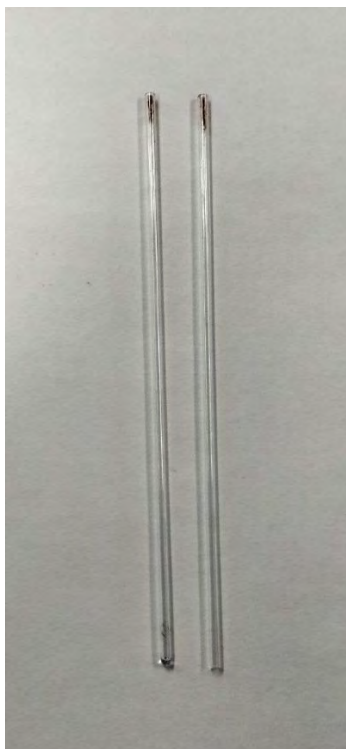


Figure 5.3: Burnt hydrogel samples during the attempt to find out the melting point.

Chapter 6: Conclusion

6.1 Summary of the findings

3D printing is a powerful tool when combined with medical imaging. It has already had a significant effect in many fields of medicine. On the other hand, the low cost, nontoxic, high-performance, swellable and biocompatible hydrogels have promising applications in biomedical fields. So, when hydrogels are printed, they can provide certain advantages. This project has tried printing hydrogels. Here are some major findings regarding the thesis work-

- PAA-cysteine conjugated hydrogel can contain a huge amount of water (~ 290% of its own weight).
- It is difficult to print hydrogels due to their low mechanical strength and porous structure.
- PAA-cysteine conjugates are inkjet printable, but FDM printing isn't possible due to the difficulty in finding the melting point of the hydrogel.
- Different biomolecules can be delivered in vivo through inkjet printable hydrogels.

6.2 Answers to the Research Questions

RQ1: Does hydrogel show required properties that can be used to inform the feasibility of biomedical applications?

Hydrogels possess a good swelling property. This is pretty useful for carrying biomolecules. It has some other properties too that can facilitate the use of hydrogels. So, hydrogels are strong contenders for biomedical applications.

RQ2: Should hydrogel be 3D printable?

Hydrogels are difficult to print. In this project inkjet printing of hydrogels was successfully done. Fused deposition modeling (FDM) printing couldn't be done due to some difficulties. In future, some modification in the synthesis process may help in this regard.

6.3 Testing research hypothesis

Based on the results presented in this thesis, the research hypothesis can be tested and evaluated as follows-

RH1: Hydrogels have swelling properties.

Hydrogels synthesized in this project have been undergone through swelling test. They were found to be swollen when immersed in water a systematic way. In fact, they showed a swelling ratio of ~290% which is quite encouraging.

RH2: Hydrogels can be printed.

Hydrogels synthesized in this project were found to be viscous. Viscosity facilitates the adhesion of the layers and maintain the shape of the printed layers. So, hydrogels were inkjet printable. And the properties found are encouraging to continue the attempt to print hydrogels with FDM printer.

6.4 Limitations and Future Directions

There was an issue in obtaining the melting point of PAA-cysteine conjugates. That is why FDM printing of hydrogels has to be sacrificed in this project. Reduced pressure could be an option for finding the melting point. But it was not possible due to laboratory set up.

In future, reduced pressure can be applied to investigate the melting point of the hydrogel sample. Different hydrogels may be investigated also in the future to obtain FDM printable hydrogels. Some mechanical tests and characterization of the hydrogels may help to prepare better printability.

To date, there has not been randomized trial to check the effectiveness of a 3D printed model of hydrogels used for medical purposes. Most of the studies discussing 3D printed applications in medicine have been in case studies and review articles. Larger studies are needed with long-term follow-up to evaluate the use of 3D printable hydrogels. Hydrogel samples carrying different biomolecules like enzymes, proteins, amino acids etc. can be transported to the guinea pig or rat to check the biocompatibility. This can open a new horizon in the *in vivo* application of hydrogels in biomedical applications such as cell growth, tissue engineering, drug delivery etc.

Chapter 7: References

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