Biofabrication of 3D cell-encapsulated tubular constructs using dynamic optical
projection stereolithography

by

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A Thesis

In

Industrial, Manufacturing, and Systems Engineering

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCES

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ACKNOWLEDGMENTS

First and foremost, I would like to express my sincere appreciation and gratitude to Dr. Changxue Xu for providing me this special opportunity to pursue a thesis under him and for sharing opulent amounts of knowledge and experience. Completion of this study would not have been possible without his orientation, focus, guidance, advising, hard work and consistent support, which I will always look up to in my professional future.

I would also like to thank my colleagues Srikumar Krishnamoorthy and Heqi Xu for their friendship, experimental guidance, useful suggestions, and valuable discussions that allowed me to understand the whole process better. They always provided me with technical support and advice to help me learn about new things.

Finally, I would like to thank the Department of Industrial, Manufacturing, and Systems Engineering at Texas Tech University. I richly benefited from an eminently dedicated faculty, extensive research facilities and an abode endowed with a profusion of academic activities.

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ABSTRACT

It has been widely recognized that one of the critical limitations in biofabrication of functional tissues/organs is lack of vascular networks which provide tissues and organs with oxygen and nutrients. Biofabrication of 3D vascular-like constructs is a reasonable first step towards successful printing of functional tissues and organs. In this thesis, a dynamic optical projection stereolithography system has been implemented to successfully fabricate 3D Y-shaped tubular constructs with living cells encapsulated. The effects of operating conditions on the cure depth of a single layer have been investigated, such as UV intensity, exposure time, and cell density. A phase diagram has been constructed to identify optimal operating conditions. Cell viability immediately after printing has been measured to be around 75%. Post-printing mechanical properties, swelling properties, and microstructures of the gelatin methacrylate hydrogels have been characterized. The resulting fabrication knowledge helps to effectively and efficiently print tissue-engineered vascular networks with complex geometries.

The cure depth is one of the most important parameters in photopolymerization, which is dependent of several factors. Having the desired cure depth for a single layer is critical towards ensuring acceptable and satisfactory resolution in the 3D fabricated constructs. This thesis has also investigated the effect of the photoinitiator concentration on the cure depth. Finally, working curves of the GelMA solutions with different concentrations have been constructed. It is concluded that 1) with the increase of the photoinitiator concentration the cure depth decreases; and 2) with the increase of the GelMA concentration, the depth of UV penetration decreases.

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CHAPTER ONE

INTRODUCTION

1.1 Motivation and Background

Organ transplantation is one of the biggest successes in the field of medical sciences which is an everyday practice nowadays. Currently, organ transplantation is a viable solution to overcome medical conditions such as organ failure because of different diseases or disorders. There has been a substantial progress in organ transplantation procedures [1]. Better surgical techniques, efficient immunosuppression, superior aesthetics and intensive care are some of the elements that are accountable for the present achievements in organ transplantation. In spite of these advancements in organ transplantation, the world faces a tremendous organ shortfall and varied measures have been taken to broaden the organ donor pool. Organ transplantation duration, inefficient donation procedures and absence of public awareness [2]. The advanced techniques and procedures have not solved the issues related to adverse effects of immunosuppression, increased risk of infections, higher possibility of contracting cardiovascular and cerebrovascular disorders, hepatitis and renal failure. It is seen that a greater number of transplant recipients have lower life expectancy with respect to the normal people [3].

The damaged organs of the human body can be restored, repaired and replaced with the use of tissue engineering and regenerative medicine approaches and therefore they appear to be the most promising alternatives. Bioprinting of artificial organs can be achieved by using 3D biofabrication techniques to efficiently engineer biological tissue networks in a layer by layer approach with the use of specialized 3D printers [4]. These

approaches offer customized organs for particular patients and can also introduce the prospects of mass production of tissue constructs.

Regenerative medicine is considered as an interdisciplinary field for the development in biological substitutes that can effectively improve, sustain and reinstate the functions of a tissue or whole organ. Engineered scaffold constructs engross the tissue voids, subsequently offering structural support and required growth elements for the cells to form tissue within the body after transplantation [5]. Currently, tissue engineering faces certain challenges in printing thick tissue constructs since only some organs such as tracheas [6] and skin have been fabricated successfully for clinical use. A tremendous amount of research is being carried out to achieve successful organ printing.

Figure 1. 1 Organ transplantation waiting list [7]

The past 20 years have seen a dramatic surge in the demand for transplantable organs mostly due to higher lifespans and better healthcare for the organ recipients [8, 9]. For example, it was reported by the Organ Procurement and Transplantation Network (OPTN) in 2016 that, the total number of people on the waiting list of nation transplant in the United States of America were 114500, however only 34780 transplants were carried out in that year[10]. The US Department of Health and Human Services (HHS) reported that more that, 20 people die every day due to the shortage of the organs[7]. Public awareness campaigns, monetary compensations, fast transportation of organs are some of the measures that are proposed to overcome the limitations associated with the organ shortfall [11].

The public awareness campaigns have helped to increase the total number of donors. For instance, in the year 2003, organizations such as the US Department of Health and Human Services (HHS) and Health resources and Services administration (HRSA) have provided support to programs related to the donation of organs and there has been a substantial increase of around 8% in the number of organ donors [12]. Another example is the initiative taken by the American society of Transplant to highlight the available organs [13]. There has been a profound effect on people signing up as organ donors because of the awareness campaign undertaken by the Organ donation and African American Church [11, 14]. However, this increase in the number of available donors is not enough to overcome the shortage requirements [11].

In the recent times, there are varied limitations to use the donor's organs for transplantation - Standard Criteria Donors (SCDs) are the suitable donors which incorporates all the donors who are below 50 years of age, and who do not possess any

medical problems for example history of hypertension, high blood pressure, hepatitis etc [15]. In the past couple of decades, organs acquired from other donors which are also known as Extended Criteria donors (ECD's) have seen an increase in the popularity. These types of donors include people who are above 50 years of age, diabetic donors, pediatric donors and donors who have other medical conditions [16]. However, this has seen a number of limitations - A poor survival rate is observed amongst the pediatric donors. Organs acquired from the elderly donors have been quite a times biologically and functionally ineffective leading to reduced survival rates [17]. Although these changes are not observed in the elderly donors, their cumulative long-term survival is unpredictable [18]. The survival rate for the donors without medical conditions has been much higher compared to the survival rate for the donors having medical conditions [19].

A lot of organizations have supported the system of monetary compensation for organ transplantation, however this system is vulnerable to the ethical concerns and exploitation of the donors thereby creating voids in the structural framework [20]. There are other critical problems such as varied legal definitions for death and different ages of consent in various countries, which is further susceptible to ethical issues with organ harvesting, particularly in the worldwide market [21]. Three dimensional biofabrication has been believed to be a promising field to help resolve the limitations of organ transplantation [22]. 3D fabrication refers to the use of rapid prototyping technology for building of functional tissue structures and organ constructs [23].

1.2 Current State of Research

1.2.1 3D Bioprinting

3D bioprinting is the engineering of 3D objects using the technology of additive manufacturing [24]. 3D bioprinting refers to the layer-by-layer fabrication of an appropriate tissue engineering material and requires the use of computer-aided design methodologies to virtually design a 3D model and fabricate them. There are two predominant materials which are involved in 3D bioprinting. The biomaterial which is also called as the non-living scaffold which supports and provides indication to the living material and the bioink which involves the living cells of the structure [25]. The use of the bioink and the biomaterial is mainly dependent on the function, however generalities exist. One of the examples of the biomaterial is the hydrogel, whose purpose is to interact with the living system. Bioink usually contains one or more type of cells depending upon the need [26] . It involves decellularization of the extracellular matrix scaffolds and further introduction of the cells and bioinks. Researchers have had success in better engineering of the micro environments by studying the arrangement of cells in these microenvironments [27]. This application is a very promising one considering the shortage of the organs available for the organ transplant. The second application is self-assembly which involves organoids with 3D biofabrication. Before printing, biomaterial and bioink are mixed together as a spheroid thereby allowing the cells to grow in the generated environment [28, 29]. This technology has promising applications in the field of personalized medicine. In case of the mini tissues, 3D biofabrication is combined with another technology of microfluids. It is based on the principal that the new tissues/ organs are formed from the fundamental building blocks [30].

Figure 1. 2 Procedure for 3D bioprinting of viable organs [82]

3D bioprinting is one of the applications of tissue engineering which involves the combination of engineering techniques with the biological system. Generally, cells were adhered to scaffolds which worked as a substrate for the cells so that the cells could attach and expand to form tissues [31]. Although, there were limitations associated with this procedure such as incapacity to form complex 3D structures, ineffectiveness to maintain the attachment and proliferation of the cells and challenges faced in engineering 3D vascular network which is very important in 3d bioprinting [28]. 3D bioprinting applications are focused on 3D printing of artificial organs by acquiring patient specific data, samples of cells from the patient, utilizing the appropriate biomaterial to perform 3D biofabrication [32].

Figure 1. 3 Graphical representation of tissue engineering [31]

3D biofabrication is a fairly recent innovation, that has been gaining steady research interest, as indicated by the increase in number of publications, which is illustrated in Figure 1.4. This new technology has acquired a lot of attention from the researchers, scientist and general public. The main purpose of 3D bioprinting is to study and mimic every possible organ of the human body. Although there are a lot of limitations in producing artificial organs 3D printing for the eventual use of humans seems to be a very promising alternative. Till now no functional artificial organs have been produced with the technology of 3D bioprinting [33], however an extensive amount of research is being carried out and we can predict groundbreaking advancement in this.

Figure 1. 4 Use of 3D bioprinting in research [83]

There is wide variety of the bioprinting technologies used, for example stereolithography (SLA), laser printing, inject printing and fused deposition modelling. Each of the process has its own pros and cons with respect to ease of use, fabrication resolution, biomaterial compatibility and so on. Generally, 3D bioprinting with or without living cells can be accomplished in 2 approaches: Scanning method and projection method. Scanning method is to utilize an orifice or a laser beam to scan the building surface to precisely dispense biological materials and living cells, in order to generate a desired pattern of a single layer [34, 35]. Through a layer-by-layer building process, a structure with 3D geometry will finally be obtained. Most commonly used scanning method bioprinting are droplet-based bioprinting and filament-based bioprinting. Droplet-based bioprinting is represented by inkjet printing and laser assisted bioprinting, which generate droplets as building blocks to fabricate 3D structures [4, 24, 36] filament-based bioprinting

is represented by extrusion deposition, which generate continuous filament to build the desired structure [35]. Various studies have been conducted to investigate the scanning printing process and applications in tissue engineering [37, 38]. However, these manufacturing techniques have limitations related to resolution, printing speed and 3D fabrication capability. 3D Structures with complex geometry can be time-consuming to engineer with these precision manufacturing methods [34].

An alternative to scanning method, the projection method utilize sa mask to pattern the light, and the light is guided to the resin surface to polymerize the material, which allows for a whole layer to be cured simultaneously [39]. Compared to scanning method, the projection method can project an entire layer at a single time instead of scanning the surface, which means it can dramatically reduce the printing time compared to the scanning method.

Figure 1. 5 Classification of 3D printing technologies

1.2.2 Hydrogels: Gelatin Methacrylate (GelMA)

Biomaterials such as hydrogels have been of critical importance in the recent years in tissue engineering 3D biofabrication because of their similarity with the indigenous connective tissues [40]. However, there are a number of limitations in generating complex tissues relying on explicit cell and ECM organization. Developing complex vascular networks conducive for regeneration of organs like liver and kidney still remains a challenge to overcome. Recent studies in hydrogel chemistry and cell biology have led to development of scaffolding materials targeting to mimic spatial structure of cells and ECM molecules found *in vivo*. Also, encapsulation of cells and biological molecules inside hydrogel along with cell and molecule arrangement control at microscale has been successfully carried out by using photopolymerizable materials. Presently, tissue engineering has a limited scope since only a few organs such as skin [6, 41] and tracheas [42, 43] have been engineered successfully for clinic applications. Generation of thick tissue constructs still remains a challenging endeavor. One of the critical limitations in the fabrication of fully functional and effective tissue constructs is the engineering of vascular structures, which are an integral part of the biological systems, such as the blood vessels supplying tissues with oxygen and nutrients further removing metabolic wastes [44, 45]. Unlike living tissues *in vivo*, cell viability and functions cannot be sustained at a distance of more than a few hundred micrometers from the blood supply, which finally results in cell death, limiting the engineered tissue to grow into more complex structures [46, 47]. Therefore, biofabrication of vascular networks indicating the feasibility of envisioned organ printing technology is an important first step towards successful organ printing.

Gelatin Methacrylate being a photocrosslinkable derivative of gelatin was reported for the first time by Van Den Bulcke *et al.* [48]. The synthesis of GelMA is achieved by reacting the primary amine groups of gelatin with methacrylic anhydride at an elevated temperature, which eventually results in the addition of methacrylate groups onto the macromers of gelatin. This further results in addition methacrylate groups onto the macromers of gelatin. The Degree of Functionalization (DoF) which is also known as the degree of methacryloyl substitution can be changed by adjusting the amount of methacrylic anhydride added to the initial reaction [49]. The following process can be also used to change the physical properties of GelMA [50]. GelMA is a synthetically crosslinkable hydrogel, that can be crosslinked by introduction to various explicit wavelengths of light, for example, UV radiation within the presence of a suitable photoinitiator [51]. The photoinitiator facilitates the release of free radicals upon the assimilation of light, which at that point in this way results in the polymerization of GelMA[48, 51]. GelMA holds a large portion of the amino acids of gelatin [52], and shows tailorability of its physiochemical characteristics by varying of synthesis parameters, for example, changing the DoF, photoinitiator concentration, and GelMA concentration [49, 51-53]. Zhao *et al.* [52] used GelMA in epidermal tissue building, and revealed that with the increase of the GelMA concentration, the mechanical strength improves, the swelling and degradation are reduced. Schuurman *et al.* [54] used diverse concentrations of GelMA for the engineering of tissue fabricated cartilage develops and detailed that with the increase of the GelMA concentration, the mechanical quality increases, and the swelling diminishes. Chen *et al.* [49] utilized GelMA and it has been found out that with increase of the GelMA degree of functionalization, there is a decrease in the pore size of the hydrogel and, on the other hand

the compressive modulus increases. Colosi *et al.* [55] examined the impacts of the UV light intensity and exposure time on the mechanical properties of GelMA, and concluded that the mechanical strength for GelMA increments with the increase of the UV light intensity and the exposure time.

By the virtue of the native bioactivity of the gelatin which leads to reservation of amino acid motifs such as RGD motifs and MMP-degradable motifs in GelMA post substitution, GelMa is considered to be highly biocompatible [56, 57] and therefore it is used in different 3D biofabrication applications. Gauvin *et al.* [58] used a stereolithography system for 3D bioprinting of GelMA hydrogels with tunable characteristics and microstructures. Soman *et al.* [59] could accomplish quick biofabrication of 3D microstructures that were very similar to native tissues and took into consideration viable cell survival. A digital micromirror device (DMD) was utilized by Grogan *et al.* [60] to manufacture meniscus-like tissue utilizing GelMA. Yin *et al.* [61] proposed the utilization of a two-step gelation procedure to successfully print GelMA of low-concentration utilizing a microextrusion setup. It was seen that GelMA hydrogels have a capacity to contain the local microcellular environment. Chen *et al.* [49] used GelMA for the creation of functional vascular systems. It was accounted for that GelMA had properties that could solve the deficiencies of other pervasive hydrogels, for example, insufficient cell viability and binding, poor mechanical properties, and the inefficient control of microarchitecture of the hydrogel [49]. Gao *et al.* [62] proposed the use of photocrosslinkable GelMA to improve the properties of bone and cartilage tissue using ink-jet-bioprinting and it was observed that GelMA scaffolds demonstrated improved mechanical properties and osteogenic and chondrogenic differentiation indicating its favorable use in bone and

cartilage tissue engineering. Wang *et al.* [63] in his study, utilized GelMA-DexMA copolymer hydrogels with tunable mechanical properties for tissue engineering and it can be concluded that GelMA can be used with other biopolymers to enhance tuning characteristics such as porosity swelling ratio and degradability along with the mechanical properties. Nichol *et al.* [51] exhibited the use of cell-laden GelMA hydrogels for the arrangement of micropatterns. It was accounted that GelMA is certainly appropriate for applications that need rapid cell adhesion, migration and proliferation. Bertassoni *et al.* [64] showed the usage of cell-laden GelMA at various concentrations and cell densities by utilizing a microextrusion framework. Great printability and high cell survival in the printed constructs were reported. Colosi *et al.* [62] showed the use of an inkjet printing system for the engineering of a PEG-GelMA scaffold for bone tissue building. Superior mechanical properties by the use of the hydrogel was reported by them.

1.2.3 DMD based Projection Stereolithography

As one of the projection methods, DMD based dynamic optical projection stereolithography utilize a digital micromirror device (DMD) as a dynamic mask to create a layer of any desired pattern on the resin surface, and structures are built in a layer by layer manner by photocrosslinking. The DMD based dynamic optical projection stereolithography is favored of its micrometer printing resolution [65], superior printing speed, a high degree of pattern flexibility and scalability [66].

The importance of DMD based dynamic optical projection stereolithography has been widely recognized in the different biofabrication applications [67]. The DMD can generate a layer of desired pattern at a single time, which means this technology is

especially suitable for fabrication of 3D structures with complex geometry, like cell scaffolds [58, 60, 68] and vascular networks [67]. Different cell scaffolds with different geometry features have been designed and fabricated, and cell behavior have been evaluated in these cell scaffolds [59, 66]. In bone tissue engineering, bone scaffolds with different porosity and pore sizes has been evaluated to assure good vascularization, nutrient delivery and cell attachment/ingrowth [69-71]. A large-volume tissue mimics which combined perfusable vascular network with cell scaffolds has been fabricated and evaluated [47, 72]. DMD based microlithography has also been used for further bioprinting applications, for example, Choi reported microneedles which can be used as an alternative drug delivery device for those with a low-pain threshold, or for time release application [65].

Figure 1. 6 (a) Graphical Representation of a Vascular Network; (b) Single drops of cell laden hydrogel are dispensed layer- by-layer to form a three-dimensional tissue construct [53].

The appearance and structural organization of the micro-vascular structures are different in different organs and tissues. Hence, a multiscale vascular network which can

mimic both artery and vein structures and capillaries need to be designed and fabricated. Schuller-Ravoo et al has successfully designed and fabricated a vascular network with different scale using stereolithography, and the viability of the cells that seeded on the same material of printed vascular network has been tested [54]. However, this multiscale vascular network doesn't have any cells inside the structure. Unfortunately, 3D vascular network with cells encapsulated inside the structure, has yet to be reported, which is a subject of research in this study.

1.2.4 Cure Depth and Working Curve

The cure depth is one of the most important parameters in photopolymerization, which is dependent of several factors. Having the desired cure depth for a single layer is predominantly, a critical factor towards ensuring acceptable and satisfactory resolution in the 3D fabricated constructs [73]. The cure depth determines the thickness of the layers that can be built thereby predicting the number of layers required to print the structure. The thickness of the printed line is significantly influenced by two major operating conditions namely the UV light intensity and the exposure time [59]. Along with these factors the concentration of the resin and photoinitiator are also detrimental towards the thickness of the line. The amount of UV of energy E will cause the polymerization to a depth C_d as suggested by the Jacob's equation as $C_d = D_p \ln(E/E_c)$, where E_c is the critical energy and D_p is the sensitivity of the photopolymerizable suspension [74]. A working curve is presented in terms of the cure depth and and critical exposure. Previous research has been done to study the effect of photopolymer concentration, photoinitiator (PI) type and concentration on the hydrogel thickness. Cure depth analysis for different hydrogel concentrations as a function of the type and concentration of the photoinitiator has been

presented. Arcaute *et al.* [75] used polyethylene glycol (PEG) to 3D fabricate complex structures having internal channels of various orientations using stereolithography. It was noted that cure depth of the hydrogel was a strong function of the PEG concentration and photoinitiator concentration and therefore photocrosslinkable biomaterials such as PEG hydrogels are feasible for fabricating complex bioactive scaffolds. This paper aims to examine the variation in the E_c and D_p with change in the polymer and photoinitiator concentration. This study covers the working of the dynamic projection stereolithography and its experimental setup followed by the mechanism of photocrosslinking. Relationship between the cure depth for different concentrations of GelMA is established. The paper also aims at studying the relationship between the cure depth and photoinitiator concentration in the resin. Consequently, the conclusions are drawn, and the future scope is proposed.

1.2.5 Properties

Suitable biocompatible materials are needed in the process of 3D bioprinting to mimic the functions of a cell's natural extracellular matrix (ECM), for it to serve as a perfect 3D cell culture medium [40]. Biomaterials such as hydrogels have been of critical importance in the recent years in tissue engineering 3D biofabrication, because of their similarity with the indigenous connective tissues. The unique hydrophilic properties which are inherited by the hydrogels make them the most appropriate material for cell adhesion and proliferation [40, 76]. Hydrogels are crosslinked polymers which depict the property of swelling when introduced to water and retain large quantity of water inside the 3D networks without dissolving in the water [77]. Characteristics of hydrogels such as high-water retention compatibility, biocompatibility, and biodegradability make them truly

promising for biomedical applications [78]. These characteristics are directly related to the characteristics observed in natural ECM [79]. Additionally, soft nature of the hydrogel results in minimization of irritation to the surrounding tissues, and generally, cell adhesion of hydrogels can be improved by adjustments with ligands [79]. When the hydrophilic network (hydrogel precursor solution) is crosslinked in a specific way, synthetization of the hydrogels takes place [80]. The crosslinking mechanisms can be categorized into two predominant categories: chemical crosslinking and physical crosslinking [81]. The manufacturing process, mechanical characteristics of the fabricated structure can be affected by the type of the crosslinking mechanism.

1.3 Scope of Thesis

Following are the objectives of our research:

1. To find out the effect of operating conditions on the cure depth by printing single line and considering two operating conditions namely UV light and exposure time. Determine the cure depth and analyze the change in the cure depth with respect to different exposure times and UV light intensities. Generate a phase diagram to identify the optimal conditions for the filament formation.
2. To find out the effect of cell concentration on the cure depth by keeping both, UV light intensity and the exposure time constant. Introduce living cells in the solution and keep different levels of cell concentration while printing a single line. Make observations about the quality of the line and its thickness for solutions having different levels of cell concentration. List the findings of the cell viability test. Obtain the cell viability percentage for the 3D printed structure at different days

after printing. Make comparisons of the cell viability percentage in accordance with the respective days. Obtain cell viability percentages for the 3D printed structures having different operating conditions (UV light intensity). Compare the results and determine the optimum operating condition for the survival of cells.

3. To 3D print vascular construct and find out its mechanical properties. Determine the physical characteristics of the hydrogel such as swelling properties. Obtain the stress-strain graph by performing tensile test on dog bone shaped samples to find out the tensile and Young's moduli. Study the microstructure by finding out the pore size for the 3D printed structure with cells encapsulated in it using the SEM and evaluate the effect of cellular incubation on the hydrogel properties.
4. To study working of the dynamic projection stereolithography and its experimental setup followed by the mechanism of photocrosslinking. Establish a relationship between the cure depth for different concentrations of GelMA. Determine the relationship between the cure depth and photoinitiator concentration in the resin. Examine the variation in the E_c and D_p with change in the polymer and photoinitiator concentration.

1.4 Organization of Thesis

This thesis is organized in a manner as to impart a sequential understanding of the various aspects of biofabrication, with a specific focus on hydrogel and hydrogel properties.

- In Chapter 1, first, the motivation behind this work is laid out. Second, the current state of research is reviewed, namely, hydrogels, GelMA, and cure depth. Third, the objectives for this work are provided.
- In Chapter 2, the fabrication of 3D structures with cells encapsulated based on 5% GelMA using DMD-based dynamic optical projection stereolithography is demonstrated. The cure depth of the filament is investigated in terms of the operating conditions namely UV light intensity, exposure time and cell concentration. Based upon these results a phase diagram is constructed to identify the optimal conditions to print a uniform line. The DMD based stereolithography is used to fabricate a hollow Y-shaped cellular construct and the cell viability was found out after different time intervals. The post printing property characterization is done which included, mechanical properties, swelling properties and the pore size. 5% GelMA hydrogels with and without cells are compared. The swelling properties and microstructure of the hydrogel are studied and finally conclusions are listed based on the results.
- In Chapter 3, first, the experimental materials and methods are introduced in detail. Second, the GelMA degree of substitution is measured and discussed. Third, the variation in the E_c and D_p with change in the polymer and photoinitiator concentration is examined. This study covers the working of the dynamic projection stereolithography and its experimental setup followed by the mechanism of photocrosslinking. Relationship between the cure depth for

different concentrations of GelMA is established. The relationship between the cure depth and photoinitiator concentration in the resin is also studied. Consequently, the conclusions are drawn, and the future scope is proposed.

- In Chapter 4, overall important conclusions of the entire study based upon the detailed analysis have been listed and based upon the scope of the project the future work has been proposed.

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CHAPTER TWO

BIOFABRICATION OF 3D CELL-ENCAPSULATED TUBULAR CONSTRUCTS USING DYNAMIC OPTICAL PROJECTION STEREOLITHOGRAPHY

2.1 Introduction

Tissue engineering and regenerative medicine approaches have been emerging as promising technologies for restoring, repairing or replacing damaged or lost tissues/organs of human body. Of various tissue engineering advances, bioprinting precisely deposit extracellular matrix (ECM) and living cells onto a substrate in a computer-controlled pattern to construct artificial tissues and organs based on a layer-by-layer manufacturing mechanism. [1, 2]. Compared with other tissue engineering technologies, 3D bioprinting is very versatile in the choices of biological materials, cell types, and growth and differentiation factors, despite technical challenges related to the sensitivities of living cells and the tissue/organ construction [3, 4].

Vascular networks supply tissues and organs with oxygen and nutrients and remove metabolic byproducts in the body [5, 6]. Without perfusable vascular networks, 3D engineered tissues/organs with populated cells encapsulated quickly develop a necrotic core [7]. Hence, vascularization and blood vessels have been widely recognized as a main technological barrier for building thick tissues and organs. Biofabrication of 3D tubular constructs with living cells encapsulated is not only a reasonable first step towards successful organ printing but also a critical indicator of the feasibility of envisioned organ printing technology.

Additive manufacturing fabricates 3D structures layer by layer. A variety of processes based on lasers, printing heads and extruder heads have been utilized to create each layer. During the layer-forming processes, three modes of operation are used: a moving spot, a moving line, and a layer mode [8]. In a moving spot mode, a moving point or spot is directed through a path to scan a 2D pattern generated from a CAD model of a 3D structure. The representative processes include selective laser sintering [9] and microextrusion [10, 11]. In a moving line mode, a linear array of spots sweeps across the entire layer in one translational motion, such as inkjet printhead with multiple channels [12]. In a layer mode, a mask is utilized to create the entire layer at the same time. A representative process is projection stereolithography [13] [14] [15], where the entire layer of liquid photopolymer resin is exposed to an ultraviolet light source through a mask. The main advantage of the layer mode is significant reduction of printing time, which is critical for bioprinting applications. Digital micromirror devices (DMD) and liquid crystal displays are two main light projection devices, which possess dynamic mask projection capabilities [16]. DMD is favored due to its higher contrast and transmittance [17], higher resolution, and faster fabrication speed [18].

DMD developed by Texas Instruments is an array of micromirrors actuated by electrostatic forces. These micromirrors can be individually rotated $\pm 10^\circ$, which controls pixels in the image to appear bright or dark. DMD is utilized as a dynamic mask to create a layer of any desired 2D pattern on a substrate, and 3D constructs are fabricated layer by layer. DMD-based projection stereolithography has been widely employed to fabricate 3D constructs for a variety of applications. Ha *et al.* [17] proposed a DMD-based projection microlithography to achieve mass production of 3D microstructures and the resolution was

a few microns. Zhou *et al.* [19] presented a novel DMD-based stereolithography process to fabricate 3D construct with desired material properties by adjusting two base material concentrations. Lu *et al.* [20] developed a magnetic field-assisted projection stereolithography to selectively deposit the magnetic particles in a liquid resin with various patterns. DMD-based stereolithography has also found wide biomedical applications which involve highly intricate and complex geometry, such as scaffolds [21-23] and vascular networks [24]. Zhang *et al.* [25] utilized the DMD-based projection stereolithography to fabricate complex biomimetic scaffolds using poly(ethylene glycol) diacrylate and gelatin methacrylate. Such scaffolds with complex topographic features were used to study cell interactions with microenvironments in vitro. Choi [26] fabricated microneedles as a drug delivery device with important advantages including high mechanical strength, low insertion force, and proper drug dosage. Schüller-Ravoo *et al.* [24] used the DMD-based stereolithography to fabricate a 3D microvascular network with a high density of small open channels. Human umbilical vein endothelial cells were seeded on the 2D film to test cell attachment, and water was perfused through the fabricated microvascular structure at physiological pressures. While the DMD-based projection stereolithography has been reported for fabrication of a variety of 3D structures for wide applications, biofabrication of 3D tubular constructs with living cells encapsulated is still missing.

The objective of this study is to fabricate 3D tubular constructs with living cells encapsulated. The rest of the paper is organized as follows. First, experimental materials and setup are introduced in detail. Second, the effects of UV intensity, exposure time, and cell density on cure depth are investigated, respectively. A phase diagram is constructed to identify optimal operating conditions. Third, 3D vascular-like structures with cells

encapsulated are fabricated, and post-printing mechanical and physical properties and microstructures are characterized. Finally, main conclusions are drawn, and future works are proposed.

2.2 Materials and Methods

2.2.1 Materials

2.2.1.1 Gelatin Methacrylate (GelMA)

Extracellular matrix (ECM) is a structural support scaffold facilitating cell migration, proliferation and differentiation. It is composed of a variety of matrix proteins, such as collagens, elastin, and fibronectin, to name a few. Collagens are the most important structural protein within ECM. GelMA is a derivative of gelatin which is a denatured collagen. GelMA retains much of the biocompatible properties of gelatin. In addition, GelMA is inexpensive and easy to synthesize. Physical and mechanical properties are tunable for different applications by modifying methacrylation degree and gel concentration [27-29]. GelMA as a versatile has been widely used for various biomedical applications [28-32]. Therefore, GelMA has been selected in this study to mimic the ECM.

GelMA was synthesized in the lab using the following protocol. Type A gelatin (MP Biomedicals, Santa Ana, CA) was dissolved into Dulbecco's Phosphate Buffered Saline (DPBS) at 50 °C to obtain 10% (w/v) gelatin solution. Methacrylic anhydride was added into the gelatin solution at a flow rate of 0.5 ml/min. The mixture was subjected to vigorous stirring at 50 °C for 2 hours when the methacrylic anhydride reacts with the gelatin resulting in the addition of methacrylamide groups onto the gelatin macromers [27].

Then, warm DPBS was added to stop the reaction. Dialysis was performed at 40°C against deionized water using 12-14 kDa cutoff dialysis tubing for seven days to extract the dissolved salts and methacrylic acid. Finally, GelMA white porous foam was produced after lyophilizing the solution for seven days. GelMA foam was stored in the freezer at -80°C [53].

The GelMA solution used this study was prepared by dissolving the lyophilized GelMA foam into DPBS at a concentration of 5% (w/v). The photoinitiator Irgacure 2959 (TCI America, Portland, OR) was added into the GelMA solution at a concentration of 0.5% (w/v). The photoinitiator concentration 0.5% (w/v) has been widely used in various biomedical applications in the literature, and the corresponding cell viability has been reported as good using this photoinitiator concentration[33, 34].

2.2.1.2 Cell Culture and Cell Viability Test

Fibroblasts are one of the main cell types in blood vessels and have been extensively employed as model cells in the literature [35-38]. NIH 3T3 mouse fibroblasts (ATCC, Rockville, MD) were grown at 37 °C in a humidified atmosphere containing 5% CO₂. The cell growth medium was Dulbecco's Modified Eagles Medium (DMEM; Sigma-Aldrich, St. Louis, MO) with 10% Calf Bovine Serum (CBS; HyClone, Logan, UT) and 1% antibiotic-antimycotic solution (Thermo Fisher Scientific, Waltham, MA). This cell growth medium was replaced every three days. 0.25% Trypsin/EDTA (Sigma-Aldrich, St. Louis, MO) was added to detach the cells from the culture flask. The formed cell suspension was subject to centrifuging at 1000 rpm for 5 minutes to harvest the cell pallet.

Finally, the cell pallet was mixed with the GelMA solutions to make the final bioink with a cell concentration range from 0 - 10×10^6 cells/ml [39-41].

The post-printing cell viability was evaluated using a fluorescence-based Live/Dead assay (Biotium, Fremont, CA). Live cells were stained with calcein AM (green), and the dead cells were stained with ethidium homodimer-1 (red). The fabricated cellular constructs were incubated together with the assay at 37 °C for 20 minutes. A fluorescent microscope (EVOS FL, Thermo Fisher Scientific, Waltham, MA) was utilized to capture the images of the live and dead cells, and the post-printing cell viability was calculated based on the live cell percentage [53].

2.2.2 Methods

2.2.2.1 Experimental Setup

In Figure 2.1, the dynamic optical projection stereolithography system was set up to fabricate cell-encapsulated tubular constructs in this study. This stereolithography system was mainly composed of five important components: a UV source (Omnicure S2000, Excelitas Technologies, Ontario, Canada), a collimator (Lumen Dynamics, Excelitas Technologies, Ontario, Canada), a digital micromirror device (DMD, DLP Discovery 4100, Texas Instruments Incorporated, Dallas, TX), a motorized Z stage (Thorlabs, Newton, NJ) attached with a platform, and a container filled with photocrosslinkable bioink. The UV source (Omnicure S2000, Excelitas Technologies, Ontario, Canada) had a wavelength of 365nm. The UV source provided the UV light with different intensities to crosslink the cell-laden GelMA bioink. The collimator aligned the UV light and made it parallel or collimated. The motorized Z stage was controlled to move

the platform down vertically to define the layer thickness after one layer was finished. The container was the reservoir of cell-laden bioink. The DMD was the key component of the stereolithography system used as a dynamic mask, which could easily create any desired 2D pattern [53]. As the DMD can generate one entire layer of a desired pattern at a single time, the dynamic optical projection stereolithography is suitable for fabrication of 3D constructs with complex geometries with a high printing speed, good pattern flexibility and scalability [25, 42, 43].

Figure 2. 1 Schematic representation of the experimental setup

The procedure to fabricate 3D constructs is as follows. 3D CAD model was built using SolidWorks, and saved as an STL file. Slic3r (free slicing software) was used to slice the STL file into many layers and each layer was a binary image representing the 2D

pattern/mask of this layer. The binary images of all layers were loaded into the DMD in sequence, and the DMD started to display the 2D patterns/masks one by one. When the parallel UV beam after the collimator illuminated the DMD, the 2D pattern/mask was reflected and guided to the bioink surface through two focusing lenses (Thorlabs, Newton, NJ, USA) and a 90° prism (Thorlabs, Newton, NJ, USA). The photoinitiator in the exposed areas was decomposed to generate free radicals, which polymerized the GelMA to form one layer on the platform. Then, the Z stage controlled the platform to move down vertically (200 μm), and a new layer was deposited on the top of the existing structure. The resolution of the motorized Z stage is 0.1 μm . 3D cellular constructs were fabricated based on a layer-by-layer manner [53].

The tensile test has been performed in this study to characterize the post-printing mechanical properties. Two different bioinks were utilized to prepare dog-bone specimens. One bioink only contained the 5% (w/v) GelMA, and the other bioink contained the 5% (w/v) GelMA and living cells (cell density 2.5×10^6 cells/ml). The UV light intensity and the exposure time used were 10 mW/cm^2 and 35 seconds, respectively. The specimens were subject to uniaxial stretching at a constant rate of 1 mm/min until failure on a Dynamic Mechanical Analyzer (DMA Q800, TA Instruments, New Castle, DE) [53].

In this study, swelling properties of the bioink with 5% (w/v) GelMA have been characterized. The specimens were prepared by exposing the bioink with 5% (w/v) GelMA to the UV light at a UV intensity of 12 mW/cm^2 for 35 seconds. The specimens were cultured in DPBS for 0, 24, 48, and 72 hours, and weighted to obtain the swollen weight as W_s . Then, the specimens were lyophilized at -50°C for 12 h, and the corresponding dry

weight of the specimens were measured as W_d . The swelling ratio can be estimated as $(W_s - W_d)/W_d$ [53].

In this study, the pore size of the 5% (w/v) GelMA hydrogels has been measured. The specimens were prepared by exposing the bioink with 5% (w/v) GelMA to the UV light at a UV intensity of 12 mW/cm^2 for 35 seconds. The specimens were cultured in DPBS for 0, 24, 48, and 72 hours [53]. Then, the specimens were lyophilized at -50°C for 12 hours and viewed under a scanning electron microscopy (SEM; ProX, Phenom, Phoenix, AZ).

2.2.2.2 *Experimental Conditions*

Different operating conditions were adjusted in this study to investigate the cure depth of the bioink. In Figure 2.2, a 3D structure with four columns and four crossbeams was designed to study cure depth of a single layer. Two binary images were generated for the columns and the crossbeams, respectively. The columns had thirteen layers, and the crossbeams only had one layer. The adjustable operating conditions included the UV intensity, exposure time, and cell density. The UV intensity was in the range of $7 - 16 \text{ mW/cm}^2$ with an interval of 3 mW/cm^2 . The exposure time was in the range of $15 - 45$ seconds with an interval of 10 seconds. The cell densities were 0, 5, and 10×10^6 cells/ml.

Figure 2. 2 3D structure designed to study cure depth

2.3. Results

2.3.1 Cure Depth

3D constructs are fabricated based on a layer-by-layer mechanism. In this section, cure depth of a single layer has been investigated under different process conditions. Specifically, the effects of the UV intensity, exposure time, and cell density have been studied. Then, a phase diagram has been constructed to identify the optimal process conditions based on the uniformity of the fabricated lines.

2.3.1.1 Effects of UV intensity and exposure time on cure depth

When the bioink is exposed to the UV light, the photoinitiator generates free radicals which cure the bioink. The cure depth primarily depends on the UV energy and the optical properties of the bioink. The UV light energy follows a Gaussian distribution on the bioink surface [44]:

$$E = \frac{2PT}{\pi W_0^2} e^{\left(-\frac{2r^2}{W_0^2} - \frac{z}{D_p}\right)} \quad (1)$$

where E is the UV energy at any point (r, z) on the bioink surface, P is the UV power, T is the exposure time, W_0 is the $1/e^2$ Gaussian half-width of the UV spot, and D_p is the depth of UV penetration into the bioink until a reduction in irradiance of $1/e$ is reached. At $r = 0$ and $z = 0$, the UV energy is maximum $E_{max} = E(0, 0) = 2PT/\pi W_0^2$. The maximum cure depth $C_d = z$ can be derived, when $r = 0$ [44, 45]:

$$C_d = D_p \ln(E_{max}/E_c) \quad (2)$$

where E_c is the critical UV energy to initiate the bioink crosslinking. The Equation 2 is also known as “working curve” [21, 46]. It is seen from Equation (2) that the cure depth depends on D_p , E_{max} and E_c . D_p and E_c are related to the bioink and E_{max} is related to the input UV energy.

In Figure 2.3 & Figure 2.4, it shows the effects of the UV intensity and exposure time on the cure depth, respectively. The curves in the mentioned figures are derived from the experimental data. In Figure 2.3, the UV intensity is adjusted between 7 – 16 mW/cm² at a fixed exposure time of 35 seconds. It is seen that the cure depth increases with the increase of the UV intensity. In Figure 2.4, the exposure time is adjusted from 15 – 45 seconds at a fixed UV intensity of 10 mW/cm². It is observed that the cure depth also increases with the increase of the exposure time. The increase of either the UV intensity or the exposure time results in the increase of the input UV energy. Based on Equation (2), the cure depth increases with the increase of the input UV energy. Both observations are consistent with the reported results using other photocrosslinkable materials [21, 45]. It is

noted that the cure depth in this study is on the order of 1 mm. It is noted that the cure depth is the maximum thickness of a single layer, which can be incorporated during printing process. The typical layer thickness used in this study is 150 - 200 μm during biofabrication of 3D cellular constructs. The cross section of the fabricated line is shown a parabolic shape [44], and the cure depth in this study is the maximum cure depth [53].

Figure 2. 3 Effect of UV light intensity on cure depth under an exposure time of 35 s

Figure 2. 4 Effect of exposure time on the cure depth under a UV intensity of 10 mW/cm^2

It is seen from Equation 2 that the cure depth depends on D_p , E_{max} , and E_c . The input UV energy E_{max} is determined by the UV intensity and the exposure time. In this study, different UV intensities and exposure times were selected resulting in the input UV energy in the range of 100 – 1000 mJ/cm². D_p and E_c are determined by the bioink. D_p represents the depth of UV penetration into the bioink until a reduction in irradiance of $1/e$ is reached, and E_c represents the critical UV energy to initiate the bioink crosslinking. In Figure 2.5, it shows the cure depth of the 5% (w/v) GelMA as a function of the input UV energy. The working curve is fitted based on Equation 2: $C_d = 0.7\ln(E_{max}/64)$. It is seen that the cure depth is almost proportional to natural log of the input UV energy. The slope of the working curve in Figure 2.5 is D_p . For the 5% (w/v) GelMA bioink used in this study, D_p is 0.7 mm, and E_c is 64 mJ/mm².

Figure 2. 5 Working curve of 5% (w/v) GelMA

2.3.1.2 *Effect of cell density on cure depth*

The bioink used in this study incorporates living cells. Different cell densities in the bioink affect the cure depth. The cell densities used in this study include 0, 5×10^6 cells/ml, and 10×10^6 cells/ml. The UV intensity and exposure time are fixed at 10 mW/cm^2 and 30 seconds, respectively. In Figure 2.6, it is seen that with the increase of the cell density the cure depth decreases. For the bioink without cells, the cure depth is 1.0 mm. When the cell density increases to 10×10^6 cells/ml, the cure depth decreases to 0.9 mm. When the living cells are added into the bioink, some GelMA solution is replaced by the living cells. Compared to the GelMA solutions, living cells are more difficult for the UV to penetrate and have smaller D_p . As the cell density increases, D_p of the bioink decreases resulting in the decrease of the cure depth. It is noted the decrease of the cure depth is not significant as the cell density increases. The maximum cell density used is 10×10^6 cells/ml, which is already a very high cell density used in 3D bioprinting. However, the associated cell volume fraction is only 1.76%. Hence, the effect of cell density on the cure depth is not significant [53].

Figure 2. 6 Effect of cell density on cure depth under a fixed UV intensity of 10 mW/cm^2 and exposure time of 30 s

2.3.1.3 Phase diagram

Selecting the optical process conditions is very important for effective and efficient fabrication of 3D constructs. This section investigates the quality of the fabricated lines under different process conditions. The UV intensity is between $7 - 16 \text{ mW/cm}^2$ and the exposure time is between 15 - 45 seconds. Three different types of lines are classified based on their morphology shown in Figure 2.7: no presence of lines, non-uniform lines, and uniform lines. When the UV intensity and exposure time are insufficient, no lines are fabricated. When the UV intensity and exposure time are medium, the fabricated lines are uniform. When the UV intensity and exposure time are large, the fabricated lines are non-uniform due to over crosslinking. It is observed that the fabricated lines have the maximum thickness at both ends and the minimum thickness in the middle. The phase diagram in Figure 2.7 has been constructed to classify three different regimes corresponding to the different line morphologies [53]. The optimal operating conditions for printing a uniform line can be easily identified from the phase diagram.

Figure 2. 7 Phase Diagram in terms of UV intensity and exposure time

2.3.2 Fabrication of 3D Cellular Constructs

This section focuses on the fabrication of 3D cellular constructs and the post-printing cell viability evaluation. The bioink used contained 5% (w/v) GelMA and 3T3 fibroblasts with a cell concentration of 2.5×10^6 cells/ml. The UV light intensity and the exposure time used during the fabrication process were 10mW/cm² and 35 seconds, respectively. After one layer was completed, the platform moved down by 0.2 mm to define the layer thickness, and the new layer was deposited on the top of the previous layer. 3D cellular constructs were successfully fabricated based on a layer-by-layer manufacturing mechanism. In Figure 2.8, it shows the fabricated Y-shaped hollow construct with living cells encapsulated, which mimics the typical unit in vascular networks. The overall height of the cellular construct is 14 mm including the straight part of 8 mm and the branch part of 6 mm. The outer diameter and the inner diameter of the tube are 3.2 mm and 2 mm,

respectively. The wall thickness is 0.6mm. The cellular construct has 70 layers including the straight part of 40 layers and the branch part of 30 layers [53]. The typical layer thickness used in this study is 150 - 200 μm during biofabrication of 3D cellular constructs.

Figure 2. 8 Fabricated 3D Y-shaped hollow construct with cells encapsulated

Figure 2. 9 Cell viability test immediately after printing

Cell viability has been evaluated to make sure that the dynamic optical projection stereolithography is a viable technology for cell encapsulation and biofabrication. The fabricated tubular construct with cells encapsulated were incubated together with the fluorescent calcein AM and Ethidium homodimer III, which stained live cells green and dead cells red, respectively. The fluorescent microscope (EVOS FL, Thermo Fisher Scientific, Waltham, MA) was utilized to capture the images shown in Figure 2.9 it is found that the cell viability immediately after printing is around 75%, which is comparable to the reported results in the literature [27, 31]. After 48h and 96h incubation, the cell viabilities are 80% and 70%, respectively. The number of live cells and dead cells was counted manually. The cell viability was calculated by the number of the live cells divided by the total number of both live and dead cells [53]. The cell viability results show that most cells survive during the dynamic optical projection stereolithography and remain viable in the post-printing incubation.

2.4 Discussion

The post-printing characterization has been performed in terms of mechanical properties, swelling properties, and microstructures of the fabricated constructs. These results have been further discussed and compared with the published results in the literature. The future study may include: 1) Effects of GelMA concentration on the mechanical and photocrosslinkable properties and 2) effects of cell concentration on the degradation of GelMA hydrogels with different concentrations.

2.4.1 Swelling properties

Swelling properties of hydrogels play an important role in water absorption and nutrient diffusion as well as cell viability and proliferation [47, 48]. In this study, swelling properties of the bioink with 5% (w/v) GelMA have been characterized. The specimens were prepared by exposing the bioink with 5% (w/v) GelMA to the UV light at a UV intensity of 12 mW/cm^2 for 35 seconds. The specimens were cultured in DPBS for 0, 24, 48, and 72 hours, and weighted to obtain the swollen weight as W_s . Then, the specimens were lyophilized at -50°C for 12 h, and the corresponding dry weight of the specimens were measured as W_d . The swelling ratio can be estimated as $(W_s - W_d)/W_d$. In Figure 2.10, it shows the measured swelling ratio for the specimens with 5% (w/v) GelMA at different incubation times. It is seen that the swelling ratio increases rapidly from 11 to 16 in the first 24 hours. However, from 24 - 72 hours, it increases slightly from 16 to 18 [53]. The measured swelling ratio of the 5% GelMA hydrogel is comparable to the results in the literature [27].

Figure 2. 10 Swelling ratio for 5% GelMA hydrogel at different incubation times

2.4.2 Mechanical properties

Generally, mechanical properties of the GelMA hydrogel are characterized using a compression test [27, 31, 49]. However, in this paper, tubular constructs with living cells encapsulated have been fabricated to mimic artificial blood vessels. One critical mechanical property of the artificial blood vessels is burst pressure, which is related to the ultimate tensile stress [50]. Hence, the tensile test has been performed in this study to characterize the post-printing mechanical properties. Two different bioinks were utilized to prepare dog-bone specimens. One bioink only contained the 5% (w/v) GelMA, and the other bioink contained the 5% (w/v) GelMA and living cells (cell density 2.5×10^6 cells/ml). The UV light intensity and the exposure time used were 10 mW/cm^2 and 35 seconds, respectively. The specimens were subject to uniaxial stretching at a constant rate of 1 mm/min until failure on a Dynamic Mechanical Analyzer (DMA Q800, TA Instruments, New Castle, DE). The associated stress-strain curves were obtained shown in Figure 2.11.

For the specimen without cells, the maximum strain at failure is 18.7% and the ultimate tensile strength is 2.4 kPa while for the specimen with cells encapsulated, the maximum strain at failure is 14.6% and the ultimate tensile strength is 1.7 kPa. It is seen that when living cells are encapsulated inside the GelMA hydrogels, both maximum strain and ultimate tensile strength decrease [53]. In addition, the deformation of the specimens can be separated into two stages: elastic deformation and plastic deformation. In Figure 2.11, the elastic deformation range is much larger than the plastic deformation range for both specimens. For example, the elastic deformation occurs at a strain of 0 – 13.7% while the plastic deformation occurs at a strain of 13.7 – 18.7% for the specimen without cells. The tensile modulus can be estimated based on the linear relationship of the stress and strain in the elastic deformation range. The measured tensile moduli are 9.8 and 9.6 kPa for the specimens with and without cells, respectively. It is seen that when the living cells are encapsulated inside the GelMA hydrogel, the tensile modulus decreases slightly. This observation is consistent with the results reported by Buckley *et al.*, which investigated the effect of chondrocyte density on the mechanical properties of the agarose hydrogels [51].

Figure 2. 11 Stress-strain curve for 5% GelMA with cells and without cells encapsulated

2.4.3 Pore Size

The pore size of hydrogels significantly affects the effective transfer of oxygen and nutrients as well as the cell attachment and proliferation. In this study, the pore size of the 5% (w/v) GelMA hydrogels has been measured. The specimens were prepared by exposing the bioink with 5% (w/v) GelMA to the UV light at a UV intensity of 12 mW/cm² for 35 seconds. The specimens were cultured in DPBS for 0, 24, 48, and 72 hours. Then, the specimens were lyophilized at -50°C for 12 hours and viewed under a scanning electron microscopy (SEM; ProX, Phenom, Phoenix, AZ). The SEM images were captured shown in Figure 2.12 and the pore size was subsequently measured. Figure 2.13 shows the measured pore size of the 5% GelMA hydrogel at the incubation times from 0 – 72 hours. It is seen that the pore size increases significantly from 93 to 120 μm within the first 24 hours, which is mainly due to swelling. However, from 24 – 72 hours the pore size don't change significantly, showing the stable GelMA network. The reported pore size of 5%

(w/v) GelMA hydrogel in the literature is around $44\ \mu\text{m}$, which is much smaller than our measurement [52]. The microstructure of the GelMA hydrogel is dependent on several important factors, such as UV intensity, exposure time, degree of functionalization, to name a few. The study in the literature [52] utilized higher UV energy and longer exposure time, which results in much smaller pore size.

Figure 2. 12 SEM images displaying microstructures for the 5% GelMA hydrogel at different incubation times: (a) immediately after printing, (b) 24 hours, (c) 48 hours, and (d) 72 hours

Figure 2. 13 Pore sizes for 5% GelMA having different incubation times

2.5. Conclusion

In this paper, the DMD-based dynamic optical projection stereolithography has been utilized to fabricate 3D tubular constructs with living cells encapsulated. The cure depth has been investigated under different UV intensities, exposure times and cell densities. The cell viability has been tested, and the post-printing characterization has been performed in terms of mechanical properties, swelling properties, and microstructures. The main conclusions are: 1) the cure depth increases as the UV intensity and exposure time increase while the cure depth decreases as the cell density increases; 2) a phase diagram has been constructed to identify optimal printing conditions; 3) cell viability immediately after printing is 75%; 4) when living cells are encapsulated within the GelMA hydrogel, both the maximum strain and ultimate tensile strength decreases; and 5) within the first 24 hours, both the swelling ratio and the pore size increase significantly. The future study may include: 1) effects of cell density on the mechanical properties, physical properties and microstructures of the GelMA hydrogels with living cells encapsulated, and 2) effects of cell-mediated enzymatic degradation on the shape fidelity and mechanical properties of the GelMA hydrogels with living cells encapsulated.

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CHAPTER THREE

INVESTIGATION OF GELMA WORKING CURVE USING DYNAMIC OPTICAL PROJECTION STEREOLITHOGRAPHY

3.1 Introduction

Extracellular matrix (ECM) is a complex meshwork within all tissues and organs, composed of structural proteins (e.g., collagen, fibrillin, and elastin), specialized proteins (e.g., fibronectin, and various laminins and integrins) and proteoglycans. ECM provides essential physical support to connect cells together to form tissues. Moreover, ECM is critical in regulation of cell behaviors in multicellular organisms, such as tissue morphogenesis, cell differentiation, and homeostasis [1]. Suitable biocompatible materials are needed in the process of 3D bioprinting to mimic the natural ECM functions [2]. Hydrogels have been of critical importance in the recent years in tissue engineering 3D biofabrication, because of their similarity with the indigenous connective tissues. The unique hydrophilic properties which are inherited by the hydrogels make them the most appropriate material for cell adhesion and proliferation [2, 3]. Hydrogels are crosslinked polymers which depict the property of swelling when introduced to water and retain large quantity of water inside the 3D networks without dissolving in the water [4]. Characteristics of hydrogels such as high-water retention compatibility, biocompatibility, and biodegradability make them truly promising for biomedical applications [5]. These characteristics are directly related to the characteristics observed in natural ECM [6]. Additionally, hydrogel's soft nature results in minimization of irritation to the surrounding tissues, and generally, cell adhesion of hydrogels can be improved by adjustments with ligands [6]. When the hydrophilic network (hydrogel precursor solution) is crosslinked in

a specific way, synthetization of the hydrogels takes place [7]. The crosslinking mechanisms can be categorized into two predominant categories: chemical crosslinking and physical crosslinking [8]. The manufacturing process, mechanical characteristics of the fabricated structure can be affected by the type of the crosslinking mechanism.

The synthesis of GelMA is achieved by reacting the primary amine groups of gelatin with methacrylic anhydride at an elevated temperature, which eventually results in the addition of methacrylate groups onto the macromers of gelatin. Gelatin methacrylate being a photocrosslinkable derivative of gelatin was reported for the first time by Van Den Bulcke et al [9]. Reaction between the primary amine groups of gelatin and the methacrylic anhydride at an elevated temperature leads to the synthesis of GelMA. This further results in addition methacrylate groups onto the macromers of gelatin. The Degree of Functionalization (DoF) which is also known as the degree of methacryloyl substitution can be changed by adjusting the amount of methacrylic anhydride added to the initial reaction [10]. The following process can be also used to change the physical properties of GelMA [11]. GelMA is a synthetically crosslinkable hydrogel, that can be crosslinked by introduction to various explicit wavelengths of light, for example, UV radiation within the presence of a suitable photoinitiator [12]. The photoinitiator facilitates the release of free radicals upon the assimilation of light, which at that point in this way results in the polymerization of GelMA[9, 12]. GelMA holds a large portion of the amino acids of gelatin [13], and shows tailorability of its physiochemical characteristics by varying of synthesis parameters, for example, changing the DoF, photoinitiator concentration, and GelMA concentration [10, 12-14]. Zhao *et al.* [13] used GelMA in epidermal tissue building, and revealed that with the increase of the GelMA concentration, the mechanical strength

improves, the swelling and degradation are reduced. Schuurman *et al.* [15] used diverse concentrations of GelMA for the engineering of tissue fabricated cartilage develops and detailed that with the increase of the GelMA concentration, the mechanical quality increases, and the swelling diminishes. Chen *et al.* [10] utilized GelMA and it has been found out that with increase of the GelMA degree of functionalization, there is a decrease in the pore size of the hydrogel and, on the other hand the compressive modulus increases. Colosi *et al.* [16] examined the impacts of the UV light intensity and exposure time on the mechanical properties of GelMA, and concluded that the mechanical strength for GelMA increments with the increase of the UV light intensity and the exposure time.

By the virtue of the native bioactivity of the gelatin which leads to reservation of amino acid motifs such as RGD motifs and MMP-degradable motifs in GelMA post substitution, GelMa is considered to be highly biocompatible [17, 18] and therefore it is used in different 3D biofabrication applications. Gauvin *et al.* [19] used a stereolithography system for 3D bioprinting of GelMA hydrogels with tunable characteristics and microstructures. Soman *et al.* [20] could accomplish quick biofabrication of 3D microstructures that were very similar to native tissues and took into consideration viable cell survival. A digital micromirror device (DMD) was utilized by Grogan *et al.* [21] to manufacture meniscus-like tissue utilizing GelMA. Yin *et al.* [22] proposed the utilization of a two-step gelation procedure to successfully print GelMA of low-concentration utilizing a microextrusion setup. It was seen that GelMA hydrogels have a capacity to contain the local microcellular environment. Chen *et al.* [10] used GelMA for the creation of functional vascular systems. It was accounted for that GelMA had properties that could solve the deficiencies of other pervasive hydrogels, for example, insufficient cell viability

and binding, poor mechanical properties, and the inefficient control of microarchitecture of the hydrogel [10]. Gao *et al.* [23] proposed the use of photocrosslinkable GelMA to improve the properties of bone and cartilage tissue using ink-jet-bioprinting and it was observed that GelMA scaffolds demonstrated improved mechanical properties and osteogenic and chondrogenic differentiation indicating its favorable use in bone and cartilage tissue engineering. Wang *et al.* [24] in his study, utilized GelMA-DexMA copolymer hydrogels with tunable mechanical properties for tissue engineering and it can be concluded that GelMA can be used with other biopolymers to enhance tuning characteristics such as porosity swelling ratio and degradability along with the mechanical properties. Nichol *et al.* [12] exhibited the use of cell-laden GelMA hydrogels for the arrangement of micropatterns. It was accounted that GelMA is certainly appropriate for applications that need rapid cell adhesion, migration and proliferation. Bertassoni *et al.* [25] showed the usage of cell-laden GelMA at various concentrations and cell densities by utilizing a microextrusion framework. Great printability and high cell survival in the printed constructs were reported. Colosi *et al.* [23] showed the use of an inkjet printing system for the engineering of a PEG-GelMA scaffold for bone tissue building. Superior mechanical properties by the use of the hydrogel was reported. Previous research has been done to study the effect of photopolymer concentration, photoinitiator (PI) type and concentration on the hydrogel thickness. Cure depth analysis for different hydrogel concentrations as a function of the type and concentration of the photoinitiator has been presented. Arcaute *et al.* [26] used polyethylene glycol (PEG) to 3D fabricate complex structures having internal channels of various orientations using stereolithography. It was noted that cure depth of the hydrogel was a strong function of the PEG concentration and

photoinitiator concentration and therefore photocrosslinkable biomaterials such as PEG hydrogels are feasible for fabricating complex bioactive scaffolds.

The cure depth is one of the most important parameters in photopolymerization, which is dependent of several factors. Having the desired cure depth for a single layer is predominantly, a critical factor towards ensuring acceptable and satisfactory resolution in the 3D fabricated constructs [27]. The cure depth determines the thickness of the layers that can be built thereby predicting the number of layers required to print the structure. The thickness of the printed line is significantly influenced by two major operating conditions namely the UV light intensity and the exposure time [20]. Along with these factors the concentration of the resin and photoinitiator are also detrimental towards the thickness of the line. The amount of UV of energy E will cause the polymerization to a depth C_d as suggested by the Jacob's equation as $C_d = D_p \ln(E/E_c)$, where E_c is the critical energy and D_p is the sensitivity of the photopolymerizable suspension [28]. A working curve is presented in terms of the cure depth and critical exposure. This paper aims to examine the variation in the E_c and D_p with change in the polymer and photoinitiator concentration. This study covers the working of the dynamic projection stereolithography and its experimental setup followed by the mechanism of photocrosslinking. Relationship between the cure depth for different concentrations of GelMA is established. The paper also aims at studying the relationship between the cure depth and photoinitiator concentration in the resin. Consequently, the conclusions are drawn, and the future scope is proposed.

3.2 Materials and Methods

3.2.1 Materials

3.2.1.1 Gelatin methacrylate (GelMA)

Gelatin methacrylate (GelMA) is one of the most promising hydrogels which is extensively used for different biomedical applications because of its appropriate physical and biological characteristics. GelMA is a derivative of gelatin which is synthesized after the reaction of primary amine groups with gelatin at elevated temperatures. Being a synthetically crosslinkable hydrogel [29], GelMA can be photocrosslinked by introduction of various explicit wavelengths of light with the presence of a suitable photoinitiator. GelMA hydrogels are highly preferred for biomedical applications due to their hydrophilic nature which makes them appropriate for cell adhesion and proliferation [2, 3]. Being an inexpensive, cell responsive and easy to synthesize hydrogel, GelMA is suitable for photomasking, bioprinting and micromolding applications. Mechanical characteristics and the hydration of GelMA are found out to be tunable for different applications by modifying methacrylation degree and gel concentration [12]. In addition to this, the extracellular matrix (ECM) is closely depicted by the GelMA [24, 30], which makes it a versatile matrix which can be used for various biomedical applications to engineer microvascular networks including the fabrication of complex engineered tissues[10, 14].

Synthesization of GelMA took place in the following order. Type A gelatin solution (MP Biomedicals, Santa Ana, CA) was dissolved into Dulbecco's Phosphate Buffered Saline (DPBS) at 50°C to obtain 10% (w/v) gelatin solution. This was followed by incorporation of the methacrylic anhydride into the gelatin solution at the flow rate of 0.5

ml/min. After addition of Methacrylic anhydride and its reaction with gelatin macromers, this mixture was subjected to vigorous stirring for 120 minutes. At this instance, the methacrylic anhydride reacts with gelatin which results in the addition of methacrylamide groups onto gelatin macromers [12]. Further reaction was avoided by adding warm DPBS and attaining 5X dilution. Dialysis was performed at 40°C against deionized water using 12-14 kDa cutoff dialysis tubing for seven days to extract the dissolved salts and methacrylic acid. Eventually, GelMA white porous foam was produced by lyophilizing the solution for seven days. It was stored in the freezer at -80°C [40]. The ratio of methacrylic anhydride in comparison to the gelatin solution was 8%.

The GelMA solution was synthesized by adding and dissolving the lyophilized GelMA foam into Dulbecco's phosphate-buffered saline (DPBS) and photoinitiator having the desired concentration was later added to the solution. The photoinitiator used was Irgacure 2959 (TCI America, Portland, OR). The solution was mixed rigorously to achieve homogeneous characteristics.

3.2.2 Methods

3.2.2.1 Experimental setup

In Figure 3.1, the dynamic optical projection stereolithography system was set up to engineer a single printed line. As one of the projection methods, DMD based dynamic optical projection stereolithography utilizes a digital micromirror device as a dynamic mask to create a layer of any desired pattern on the resin surface, and structures are built by photocrosslinking. As DMD can generate a layer of desired pattern at a single time, this technology is suitable for fabrication of 3D structures with complex geometries and is

preferred due to its micrometer printing resolution, higher printing speed, better pattern flexibility and scalability [27, 31, 32]. The importance of DMD based dynamic optical projection stereolithography has been widely recognized in different biofabrication applications. The stereolithography system was composed of 5 critical components namely UV source (Omnicure S2000, Excelitas Technologies, Ontario, Canada) having a wavelength of 365 nm, collimator (Lumen Dynamics, Excelitas Technologies, Ontario, Canada), digital micromirror device (DMD, DLP Discovery 4100, Texas Instruments Incorporated, Dallas, TX), a motorized Z stage (Thorlabs, Newton, NJ) attached to a platform and a reservoir contained with photocrosslinkable bioink [40]. The UV light with various intensities was supplied by the UV source. This UV light was made parallel by the collimator. The vertical movement of the platform was controlled by the motorized Z stage. The DMD was the most critical component used in the system as the dynamic mask and the function was to generate any desired 2D pattern.

The procedure to fabricate the desired single line is as follows. A suitable CAD model is sliced with a series of closely spaced planes to determine the shape of the built layers. The mask patterns are progressively developed in the form of bitmap images on a computer programmable exposition of digital micro-mirrors on the DMD chip. The light which is irradiated on the DMD chip is modeled with respect to the defined mask pattern. This modulated light is then passed through a couple of reduction lenses and a prism. Eventually, the desired image is formed onto the curable resin surface. For every layer, the illuminated area is cured under one exposure and the dark region on the resin surface remains unaffected. After the solidification of the first layer, the Z stage controlled the platform to move down vertically (200 μm), and a new layer was deposited on the top of

the existing structure. Geometrically intricate microstructures can be built by fabricating layers in a sequential order from top to bottom [32].

Figure 3. 1 Schematic representation of the experimental setup

3.2.2.2 Experimental Conditions and Design

Different photoinitiator and bioink concentrations were used to determine the cure depth of a single printed line. In Figure 3.2, a table shaped structure with four columns and a frame at the top was designed. Two binary images were generated for the columns and the frame respectively. The first image having four columns was used to print 13 layers. The purpose of the 4 columns was to provide support to the printed, single line. The second image comprising of the frame was used to fabricate the top most layer depicting a single

line. The UV intensity was in the range of $7 - 16 \text{ mW/cm}^2$ with an interval of 3 mW/cm^2 .

The exposure time was in the range of $15 - 45$ seconds with an interval of 10 seconds.

Figure 3. 2 3D structure designed to study cure depth

3.3 Results

3.3.1 Cure Depth

3D fabrication using DMD – based stereolithography is achieved with layer by layer fashion. In this section cure depth of the single layer has been investigated using different process conditions. In particular, the effect of photopolymer concentration and photoinitiator concentration have been studied. Subsequently working curves are constructed to analyze the cure depth since it is one of the most important parameters in photopolymerization, which is dependent of several factors. Having the desired cure depth

for a single layer is predominantly, a critical factor towards ensuring acceptable and satisfactory resolution in the 3D fabricated constructs [27].

After being exposed to the UV light, the photoinitiator generates free radicals which photocrosslink the bioink. The energy and other process parameters play a very critical role in determining the cure depth. The UV light energy follows a Gaussian distribution on the bioink surface [33]:

$$E = \frac{2PT}{\pi W_0^2} e^{\left(-\frac{2r^2}{W_0^2} - \frac{z}{D_p} \right)} \quad (1)$$

where E is the UV energy at any point (r, z) on the bioink surface, P is the UV power, T is the exposure time, W_0 is the $1/e^2$ Gaussian half-width of the UV spot, and D_p is the depth of UV penetration into the bioink until a reduction in irradiance of $1/e$ is reached. At $r = 0$ and $z = 0$, the UV energy is maximum $E_{max} = E(0, 0) = 2PT/\pi W_0^2$. The maximum cure depth $C_d = z$ can be derived, when $r = 0$ [33, 34]:

$$C_d = D_p \ln(E_{max}/E_c) \quad (2)$$

where E_c is the critical UV energy to initiate the bioink crosslinking. The Equation 2 is also known as “working curve” [35, 36]. It is seen from Equation (2) that the cure depth depends on D_p , E_{max} and E_c . D_p and E_c are related to the type and concentration of bioink and E_{max} is related to the input UV energy [40].

3.3.1.1 Effect of Photopolymer Concentration on Cure Depth

Having the desired cure depth for a single layer is predominantly, a critical factor towards ensuring acceptable and satisfactory resolution in the 3D fabricated constructs.

The thickness of the printed line is significantly influenced by two major conditions namely the UV photopolymer concentration and photoinitiator concentration [37, 38]. A single line was printed using the desired operating conditions having different levels and intensities. A table like structure consisting of four columns, 13 layers each and a frame comprising of only layer was fabricated to study the effects of operating conditions on the thickness of printed line. The printed structure was carefully removed from the substrate and observed under the microscope to measure the cure depth.

Table 3. 1 Cure depth for 5%, 10% and 15% GelMA for different UV energies

UV Energy (mJ/cm ²)	Cure Depth (mm)		
	5% GelMA	10% GelMA	15% GelMA
150	0.73	0.68	0.51
195	0.85	0.77	0.60
245	1.08	0.79	0.78
250	0.95	0.90	0.87
350	1.14	1.09	0.90
450	1.34	1.10	1.12
455	1.39	1.29	1.15
560	1.60	1.53	1.25

Experiments were conducted to validate the cure depth outcomes for different concentrations of GelMA under different operating conditions. The UV light intensity used was in the range from 7 mW/cm² to 16 mW/cm² whereas the exposure time was 15, 25, 35 and 45 seconds. From Table 3.1 it was observed that the cure depth decreases with the increase in GelMA concentration. The critical energy is a function of exposure time and UV light intensity. At a constant critical energy of 150 mJ/cm² the cure depth for 5% GelMA was 0.73mm, for 10% GelMA it reduced to 0.68mm and for 15% GelMA it further reduced

to 0.51mm. The increase of D_p which is the depth of penetration or sensitivity of the material results in the increase of the input UV energy. Based on Equation (2), the cure depth increases with the increase of the penetration depth. The cure depth depends upon the D_p and E_c values therefore it becomes very important to analyze them. The observations are consistent with the reported results using other photocrosslinkable materials [37, 39]. It is noted that the cure depth in this research is on the order of 1 mm, which is the maximum height of the printed line. When the dynamic optical projection stereolithography system is utilized to print 3D structure, the general layer thickness is around 150-200 μm [40].

3.3.2 Working Curve

It is concluded from Equation 2 that D_p , E_{max} , and E_c are critical factors in deciding the depth of cure. The UV intensity and the exposure time determine the input UV energy E_{max} . In this study of using various concentrations of GelMA, different UV intensities and exposure times were used resulting in the input UV energy in the range of 100 – 1000 mJ/cm^2 . D_p and E_c are determined by the bioink. D_p represents the depth of UV penetration into the bioink until a reduction in irradiance of $1/e$ is reached, and E_c represents the critical UV energy to initiate the bioink crosslinking. In Figure 3.3, 3.4 and 3.5, it shows the cure depth of the 5%, 10% and 15% (w/v) GelMA as a function of the input UV energy. The working curve is fitted based on Equation 2: $C_d = D_p (E_{max}/E_c)$. It is seen that the cure depth is almost proportional to natural log of the input UV energy [40]. The slope of the working curve is D_p . It is observed that the D_p decreases and the E_c increases with decrease in the GelMA concentration. For the 5% (w/v) GelMA bioink used in, D_p is 0.61 mm, and E_c is 48 mJ/mm^2 . D_p and E_c values for 10% GelMA were 0.59 mm and 54 mJ/mm^2 , respectively. For 15% GelMA, D_p is 0.56 mm, and E_c is 61 mJ/mm^2 . It is seen that with

the increase of the GelMA concentration, D_p decreases while E_c increases. The decrease of D_p is mainly due to the smaller number of possible crosslinking sites and decrease in the free radical initiation which results in the decrease in the penetration depth of the photons.

Figure 3. 3 Working curve of 5% (w/v) GelMA

Figure 3. 4 Working curve of 10% (w/v) GelMA

Figure 3. 5 Working curve of 15% (w/v) GelMA

3.3.3 Effect of the Photoinitiator Concentration on Cure Depth

The photoinitiator Irgacure 2959 (TCI America, Portland, OR) was used in this study and was added to the bioink containing GelMA solution at different levels of concentration. 0.3%, 0.5%, 0.75% and 1% (w/v) are the different levels of photoinitiator present in the bioink. These levels of photoinitiator concentration have been widely used in different 3D bioprinting applications in the literature. The two operating conditions namely the UV light intensity and the exposure time were kept constant at 10mW/cm^2 and 35 seconds, respectively. The bioink comprised of 5% (w/v) GelMA. Figure 3.6 resembles a graph with cure depth on Y-axis and photoinitiator concentration on the X-axis. It can be observed that as the photoinitiator concentration increases the GelMA cure depth decreases. The cure depth was 1.11 mm for the photoinitiator concentration of 0.3% and the cure depth decreased to 0.87 mm for 1% photoinitiator concentration. The increase in the photoinitiator concentration results in more radical initiation and greater number of double bond conversion. However, the penetration depth of the photons is significantly

reduced with increase in the photoinitiator concentration. When there is significant light attenuation, irrespective of thickness of the film, the penetration distance of the photons becomes less than the depth of the medium. This further results in decrease in the free radical initiation. The observations are consistent with other published papers [28, 41].

Table 3. 2 Cure depth for different photoinitiator concentrations

Photoinitiator Concentration (%)	Cure Depth (mm)
0.3	1.11
0.5	1.09
0.75	0.97
1	0.86

Figure 3. 6 Effect of photoinitiator concentration on cure depth

3.4 Conclusions

In this paper, GelMA working curve has been systematic investigated using DMD-based dynamic optical projection stereolithography. The GelMA solutions with three concentration of 5%, 10% and 15% (w/v) have been utilized to print a single line under different UV intensities and exposure times. The cure depth has been measured based on

the images under the microscope. The main conclusion are as follows: 1) with the increase of GelMA concentration, the cure depth decreases; 2) Working curves have been constructed for different GelMA concentrations, and it is seen that with the increase of GelMA concentration the depth of UV penetration D_p decreases while the critical UV energy to initiate the GelMA solution crosslinking E_c increases; and 3) the cure depth decreases with the increase in the photoinitiator concentration. The future study may include: 1) effects of degree of methacrylation on the cure depth, and 2) comparison of the cure depth using different photoinitiators.

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CHAPTER FOUR

CONCLUSIONS AND FUTURE WORK

4.1 Conclusions

In this thesis, DMD based dynamic optical projection stereolithography has been used to 3D fabricate tubular constructs with living cells encapsulated. Different operating conditions and process conditions were used to investigate the cure depth. Operating conditions such as UV light intensity, exposure time, cell density and process conditions such as polymer concentration and photoinitiator concentration have been varied to analyze their effect on the cure depth of the filament. Relationship between the cure depth for different concentrations of GelMA and the photoinitiator is established. The DMD based stereolithography is used to fabricate a hollow Y-shaped cellular construct and its cell viability has been tested immediately after printing. The post printing property characterization is done which includes, mechanical properties, swelling properties and the pore size. Following are the important conclusions of this study:

- The cure depth increases as the UV intensity and exposure time increase while the cure depth decreases as the cell density increases.
- There is an increase of cure depth with an increase in the UV intensity.
- There is an increase of cure depth with an increase in the exposure time.
- There is a decrease of cure depth with an increase in the cell density.
- A phase diagram has been constructed to identify optimal printing conditions.

- Cell viability immediately after printing is 75%.
- When living cells are encapsulated within the GelMA hydrogel, both the maximum strain and ultimate tensile strength decreases.
- Within the first 24 hours, both the swelling ratio and the pore size increase significantly.
- There is a decrease of cure depth with an increase in the polymer concentration.
- There is a decrease of cure depth with an increase in the photoinitiator concentration.
- Working curve has been constructed for different GelMA concentrations to analyze the cure depth.

4.2 Future Work

The cure depth is one of the most important parameters in photopolymerization, which is dependent of several factors. Having the desired cure depth for a single layer is predominantly, a critical factor towards ensuring acceptable and satisfactory resolution in the 3D fabricated constructs. Process parameters such as the methacrylatin degree and photoinitiator type also play an important role in determining the cure depth. Following are the future studies that are proposed:

- Effect of cell density on the mechanical properties, physical properties and microstructures of the GelMA hydrogels with living cells encapsulated.

- Effects of cell-mediated enzymatic degradation on the shape fidelity and mechanical properties of the GelMA hydrogels with living cells encapsulated.
- Effect of degree of methacrylation on the cure depth.
- Effect of type of photoinitiator type on the cure depth.

LIST OF PUBLICATIONS

Journal Articles:

1. **Wadnap S**, Krishnamoorthy S, Zhang Z., and Xu C. Biofabrication of 3D cell-encapsulated tubular constructs using dynamic optical projection stereolithography. *Journal of Materials Science: Materials in Medicine*; 3: 36.

Conference Proceedings:

1. **Wadnap S**, Krishnamoorthy S, Xu C, Zhang Z. Biofabrication of 3D vascular constructs using dynamic optical projection stereolithography, In 29th Annual International Solid Freedom Fabrication (SFF) Symposium; 2018 August 13-15; Austin, Texas, USA

Conference posters:

2. **Wadnap S**, Krishnamoorthy S, Xu C, Zhang Z. Biofabrication of 3D vascular constructs using dynamic optical projection stereolithography, In 29th Annual International Solid Freedom Fabrication (SFF) Symposium; 2018 August 13-15; Austin, Texas, USA