FABRICATION OF PPF BASED DRUG CONTAINING MICRONEEDLE ARRAYS
BY MICROSTEREOLITHOGRAPHY

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FABRICATION OF PPF BASED DRUG CONTAINING MICRONEEDLE ARRAYS
BY MICROSTEREOLITHOGRAPHY

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ABSTRACT

Transdermal drug delivery systems are one of the impetus areas in drug delivery research due to their distinguished advantages over oral and hypodermic needles. Microneedle based transdermal drug delivery system is one of the emerging devices for drug delivery in recent years. Microneedles deliver drug into the epidermis without disrupting the nerves endings. Many researchers have designed different microneedles and have fabricated those using different materials and fabrication processes. In this current work, microneedles were fabricated using a biodegradable and biocompatible polymer poly(propylene fumarate) (PPF), which is being recently used for different biomedical applications. The fabrication process adopted in this work was microstereolithography which is an emerging 3D microfabrication process that is being explored in biomedical applications since few years.

An attempt was made to fabricate PPF based microneedle arrays containing drug (BSA) using microstereolithography process. To obtain these microneedle arrays, firstly PPF was synthesized and characterized, and then different prepolymer were prepared using PPF and different ratios of drug for the fabrication. The microstereolithography system that was used for fabrication was developed, calibrated and verified. Since these materials are new, their material properties such as viscosity, cure depth and exposure time, and mechanical properties such as elastic modulus and ultimate compressive strength were obtained by conducting different experiments. Finally, the microneedles
arrays were fabricated and were characterized using a microscope and scanning electron microscope.
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CHAPTER I

INTRODUCTION

Over the past few years the need for effectively delivering the drug into the targeted tissue or organs of the human body has been of importance in medical science. The conventional routes of drug delivery has been through hypodermic needles and orally administered pills. In case of oral administered pills it possess few limitations such as, ‘time and again’ patients forget to take their medicine and even the most acquiescent patients gets wearied of swallowing the pills, particularly if they have to take several each day. Additionally, they suffer poor absorption, must survive the harsh environment and enzymatic degradation in the gastrointestinal tract, and first pass metabolism and elimination by liver. In case of hypodermic needles, even though they effectively deliver drugs, they suffer few limitations like causing pain and trauma to patients, many patients are phobic to needles, pose risk of disease transmission by needle re-use, inducing possible infections, generate dangerous medical waste and requires expertise in performing the injection. Both these routes have further limitations as bolus delivery methods, where the full dose of drug is introduced into the body at once. To overcome the toxic and irritating effects as drug concentration decays at later times, pills and
injections at times, have to be administered multiple times per day or sustained release formulations can be made for some cases [1].

Transdermal drug delivery refers to the slow transport of drugs through the skin, eventually reaching the bloodstream for systemic effects. This is an emerging alternative for the conventional routes which can avoid their limitations. The various advantages of this approach are better patient compliance, possibility of controlled release over time, the evasion of gastrointestinal tract or first pass liver effects, painless drug delivery, and also overcome all the limitations posed by pills and injections. In addition, transdermal systems are non-invasive, can be self-administered and are generally inexpensive [2].

Transdermal drug delivery devices can be divided into active and passive devices based on the technologies used for the skin permeation. In passive devices, the significant methods of drug delivery through the skin are chemical enhancers, emulsions, lipid assemblies and peptides. The most common active methods of skin permeation reported in literature are jet injectors, ultrasound, powder injection, ablation, electroprotein and microneedles [2].

However, transdermal drug delivery is severely limited by the inability of large majority of drugs to cross skin at therapeutic rates due to the great barrier imposed by outermost layer 10-20 µm of skin, the stratum corneum (SC) [1]. Since the stratum corneum contains no nerves, the development of a hybrid of hypodermic needles and transdermal patches, arrays of micron-scale “microneedles” that are long and robust enough to penetrate across this layer, but short enough not to stimulate nerves in deeper tissues, has the potential to make transdermal drug delivery of many more drugs possible.
Over past few years, various microneedle designs varying from in-plane and out of plane to solid, hollow and biodegradable microneedles with different shapes like pyramids, beveled tips, tapered cones, chisel tips have been fabricated using various MEMS techniques.

Many MEMS based processes such as photolithography, deep reactive ion etching and LIGA (X-Ray Lithography, Electroplating and Molding) have been adopted for fabrication of microneedles over past. However, these MEMS techniques are two-dimensional (2D) processes involving multiple steps and requiring complicated processing procedures in a cleanroom environment. These 2D techniques are normally difficult, if not impossible, to fabricate arbitrary shape and composition without the use of microassembly. The investment costs of these processes are expensive, and the choice of material is somewhat limited.

Recently, microstereolithography (µSL) process emerged as a freeform process capable of creating 3D microstructures with complex geometries. Microstereolithography has its root from the stereolithography process rather than integrated circuit technology. Stereolithography is a process of creating 3D solid models in a layer-by-layer fashion from a 3D CAD model using photopolymer as a base material. A focused ultraviolet (UV) light, coupled with some optics, is used to cure the photopolymer. This process can fabricate high aspect ratio and complex 3D microstructures. A resolution of 1 – 2 µm can be achieved with this system [3].

Microstereolithography technique is being explored in biomedical, tissue engineering, robotics and many other fields. Microstereolithography has been used for fabrication of scaffolds, nerve guides and stents in biomedical field.
Microstereolithography has not yet been completely explored for the fabrication of microneedles.

Various metals and polymers have been explored for fabrication of microneedles. The MEMS based techniques mostly used metals like titanium, nickel, and palladium, polymers like polydimethylsiloxane (PDMS), polyglycolic acid (PGA), and poly(lactic-co-glycolic acid) (PLGA) and silicon. The use of metallic microneedles may lead to irritation or retention, erythema, swelling, discoloration or other side effects when traces of metal are retained beneath the skin. Therefore, the use of biodegradable polymers is advantageous for biomedical applications and mostly for microneedles, as they degrade over time and the degradation by products are non-toxic and they either dissolve or get excreted from human body easily [1].

Many novel biomaterials have been synthesized and characterized in response to the demands of various biomedical applications like scaffolds, stents and microneedles. Recently, biodegradable, unsaturated polyesters have emerged as some of the more promising materials due to their ability to cross-link in situ and degrade over time. One of these materials is poly(propylene fumarate) (PPF), a linear, unsaturated polyester which consists of alternating propylene glycol and fumaric acid units and it a photocrosslinking resin [4]. The microstereolithography system uses basically photocrosslinking liquid resins for any kind of fabrication. Hence, poly(propylene fumarate) (PPF) can be used in a microstereolithography system.

The objective of this work was to fabricate poly(propylene fumarate) (PPF) based microneedles containing drug using the microstereolithography system.
Specifically, this work attempts to:

1. Synthesize and characterize poly(propylene fumarate) (PPF).
2. Prepare PPF containing drug at different ratios and measure its viscosity.
3. Design microneedle arrays.
4. Develop, calibrate and verify the microstereolithography system.
5. Fabricate and characterize PPF and PPF containing drug based microneedle arrays using the microstereolithography system.
6. Evaluate the mechanical properties of PPF and PPF containing drug.
CHAPTER II
REVIEW OF THE LITERATURE

2.1 Microneedles

The conventional (microneedle-free) transdermal drug delivery, which relies on drugs passively diffusing across the skin, is severely hindered by the extraordinary barrier properties of the outer 10-20 µm of skin, the stratum corneum. Depending on the skin anatomy, the microneedles were designed in such a way that they were long and robust enough to penetrate across the stratum corneum, but short enough not to stimulate the nerves in deeper tissues [1].

Different researchers have used different materials, designs and fabrication process to fabricate microneedles. Microneedles were classified as in-plane and out-of-plane, solid and hollow and metallic and polymeric depending on the design and material used for the fabrication.

McAllister et al. [5] fabricated microneedles from silicon wafers using lithography and ion etching. An array 20 by 20 microneedles with base diameter 80 µm and tapered to a height of 150 µm with radius of curvature at the tip close to 1 µm were fabricated as shown in Figure 2.1 (a).
Kuo et al. [6] fabricated polymeric hollow out of plane microneedles using PDMS with sharp tips by proprietary photolithography and molding process. The microneedles had a length of 600 µm and bore diameter of 50 µm as shown in Figure 2.1 (b).

Martanto et al. [7] fabricated microneedles by laser cutting the shape of each needle into the stainless steel sheet and bending each needle at 90° out of plane of the sheet. An array of 105 needles measuring 1000 µm in length with 75 x 200 µm cross section at their base and tapering to a sharp tip shown in Figure 2.1 (c).

Matriano et al. [8] fabricated microneedle arrays (i.e. microprojections) by acid etching from a titanium sheet. The microneedle array size was either 1 or 2 cm² with a needle density of 190 needles per cm², where the length of the needles was 330 µm shown in Figure 2.1 (d).

Mikszta et al. [9] fabricated microneedles with blunt tip measuring 50 - 200 µm over a 1 cm² area as shown in Figure 2.1 (e). These “microenhancer arrays” were etched from silicon wafers using lithography and potassium hydroxide etching.

Park et al. [10] fabricated biodegradable polymer based microneedles using integrated lens technique. They have fabricated different microneedles, some of them are PGA microneedles with base diameter of 250 µm, tip diameter of 10 µm and length 1500 µm, PLA microneedles with base diameter base diameter of 150 µm, tip diameter of 5 µm and length of 75 µm as shown in Figure 2.1 (f).

Shankar et al. [11] fabricated microneedles using electroplated metals like palladium, palladium-cobalt alloys and nickel as structural materials. The microneedles were 200 mm-2.0 cm in length with a cross-section of 70-200 µm in width and 75-120 µm in height, with a wall thickness of 30-35 µm. The microneedle arrays were typically
9.0 mm in width and 3.0 mm in height with between 3 and 17 needles per array as shown in Figure 2.1 (g)

Most of these microneedles were used for drug delivery or insulin delivery and fluid extraction for biological sampling. Most of the metallic and hollow polymeric microneedles had separate drug reservoirs either attached to them or separate or had drug coated on the top. The solid biodegradable polymeric microneedles had drug encapsulated inside the microneedles, so that they were released when the microneedle degrades.

Figure 2.1 (a), (b), (c), (d), (e), (f) and (g) Different microneedle designs [5], [6], [7], [8], [9], [10] and [11]
2.2 Poly(propylene fumarate) (PPF)

Many researchers have proposed different methods for synthesizing poly(propylene fumarate) (PPF). PPF with the combination of other materials was used as a biomaterial for different biomedical applications such as for the fabrication of the scaffolds, hydrogels, bone grafts and injectable carrier for cells. They have also tried to find the photocrosslinking characteristics and mechanical properties of these materials.

Fisher et al. [12] investigated the photocrosslinking of PPF to form porous scaffolds for bone tissue engineering applications. They have synthesized PPF using the two step transesterification process where propylene glycol and diethyl fumarate were considered as the initial products for the reaction. They have synthesized porous scaffolds using PPF with sodium chloride (NaCl) porogen at 70%, 80%, 90% (w/w) ratios before cross linking by porogen leaching technique and also found out their mechanical properties like elastic modulus and compressive strength at 1% yield. The strongest scaffold had elastic modulus of 2.3 ± 0.5 MPa and compressive strength of 0.11 ± 0.02 MPa.

Zhu et al. [13] tried to determine the effects of composite formulation on the compressive modulus and ultimate strength of a biodegradable, in situ PPF and bone fiber scaffold. They have synthesized PPF using a two-step reaction process but their main materials for synthesis were fumaryl chloride and propylene glycol, along with potassium carbonate and methylene chloride. They have tried to find out the mechanical properties by varying the incorporation of bone fiber, PPF molecular weight, N-vinyl pyrrolidinone (N-VP) crosslinker amount, benzoyl peroxide (BP) initiator amount and sodium chloride porogen amount.
Fisher et al. [14] investigated the photocrosslinking of PPF dissolved in its precursor diethyl fumarate (DEF), using the photoinitiator bis(2,4,6-trimethylbenzoyl) phenylphosphine oxide (BAPO) and low levels of the ultraviolet exposure. They have studied the effects of PPF number average molecular weight, BAPO initiator content, and DEF content upon photocrosslinking characteristics and mechanical properties. So that this material can be used as tissue engineered materials for the treatment of large bone defects by fabricating bone grafts.

Lee et al. [15] have enhanced the cell ingrowth and proliferation through crosslinked 3D nanocomposite scaffolds fabricated using PPF and hydroxyapatite (HA) nanoparticles. They have used solid free form (SFF) and NaCl leaching techniques to fabricate porous scaffolds. They have performed studies using MC3T3-E1 mouse preosteoblasts and cultured the scaffolds to access cell attachment, viability, ingrowth depth, and proliferation.

Lee et al. [16] have fabricated bone tissue engineering scaffolds using stereolithography technique. They have used PPF along with DEF and BAPO as crosslinker and photoinitiator, respectively. They have considered three different weight ratios of PPF/DEF and BAPO contents and tried to find out their viscosity, thermal properties of the un-crosslinked solutions and the mechanical properties of the formed scaffolds. Depending upon theses they tried to optimize the resin composition to satisfy these properties and tried to find out the laser parameters like critical exposure and penetration depth. The fabricated scaffolds were characterized by measuring mean pore size, external dimensions, compressive moduli and porosities.
Peter et al. [17] investigated the crosslinking characteristics of an injectable composite paste of PPF, N-VP, BP, NaCl, and β-tricalcium phosphate (β-TCP). They have examined the effects of PPF molecular weight, N-VP/PPF ratio, BP/PPF ratio, and NaCl weight percent on the crosslinking temperature, heat release upon crosslinking, gel point, and the composite compressive strength and modulus. The data they have obtained suggested that the injectable paste can be prepared with handling characteristics appropriate for clinical orthopedic applications and that the mechanical properties of the cured composites were suitable for trabecular bone replacement.

Kallukalam et al. [18] have prepared carboxy terminated – poly(propylene fumarate)-co-ethylene glycol) (CT-PPF-co-PEG) and set it into crosslinked hydrogel material with acrylamide. Their studies revealed that this copolymer can be used as an injectable material. They have found out that the hydrogel exhibits a higher degree of swelling, good mechanical strength and flexibility.

2.3 Microstereolithography

Many researchers have used different microstereolithography system from commercial machines to their own developed machines. They have also worked on calibrating the microstereolithography system in many ways mostly with respect to cure depth, exposure time and using different photoinitiators and resins at different ratios. These microstereolithography systems were used for different applications like fabrication of scaffolds, stents, and hydrogels.

Choi et al. [19] developed a microstereolithography system using Digital Micromirror Device (DMD™). The system was designed to have x-y resolution of ~2 μm and z resolution of ~1 μm. They synthesized PPF using Fisher et al. method and
fabricated 3D scaffolds using PPF/DEF (70:30) with 2% BAPO in their developed system. Their fabricated parts were viewed under SEM and micro-computed tomography which illustrated that the developed SL system was a promising technology for producing biodegradable and biocompatible 3D micro-scaffolds with fully interconnected pores.

Lan et al. [20] fabricated 3D PPF/DEF (70:30) with 1% BAPO scaffolds using microstereolithography and surface modification. They have coated the fabricated scaffolds with accelerated biomimetic apatite and RGD peptide to improve the surface characteristics without altering their bulk properties. Then they have seeded these scaffolds with MC3TC-E1 pre-osteoblasts and evaluated their biologic properties. They have found that these scaffolds can be potentially used in bone tissue engineering.

Arcaute [21] tried to explore the capabilities of stereolithography to photocrosslink poly(ethylene glycol) (PEG) hydrogels and create 3D scaffolds with applications in tissue engineering. She tried to fabricate peripheral nerve guidance conduits using stereolithography and found out the cell viability of human dermal fibroblasts in response to stereolithography parameters.

Lee [22] in his work presented development and application of novel 3D digital microfabrication technology, projection micro stereolithography, to engineer soft functional materials into reconfigurable active micro devices. One of the applications that he has fabricated in his work was vascular stents that were made from PEGDA hydrogels.

Lee et al. [23] fabricated nano/microscale 3D composite scaffold using PPF/DEF containing hydroxyapatite (HA) nanopowder. They have used microstereolithography process to fabricate these scaffolds instead of solid free form fabrication because of the
high resolution of the µSL. They have observed that the pores for their fabricated were well connected and the use of HA powder effectively generated nano/microscale morphology. The cell adhesion and proliferation to these scaffolds showed better. They finally suggested that the scaffolds containing HA powder may be applicable for bone tissue engineering.

2.4 Summary

It could be seen from these previous works, that microneedles were mostly fabricated using metals, silicon and in recent years polymers such as PGA, PLGA etc., PPF proved to be a good biodegradable and biocompatible material that was mostly used for scaffold and tissue engineering related fabrications, and microstereolithography is the upcoming technology overpowering MEMS based methods and is being used as fabrication method for biomedical related applications like scaffolds and stents.

It could be seen from these previous works that till now, no one has completely explored the fabrication of microneedles using microstereolithography which is an emerging fabrication technology in recent years, also PPF was not yet completely explored as a material to fabricate microneedles and PPF based microneedles containing drug have not yet been fabricated. The viscosity and mechanical properties of PPF/DEF with drug (BSA) has not yet been explored till now in the past years.

Therefore, in this work I tried to explore the fabrication of PPF/DEF based microneedles containing drug using microstereolithography system. Also, since the prepolymer used is a new material whose properties are not yet known, so I have found out the viscosity, cure depth and mechanical properties of the material which were used in this work and which will be used in future works.
CHAPTER III
MATERIALS AND METHODS

3.1 Poly(propylene fumarate)

Poly(propylene fumarate) (PPF) is a linear polyester based upon fumaric acid, which is a naturally occurring substance and a component of the tri-carboxylic acid cycle (Krebs cycle). The main advantages of PPF are (1) contains a repeating fumarate unit that is comprised of one carbon-carbon double bond that allow for crosslinking of the polymer into a covalent polymer network, and (2) the fumarate unit consists of two ester groups that allows PPF to degrade, via ester hydrolysis, into biocompatible and excretable degradation products, primarily fumaric acid, and propylene glycol which is commonly used diluent in drug formulations. The principle disadvantage of PPF is that it is a viscous liquid at room temperature (21°C), making handling of the polymer somewhat cumbersome [24].

Although crosslinked networks may be formed from PPF alone, a variety of crosslinking agents have been explored in combination with PPF for the formation of crosslinked degradable polymer networks with tunable material properties. For example, crosslinked networks of PPF with N-vinyl pyrrolidinone, poly(ethylene glycol)-dimethacrylate, PPF-diacrylate, and diethyl fumarate have been developed.
3.1.1 Synthesis of Poly(propylene fumarate)

A number of synthetic techniques for PPF have been reported, and each results in different polymer properties. For example, synthetic routes beginning with fumarates with highly reactive end groups, such as fumaryl chloride, have been described. However, these routes have been associated with significant byproducts formation. More recently, the common method for synthesizing PPF follows a two-step procedure, beginning with diethyl fumarate and propylene glycol, and involving bis(hydroxypropyl) fumarate as intermediate. This is the current method employed in this research work [24]. The two-step synthesis of PPF is as follows:

Step 1: In this step, diethyl fumarate (Sigma-Aldrich, St.Louis, OM, US) and propylene glycol (Sigma-Aldrich, St.Louis, OM, USA) were taken in a molar ratio 1:3, along with zinc chloride (Sigma-Aldrich, St.Louis, OM, USA) and hydroquinone (Sigma-Aldrich, St.Louis, OM, USA) which act as catalyst and crosslinking inhibitor, in a 0.01:0.002:1 molar ratio to diethyl fumarate in a round bottom flask. This step of the reaction takes place in a heated vessel under mechanical stirring using overhead stirrer (VWR, Randor, PA, USA) with gradual increase in temperature from 110 °C to 140 °C with continuous flow of nitrogen gas (Praxair, Danbury, CT, USA). This stage of the procedure results in the production of the bis(hydroxypropyl) fumarate intermediate and ethanol, which is collected as a distillate. This step of the reaction is terminated when approximately 90% of the theoretical yield of ethanol is collected. Figure 3.1 shows step 1 and Figure 3.2 shows the intermediate product, bis(hydroxypropyl) fumarate.

Step 2: This step involves transesterification of the bis(hydroxypropyl) fumarate intermediate to produce PPF. Here, the alkoxy group of bis(hydroxypropyl) fumarate was
replaced with an alcohol from a second bis(hydroxypropyl) fumarate intermediate, propagating PPF polymerization and producing propylene glycol as a byproduct. This stage of reaction is conducted under pressure and mechanical stirring, with a gradual increase in temperature from 100 °C to 140 °C. The reaction proceeds until the desired molecular weight of PPF is obtained. Figure 3.3 shows step 2. The two step reaction that takes place while performing this synthesis is shown in Figure 3.4. The PPF obtained after step 2 is shown in Figure 3.5.

Then purification of the PPF product occurs through dissolution of the polymer in methylene chloride followed by several diluted hydrochloric acid (Sigma-Aldrich, St. Louis, and OM, USA) washes to remove the zinc chloride catalyst. Further purification of the polymer solution involves two washes each with distilled water and brine solution. Sodium sulfate (Sigma-Aldrich, St. Louis, and OM, USA) is then used to dry the organic polymer phase. Finally, the solvents were removed from the PPF solution through rotary evaporation. Figure 3.6 (a), (b), (c) shows the purification process with acid, water and brine washes respectively. Figure 3.7 shows the rotary evaporation process. Figure 3.8 shows the process of synthesis of PPF in a flowchart view, so that a clear understanding of the process can be obtained at a glance. Appendix A gives a clear in depth details of the synthesis procedure.
Figure 3.1: Setup for step 1

Figure 3.2: Intermediate product bis(hydroxypropyl) fumarate
Figure 3.3: Setup for step 2

Figure 3.4: Chemical reaction during PPF synthesis [24]
Figure 3.5: PPF after step 2

Figure 3.6: Purification process with (a) acid wash (b) water wash, (c) and brine wash
Figure 3.7: Rotary evaporator

Figure 3.8: Schematic of the process of synthesis of PPF
3.1.2 Characterization of Poly(propylene fumarate)

The techniques employed for PPF characterization were Nuclear Magnetic Resonance (NMR), Gel Permeation Chromatography (GPC) and Rheometry. NMR was used to obtain the spectrum of PPF. GPC was used to determine the molecular weights of PPF. The rheometer was used to determine the viscosity of pure PPF at room temperature and also the effect of temperature change on viscosity.

3.1.2.1 NMR spectrum

The NMR was carried out with NMR machine (300MHz NMR, Varian, USA) as shown in Figure 3.9. A typical $^1$H NMR was run at ambient temperature. The sample was made by dissolving the PPF (approximately 0.6 gm) in CDCl$_3$ solvent (approximately 1.8 gm) so that a 5 cm height of sample was there in the NMR test tube. The NMR spectrum obtained was verified with the spectrum obtained by Fisher et al. [24] as shown in Figure 3.10, to know whether the obtained PPF is pure.

3.1.2.2 Measurement of molecular weight

GPC was carried out with GPC system (Tosoh Ecossec HLC-8320, Tosoh Bioscience LLC, and King of Prussia, PA, USA) as shown in Figure 3.11. Columns used in the GPC were Tosoh TSK gel Hz columns with packing material of crosslinked polystyrene. Chloroform (HPLC grade) was used as the eluent for the system. The whole modular system was thermo-regulated at 40 ºC. The flow rate of the eluent was 0.4 ml/minute and each samples run time was approximately 50 minutes. Based on the idea of the molecular weight of the sample a particular concentration was made using the HPLC grade chloroform solution. It was taken care not to agitate the solution so as to keep the sample intact (i.e. no broken chains). The sample was dissolved completely by
leaving it overnight on a shaker at low speeds. The dissolved sample was then injected into the GPC autosampler vials by filtering through a 0.45 µm PTFE filter. After this the sample was loaded into the machine and 20 µl was injected into the sample columns for analysis using both refractive index (RI) and ultraviolet (UV) modes of detection. Polystyrene standards were considered to analyze PPF’s molecular weights. EcoSEC analysis software provided by Tosoh was used for analysis and characterization of the GPC results (i.e. to obtain the molecular weight of the PPF).

3.1.2.3 Measurement of viscosity

The viscosity of uncured PPF was measured using a rheometer (ARES-G2, TA Instruments, and New Castle, DE, USA) as shown in Figure 3.12. Experiments were conducted at a 10 Pa shear stress and at ramp rate of 5 °C/min varying the temperature from 25 °C to 100 °C, to obtain the variation of viscosity with change in temperature.

3.2 HDDA prepolymer

HDDA prepolymer was used to verify the fabrication from microstereolithography system. In order to obtain the desired prepolymer, 1,6 hexandiol diacrylate (HDDA) (Sigma-Aldrich, St.Louis, OM, US) along with 2% (w/w) 2,2-dimethoxy-2-phenylacetophenone (DMPA) (Sigma-Aldrich, St.Louis, OM, US) and 0.1% Tinuvin 327 (Ciba, Timonium, MD, USA) were mixed using a vortex mixer ((VWR, Randor, PA, US), for 30 minutes at room temperature. DMPA was used as photoinitiator for the prepolymer to undergo curing when subjected to UV light. Tinuvin 327 was added to reduce the curing depth of the prepolymer solution. The reason for
choosing these materials was they have been proved to be better resin in terms of manufacturability in microstereolithography system [25].

Figure 3.9: $^1$H NMR machine

Figure 3.10: $^1$H NMR spectrum obtained by Fisher et al. [24]
Figure 3.11: GPC machine

Figure 3.12: Rheometer
3.3 PPF/DEF and PPF/DEF/BSA prepolymer

In order to prepare the PPF/DEF and PP/DEF/BSA prepolymer many other materials were used which were purchased. The details and reason for considering these materials is discussed below in detail.

3.3.1 Diethyl fumarate (DEF)

Diethyl fumarate is a clear liquid with a light yellow color. At higher molecular weights, PPF becomes quite viscous, inhibiting its handling properties and, by definition, markedly reducing its ability to flow. For PPF system to possess a significantly reduced viscosity, while still retaining the advantageous characteristics of fumarate based biomaterials, PPF polymer was dissolved within diethyl fumarate (DEF), the diester precursor from which PPF is synthesized. The reason for DEF being considered was it contains the crosslinkable carbon-carbon double bond as shown in Figure 3.13 that is present within PPF and thus should be able to participate in the crosslinking reaction. Furthermore, it was investigated previously that DEF does not significantly alter the biomaterial properties of the pure PPF material. To different ratios of DEF were considered in this work as they both when added to PPF were proved to have better properties [15]. The ratios of DEF that were considered are 30% and 50% (w/w) of PPF.

![Chemical structure of DEF](image)

Figure 3.13: Chemical structure of DEF [26]
3.3.2 Irgacure 819 (BAPO)

Irgacure 819 is a commercially available photoinitiator (BASF, Ludwigshafen, Germany). Irgacure 819 chemical name is bis(2,4,6-trimethylbenzoyl)-phenylphosphineoxide which is abbreviated as BAPO. It is a versatile photoinitiator for radical polymerization of unsaturated resins upon UV light exposure. It is sensitive to visible light and any exposure to sunlight should be avoided. The chemical structure of BAPO is shown in Figure 3.14 [27].

![Chemical structure of BAPO](image)

Figure 3.14: Chemical structure of BAPO [27]

BAPO can absorb light around the wavelength of 365 nm. Hence, it can be used in the system as the wavelength of the UV light in the system is 365 nm. The ratio of BAPO was considered as 2% (w/w) of PPF/DEF in this work with reference to previous research works [20].

3.3.3 Bovine serum albumin (BSA)

The application of the microneedles is to deliver drug. So to fabricate microneedles containing drug, a model protein drug has been considered. From literature
review on drug deliveries it was found that Bovine serum albumin (BSA) (Fischer Scientific, USA) is the mostly widely used drug for testing [28]. Bovine serum albumin (BSA) is a serum albumin protein derived from cows. It is often used as a protein concentration standard and BSA has numerous biochemical applications and it is also used as a nutrient in cell and microbial culture. It is used as a stabilizer for some enzymes during digestion of DNA and to prevent adhesion of the enzymes to reaction tubes, pipet tips and other vessels. This protein does not affect other enzymes that do not need it for stabilization [29]. BSA is a water soluble drug and coagulates when heated to higher temperatures at a faster pace when compared to heating at lower temperatures [30].

Initially in this work BSA ratios of 2.5%, 5% and 10% (w/w) of PPF/DEF prepolymer were considered for fabrication of microneedles with drug, as that was the most generally used percentage of drugs by other researchers [31], but the 10% didn’t dissolve in the polymer completely so we had to choose 1% instead of 10%. So finally, 1%, 2.5% and 5% (w/w) were chosen as the drug concentrations for the microneedle material.

3.3.4 Dimethyl sulfoxide (DMSO)

The biodegradable microneedle consists of a dissolved drug and biodegradable polymeric matrix in a biocompatible, water-miscible organic solvent. The key to subcutaneous sustained release of drugs is an organic solvent, which is required to be solvent for the water-insoluble matrix-forming polymer and the drug, but must also be safe in milliliter amounts with regard to both systemic toxicity and local tissue irritation. Solvents that have been identified to meet these criteria are N-methyl-2-pyrrolidone
(NMP), propylene glycol, acetone, dimethyl sulfoxide (DMSO), tetrahydrofuran, 2-pyrrolidone, and triacetin, with NMP and DMSO being the most used [32].

As PPF is not soluble in water, DMSO was considered, as BSA is soluble in DMSO when compared to NMP.

Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, and OM, USA) is an organosulfur compound which is a colorless liquid. It is miscible in wide range of organic solvents as well as water. It could penetrate the skin and other membranes without damaging them and could carry other compounds into a biological system. In medical research, DMSO is often used as a drug vehicle for in vivo and in vitro experiments. The chemical structure of DMSO is shown in Figure 3.15 [33].

![Chemical Structure of DMSO](image)

Figure 3.15: Chemical Structure of DMSO [33]

3.3.5 **PPF/DEF and PPF/DEF/BSA prepolymer preparation**

The viscosity of PPF measured from the rheometer was pretty high as seen previously, making it not easy to work with in SL system. Therefore, a diluent was needed to lower the viscosity of the solution without changing the desired biomaterial properties. As mentioned previously DEF was considered as the diluent. Two ratios of PPF/DEF were considered in this work, those were 70:30 (w/w) and 50:50 (w/w).

The PPF/DEF prepolymer was prepared by first mixing the DEF with 2% (w/w) of BAPO for about 2 hours on the vortex mixer and then adding this solution to PPF and
the solution was mixed for 15 minutes on the vortex mixer. BAPO was used as photoinitiator for the photocrosslinking of the prepolymer.

The PPF/DEF/BSA prepolymer was prepared by first making the drug solution and then adding it to the PPF/DEF prepolymer solution which was made as described previously. The drug solution was first made by taking the required amount of drug, which is 1%, 2.5% and 5% (w/w) of PPF/DEF prepolymer in centrifuge tubes and adding autoclaved water 6% (v/v) of PPF/DEF prepolymer solution. The drug – water solution is left aside for day or two depending on how much time the drug takes to dissolve completely in water. Once the drug is completely dissolved in water, then DMSO 4% (v/v) of PPF/DEF prepolymer was added to the drug - water solution. Figure 3.16 shows the PPF/DEF (50:50) prepolymer, PPF/DEF/BSA (50:50:1), PPF/DEF/BSA (50:50:2.5) and PPF/DEF/BSA (50:50:5) prepolymers.

The reason for choosing both water and DMSO is, BSA is more soluble in water when compared to DMSO but since PPF is not soluble we had to choose DMSO. So to maintain the solubility of both PPF and BSA, both water and DMSO were considered. The reason for choosing lower concentrations of water and DMSO is to allow the PPF to be soluble and nothing affects its biomaterial properties or its chemical structure. So 10% (v/v) total of DMSO and water were considered, keeping the ratio of water more than DMSO as BSA is soluble in water and at the same time not making the ratio of water more which makes PPF not soluble. Therefore it was observed that 6% (v/v) water and 4% (v/v) DMSO were reasonable ratios to allow all the materials to be soluble.
Microstereolithography

Microstereolithography (µSL) is a technology at the interface of the microengineering and rapid prototyping domains. It is based on the principle used in stereolithography, a rapid prototyping technology that has been widely spread in many industries and technological fields like automotive, aerospace and medical, pertaining to manufacturing of 3D prototype parts. The word “Microstereolithography” is now commonly accepted by almost every user and developer of this technology, many different names micro-photoforming, spatial forming, optical forming, 3D optical modeling, and microstereophotolithography have been used at the early stage of development of this technology, depending on variations in the design of the built apparatus.

This technique allows to build small-size, high resolution 3D objects, by superimposing a certain number of layers obtained by a light-induced and space-resolved
polymerization of a liquid resin into a solid polymer. As the resolution of the microstereolithography technique is far better than the one of rapid prototyping technologies, this technique is of particular interest in the microengineering domain where its 3D capability allows the production of components which no other microfabrication technique can create [34].

The microstereolithography processes are classified into two main categories, based on how each layer is build:

1. Vector-by-vector process: a layer is formed by raster scan of a laser beam. The laser beam is stationary and focused precisely to a very fine spot on the surface of the photopolymer. The container and the material are moved horizontally in order to build the layer.

2. Integral process: each layer is cured by one irradiation only. A dynamic mask/spatial light modulator are used. For this type of process, the slicing software gives output in images files (usually monochrome bitmap files); each file contain the cross sectional image of one single layer. These files are then used to drive the dynamic mask.

The integral microstereolithography process is much faster than the vector-by-vector process. By employing a dynamic mask, e.g. Liquid Crystal Device (LCD), this process presents a technical breakthrough in the field of rapid prototyping. As an emerging dynamic mask, the performance of Digital Micromirror Device (DMD) is better than that of the LCD, since when a pixel is in “active” or “on” state, the DMD can pass light more effectively than the LCD. DMD-based microstereolithography uses a single time exposure for each layer and can result in reduced fabrication times as compared to scanning based microstereolithography [35].
The principle behind microstereolithography process is photopolymerization. Polymerizations is the term used to describe the process of linking monomers (small molecules) into polymers (larger molecules) composed of many monomer units. When UV light impinges on μSL resin, the photoinitiator in the resin undergoes a chemical transformation and become reactive with the liquid monomers to form a polymer chain. Therefore, polymer chains with strong covalent bonds are formed with subsequent reactions and crosslinking [36].

Two main types of photopolymerization process are free radical photopolymerization and cationic photopolymerization. Figure shows the schematic of free radical photopolymerization. The mechanism of free radical photopolymerization as shown in Figure 3.17, starts with the formation of a radical, on an average for every two photons (from the UV light), one radical will be produced. The radical can easily lead to polymerization of over 1000 monomers as shown in the intermediate process called propagation. The polymerization can terminate either due to recombination, disproportionation or occlusion. Recombination occurs when two polymer chains merge by joining two radicals. Disproportionation involves essentially the cancelation of one radical by another, without joining. Occlusion occurs when free radical gets trapped within the solidified polymer, causing the reaction sites from reacting with other monomers or polymers by the limited mobility within the polymer networks even though they are available. Cationic photopolymerization mechanism is similar to free radical photopolymerization, instead of free radical being generated from UV light, a cation is generated which reacts with the monomer, propagates the reaction to generate a polymer and finally terminates the complete reaction [36].
The role of photoinitiator in SL resin is to convert the physical energy of the incident light into chemical energy in the form of reactive intermediates. The photoinitiator must exhibit a strong absorption at the UV emission wavelength and undergo a fast photolysis to generate the initiating species with great quantum yield. The reactive intermediates are either free radicals or cations which initiate photopolymerization as described previously [36].

3.4.1 Microstereolithography system

The microstereolithography system developed in this work is the dynamic mask projection microstereolithography system which consists of five major subsystems as shown in Figure 3.18, including:

1. The light emission system (a lamp, an optical fiber, and a collimating lens set):

   In this subsystem, a mercury lamp (OmniCureTM S2000, EXFO Co., Canada) with output of 200 W is utilized as a light source which emits light of 365 nm wavelength. An optical fiber (EXFO Co., Canada) was used to deliver the light from the lamp to a collimating lens set (EXFO Co., Canada), which consisted of two convex lenses that were used to project collimated light toward the DMD (DMD DiscoveryTM 1100 Controller Board and Starter Kit, Texas Instruments, USA).
2. Light delivery system (a prism, a tube lens and a reflecting mirror): In this subsystem, the LightGate (Unaxis Co., USA) which was utilized acts as a prism that projects incident light from the collimating lens onto the DMD and also the reflected light from the DMD onto the tube lens. The LightGate was specially coated for UV light. Collimation between the tube lens and the objective lens was maintained by utilizing a tube lens (Achromat doublet lens, Melles Griot Co., USA) with a focal length of 120 mm and diameter 40 mm. As per the reflected light from the DMD, the tube lens was positioned at its focal length from the DMD surface. The distance between the tube lens and the objective lens could be varied without defocusing, since the light was collimated. A reflecting mirror of 50.8 mm diameter (Newport Co., USA) was also used to steer the light toward the objective lens.

3. Pattern generation system (the DMD): In this subsystem, for 2D pattern generation a DMD was used, and the pattern that was used is a binary image that was created by slicing the model using the slicing software. The DMD consists of 1024 × 768 micromirrors, and each mirror is independently tilted according to the pixel information of the binary image. That is, each mirror, which is 13.68 µm along each side, is tilted ± 12° according to the black or white color of each pixel. A + 12° tilted mirror causes the incident light to be projected onto the surface of the resin (thus creating the pattern) through the light delivery subsystem, while a – 12° tilted mirror causes the incident light to be projected into a dummy direction.

4. Image focusing system (a modular focusing unit and an objective lens): In this subsystem, the focusing of the patterned light onto the resin surface is done by utilizing a modular focusing unit (IM-4, Nikon Co., Japan) with resolution of 1 µm, and objective
lens (CFI Plan Flour 4x, Nikon Co., USA) with focal length 20 mm and numerical aperture (N.A.) of 0.13. The objective lens is installed at its focal length from the resin surface by adjusting the modular focusing unit.

5. Build system (a Z-stage, platform and resin vat): In this subsystem, The stacking of cured thin layers, refreshing the resin surface and building of complex 3D microstructures was executed with the help of the Z-stage (ATS 100-050, Aerotech, USA) with a resolution of 100 nm, stainless steel platform and vat [25].

Figure 3.18:  Developed dynamic mask projection µSL system

In this current work, two different platforms and resin vats were used depending on the type of resin used for fabrication. The original platform and resin vat which other researchers have been using is used for verification of the system where HDDA prepolymer was used as the resin.
The original vat which was used needs more resin to be filled in for the fabrication. As PPF is very costly material in terms of labor and time, using a lot of material for fabrication is not a worthy method. Therefore, a new resin vat and platform are designed that were compatible with each other and with the rest of the system. The vat as shown in Figure 3.19 was designed taking into consideration the minimum amount of PPF it can hold, that is well enough for the fabrication of any part that is micro-sized. The platform as shown in Figure 3.20 was designed taking into consideration the resin vat size and the compatibility with system. The dimensions of the vat considered are inner diameter 0.63 inches and inner height of 0.394 inches, depending on this it can hold a volume of ~ 2 ml of liquid. The platform of the base on which the parts will be fabricated is a square with dimensions 5 × 5 mm.

Figure 3.19: Vat design
3.4.2 Overall fabrication process

The overall fabrication process involves many steps. Figure 3.21 shows the layout of the process.

The brief overview of individual steps involved in the overall fabrication process is:

1. Designing 3D model using SolidWorks (Dassault Systemes, Waltham, MA, US) as shown in Figure 3.22.

2. Converting the 3D model to STL model, so that it can be used in the slicing software as shown in Figure 3.23.

3. Creating the individual layers by slicing the STL model into thin sections as shown in Figure 3.24 using the reduction scale that calculated, the layer thickness that is required and the resolution of the DMD which is 1920 × 1080 pixel, which are saved as
bitmap images (a binary black and white format) that can be used to create pattern using the DMD as shown in Figure 3.25.

4. The light pattern reflected from the DMD was focused onto each layer of the resin surface for a certain exposure time to solidify.

5. The resin surface was refreshed for the next layer after the curing of one layer, by immersing the cured layer deeper into the resin vat, so that a fresh resin flows on top of the previously fabricated layer as shown in Figure 3.26.

The steps 4 and 5 were repeated as per the required number of layers for producing the 3D structure. The steps 4 and 5 were done automatically using software, developed from LabVIEW (National Instruments LabVIEW, National Instruments Co., Austin, TX, USA) as shown in Figure 3.27.
Figure 3.21: Overall fabrication process
Figure 3.22: 3D cube design

Figure 3.23: Conversion of 3D design to STL file
Figure 3.24: Slicing of the STL file imported to bitmap images

Figure 3.25: Bitmap image of one of the layers of the cube
Figure 3.26: Mechanism of refreshing the surface after fabrication of every layer
3.5 Calibration of the system and measurement of reduction scale

Once the above system has been installed, one has to know the working distance (i.e. the distance of the platform from the home position of the Z stage) and the light intensity at that position. The image focused onto the platform should have perfect feature as it is, that is shown in the DMD at that working distance and also high intensity of light. Therefore a beam profiler (BeamGage, Ophir Optronics Solutions Ltd., North Andover, MA, US) was used to measure the working distance and intensity of the focused square 800 × 800 pixel pattern as shown in Figure 3.28.
Before finding out the intensity in the system, the beam profiler needs to be calibrated, as it doesn’t know the intensity of light to which it is subjected to, and the values are shown in counts (cnts). To do this, firstly the power of the optical fiber at 100% intensity was found out using a radiometer (R2000, OmniCure, Lumen Dynamics Group Inc., Mississauga, ON, Canada). Then the value obtained from the radiometer which was 1.54 W was taken at 1% which was 15.4 mW and this value was entered in the beam profiler software when UV light at 1% from the optical fiber was exposed onto it. Once the beam profiler is calibrated then it can be used to calibrate the developed microstereolitography system. Once the position of Z-stage from its home position at which a perfect square is obtained its value cannot be exactly measured, so square parts as mentioned below were fabricated at positions near to the position obtained. In this case the Z stage position that was obtained when using the beam profiler was 1.5 mm, but the exact position is needed since the fabrication is in micron scale. Firstly, by moving the stage ± 0.1 mm up to 4 positions both sides, a square of 800 × 800 pixels was built using HDDA prepolymer for each position. The built square that had higher accuracy with the designed one was selected and its position was considered. The same procedure is done by moving the stage ± 0.01 mm up to 4 positions both sides and then moving stage ± 0.001 mm up to 4 positions both sides, then again the perfect built square position was considered. The reason for moving only up to ± 0.001 mm is, the maximum accuracy of the stage was 0.001 mm, so this procedure was stopped once a perfect square was built. This position was considered as the starting position for any fabrication. The distance of the Z stage at this position from the objective lens gives the focal plane of the objective lens, where the parts are built with accuracy.
A square of 800 × 800 pixels was built using HDDA prepolymer and its dimensions were measured using a stereomicroscope (SteREO Discovery.V12, Carl Zeiss Microscopy, LLC, United States). The measured dimensions were used to find out the reduction scale in the system using the Equation 1, where the average length and pixel size are considered for the same side of the square. As this reduction scale will be required when slicing a 3D model to bitmap images that can be used in the DMD as pattern.

\[ \text{Reduction Scale} = \frac{\text{average length}}{\text{pixel size}} \]  \quad \text{Eq. (1)}

![800 × 800 pixel square](image)

Figure 3.28: 800 × 800 pixel square

### 3.6 Measurement of viscosity of PPF/DEF and PPF/DEF/BSA prepolymer

The viscosities of uncured PPF/DEF prepolymer (70:30 and 50:50 (w/w)) and PPF/DEF/BSA prepolymer (70:30:1, 70:30:2.5, 70:30:5, 50:50:1, 50:50:2.5 and 50:50:5 (w/w/w)) were measured using a rheometer as shown in Figure 3.29. Experiments were conducted at a 10 Pa shear stress and at ramp rate of 5 °C/min, where viscosities were measured with change in temperature. The temperature was varied from 25 °C to 100 °C. It was observed that the viscosities of prepolymer with PPF/DEF and PPF/DEF/BSA (70:30 (w/w), 70:30:1, 70:30:2.5, 70:30:5 (w/w/w)) were high when compared to
PPF/DEF/BSA (50:50 (w/w), 50:50:1, 50:50:2.5 and 50:50:5 (w/w)). Therefore, all experiments were conducted using PPF/DEF and PPF/DEF/BSA (50:50 (w/w), 50:50:1, 50:50:2.5 and 50:50:5 (w/w)).

Figure 3.29: Rheometer

3.7 Cure depth experiment

The depths of penetration of light and critical energy at photoinitiation are important parameters that are needed to be controlled for 3D micro-fabrication. Polymerization takes place when the energy to which the resin is exposed is greater than the critical energy or else no polymerization takes place.

To examine the penetration depth and critical energy, curing depth experiments were conducted using the PPF/DEF prepolymer (50:50 (w/w)), PPF/DEF prepolymer (50:50 (w/w)) with 1% BSA, PPF/DEF prepolymer (50:50 (w/w)) with 2.5% BSA and
PPF/DEF prepolymer (50:50 (w/w)) with 5% BSA, as that will be used as the material for fabrication of microneedles. The energy delivered on the solution surface ($E_{\text{max}}$) penetrates into the solution. The energy inside the solution at the depth ‘z’ ($E(z)$) is defined by Beer-Lambert law as described Equation 2, where $D_p$ is the penetration depth of the solution. By introducing the critical energy ($E_c$) into Equation 2, the curing depth ($C_d$) can be defined as in Equation 3, where $E_c$ is the energy at the gel point. The gel point is the point at which solidification begins. Therefore, two important characteristics of the photocurable solution are $E_c$ and $D_p$. These can be experimentally determined through measuring the curing depth according to the exposure energy from the determined values of $E_c$ and $D_p$, where the exposure energy and stacking thickness can be chosen. In addition, if the curing depth is small, fabrication of down-facing parts and complex structures is better [37]. To conduct the curing depth experiment, the curing model as shown in Figure 3.30 was used.

\[
E(z) = E_{\text{max}} \cdot e^{(-z/D_p)} \quad \text{Eq. (2)}
\]

\[
C_d = z(E_{\text{max}}) = D_p \cdot \ln\left(\frac{E_{\text{max}}}{E_c}\right) \quad \text{Eq. (3)}
\]

The curing model consists of four posts each of 1 mm and four crossbeams. The posts were stacked with 10 layers with layer thickness of 100 µm using 30 second’s exposure time for each layer. The crossbeams were fabricated in the last layer with the given exposure energy. The irradiance in the developed µSL was 31.37 mW/cm², and the exposure energy was controlled by opening the shutter for 1, 2, 3, 4, and 5 seconds. In case they were no crossbeams formed for any particular exposure time then, the exposure
time is increased, as 5 points of exposure time are required to plot the graphs. Once the model is fabricated it was detached from the system and rinsed with isopropyl alcohol (Sigma-Aldrich, St. Louis, and OM, US) and air dried. The thickness of the crossbeams were measured at the center using a stereomicroscope (SteREO Discovery.V12, Carl Zeiss Microscopy, LLC, United States) along with its software, as shown in Figure 3.31. Two cure depth models were fabricated for each exposure time (n = 2). The curing depth graph was plotted taking the crossbeams thickness at the center for each exposure time (taken in natural logarithmic scale), where the reported values were the mean values and the associated errors were the standard deviations.

Figure 3.30: Cure depth model
3.8 Design of microneedle arrays

The microneedles were designed using 3D CAD software, SolidWorks. The dimensions and shape of the microneedles was chosen depending on skin anatomy and past literature as discussed previously. Two different microneedle features were chosen, one with circle base design and cone top and the other with a flower base design and cone top. The dimensions of the microneedles with overall height was 1000 µm (700 µm base height and 300 µm the cone height) and tip diameter was 20 µm for both the designs, whereas the base diameter for circle base design was 200 µm and for flower base design was 260 µm. The surface area of the circle base microneedle was 0.58 mm² and that of flower base design microneedle was 1.16 mm², but the volume for both of them was maintained same which was 0.03 mm³. In this work four different arrays of microneedle
patches were considered. A 3 × 3 and 5 × 5 circle base and flower base microneedles were designed as shown in Figure 3.32.

Figure 3.32: Design of microneedle arrays (a) 3 × 3 circle base, (b) 3 × 3 flower base, (c) 5 × 5 circle base and (d) 5 × 5 flower base

3.9 Verification of the microstereolithography system using HDDA prepolymer for fabrication of 2D and 3D microstructures

The developed microstereolithography system was verified to know whether the system was capable of fabricating the desired features. To fabricate the desired parts, HDDA prepolymer was used. Firstly, the microstereolithography system with the original big vat and platform was used to fabricate 2D and 3D structures. A 2D square of 2 × 2
mm and circle of 2 mm diameter were designed to be built in the system. Then a 3D cylinder, cube and letter ‘UA’ models were designed to be built in the system.

The vat and platform were changed as mentioned previously, so that the developed system can be used for fabrication of parts using PPF material. Therefore it has to be verified whether this system is capable of producing the parts as they were designed. To verify this system the four different arrays of microneedles that were designed previously were fabricated using HDDA prepolymer. Appendix B gives details regarding the parameters and procedure for the above fabrication process for all parts.

Once the parts were fabricated they were taken out from the system and rinsed with isopropyl alcohol and then dried out by blowing air using hand blower. All the above fabricated parts were either photographed or viewed in a stereomicroscope to verify whether the system was capable to fabricate the parts as they were designed.

### 3.10 Fabrication and Characterization of PPF and PPF containing BSA microneedle arrays using microstereolithography system

Four different microneedle arrays were built using the PPF/DEF (50:50) (w/w) prepolymer, to observe whether the system was able to fabricate the parts as they were designed. Then, again four different microneedles were built for PPF/DEF/BSA (50:50:1) (w/w/w) prepolymer. Similarly, four different microneedles were built for PPF/DEF/BSA (50:50:2.5) (w/w/w) prepolymer and PPF/DEF/BSA (50:50:5) (w/w/w) prepolymer respectively.

Appendix C gives details regarding the parameters and procedure for the above fabrication process for all parts. Once the parts were fabricated they were taken out from the system and rinsed with isopropyl alcohol and then dried out by blowing air using
hand blower. All the fabricated parts were characterized using a Scanning Electron Microscope (SEM) (JSM-5310, JOEL, Tokyo, Japan) as shown in Figure 3.33 and a stereomicroscope to verify whether the parts fabricated meets the design requirements. All the images were taken at 50x magnification.

The dimensional difference between the CAD model and the fabricated microneedles was calculated for the microneedles in an array for all the cases (i.e. four designs for four material) using the stereomicroscope. The dimensional differences were measured for the overall height, height of the cone, tip diameter and base diameter of the microneedles. The differences calculated were mentioned in percentage using Equation 4, 5, 6 and 7, where $OH_{FM}$, $CH_{FM}$, $TD_{FM}$ and $BD_{FM}$ are overall height, height of the cone, tip diameter and base diameter of the fabricated microneedles respectively and $OH_{CM}$, $CH_{CM}$, $TD_{CM}$ and $BD_{CM}$ are overall height, height of the cone, tip diameter and base diameter CAD designed microneedles respectively.

$$\text{Difference in overall height (\%)} = \frac{OH_{FM} - OH_{CM}}{100} \quad \text{Eq (4)}$$

$$\text{Difference in cone height (\%)} = \frac{CH_{FM} - CH_{CM}}{100} \quad \text{Eq (5)}$$

$$\text{Difference in tip diameter (\%)} = \frac{TD_{FM} - TD_{CM}}{100} \quad \text{Eq (6)}$$

$$\text{Difference in base diameter (\%)} = \frac{BD_{FM} - BD_{CM}}{100} \quad \text{Eq (7)}$$
3.11 Mechanical testing

Compressive testing of PPF/DEF prepolymer and PPF/DEF/BSA prepolymer specimens was conducted using a mechanical testing system (UTS 5582, INSTRON, Norwood, MA, US) as shown in Figure 3.34.

Cylindrical samples were synthesized by pouring the prepolymer solutions into a cylindrical glass vial (12.5 mm × 30 mm) and curing them using UV light for 30 minutes at 25 mm distance. The UV light is emitted from UV lamp to which four fibers were fixed, which were placed radially to the cylindrical at 25 mm distance to obtain an intensity of approximately 46.04 mW/cm², when measured with the beam profiler as shown in Figure 3.35.

The cylindrical samples were then surface finished to the desired length using a 1 inch belt sander. ASTM D695-10 standards were considered for the whole compression testing specimen dimensions and procedure [38]. The samples approximately had a
length of 25.41 mm and diameter of 12.39 mm when measured with a vernier caliper as shown in Figure 3.36 (a) and (b).

The samples were placed between the solid platens as shown in Figure 3.37 and compressed at a rate of 1 mm/min while compressive force and compressive extension were monitored throughout the experiment. The experiment was halted after sample fractured. The compressive load and compressive extension were obtained from the experiment, depending on these values and the specimen dimensions. The compressive stress was calculated by dividing the compressive load by the cross sectional area of the specimen. The compressive strain was calculated dividing the compressive extension by the original length of the specimen. The stress-strain curves were plotted using the compressive stress and compressive strain values for each specimen. The initial slope of the stress-strain curve determined the elastic modulus of the material. The stress at fracture which is the highest peak in the stress-strain curve was considered as ultimate compressive strength of the material. For each prepolymer combination, five samples were tested under the above conditions (n = 5), where the reported values were the mean values and their associated errors were the standard deviations.
Figure 3.34: Instron Machine

Figure 3.35: Curing of the prepolymer to make compression test specimen
Figure 3.36: Specimen dimensions (a) length and (b) diameter

Figure 3.37: Specimen placed between the solid platens of Instron machine
CHAPTER IV
RESULTS AND DISCUSSIONS

4.1 Synthesis of Poly(propylene fumarate)

Poly(propylene fumarate) (PPF) was finally synthesized using the previously mentioned synthesis procedure. The amount of PPF that was obtained was approximately 35 gm. It was observed that a lot of PPF was lost during the purification process. This could probably be due to lower molecular weight PPF not settling down and getting separated from the liquids used to purify it. Therefore, more care should be taken while separating the PPF from the other solutions and more settling time should be given for the phases of PPF and the purifying solution to separate.

4.2 Characterization of Poly(propylene fumarate)

4.2.1 NMR Spectrum

The $^1$H NMR spectrum obtained from the NMR experiment shows that the synthesized PPF was pure without any impurities and was same as that obtained by other researchers as mentioned previously. Figure 4.1 shows the $^1$H NMR spectrum obtained for the synthesized PPF.

4.2.2 Measurement of molecular weight

The number average molecular weight ($M_n$) and weight average molecular weight ($M_w$) of the synthesized PPF obtained from GPC using RI method was 1,183 Da and 1,268 Da respectively.
The number average molecular weight ($M_n$) and weight average molecular weight ($M_w$) of the synthesized PPF obtained from GPC using UV method was 1,510 Da and 1,592 Da respectively.

It was observed that the molecular weight of PPF was almost the same in both the methods, which means both the method gave the molecular weight of PPF accurately. Therefore, for finding the molecular weights of PPF either of them can be used.

4.2.3 Measurement of viscosity

It was observed that the viscosity of PPF at room temperature was 8406 cP which was very high, which makes it robust to work with in µSL as shown in Figure 4.2. It was also observed that the viscosity of PPF reduced with increase in temperature. The viscosity at 100 °C was observed to be 101.8 cP, even though the viscosity reduced, PPF cannot be used in µSL system as overtime it gets cured because of heating and also it may affect the fabrication by distorting the fabricated parts during the fabrication process.

![Figure 4.1: ¹H NMR spectrum of synthesized PPF](image)
Figure 4.2: Variation of viscosity with temperature for pure PPF
4.3 Calibration of the system and measurement of reduction scale

It was found that at a distance of 1.511 mm from the home position of Z stage the image of the square pattern in beam profiler looked perfect and had high intensity of light. Figure 4.3 and Figure 4.4 show the image of the square and the intensity profile obtained from beam profiler, where the intensity is relatively uniform over the entire surface. The peak irradiance was 31.37 mW/cm², which was strong enough to cure the photopolymer resins.

The 800 x 800 pixel square was built using HDDA prepolymer as shown in Figure 4.5 and its dimensions were measured as mentioned previously. The measured dimensions as shown in Table 4.1 were used to find out the reduction scale in the system.

Using Equation 1, the reduction scale was calculated where the direction considered over here was horizontal. So the average length of the square is 2413.434 µm from the Table 4.1 and the pixel size of the square is 800 as 800 × 800 pixel square was considered. Therefore using these values the reduction scale obtained was 3.0167 µm/pixel. This reduction scale was used for slicing the STL file of 3D model to bitmap images that were used as patterns in the DMD for fabrication.
Figure 4.3: Image of the square in the beam profiler

Figure 4.4: Intensity of the square in the beam profiler
Figure 4.5: 800 × 800 square fabricated at Z= 1.511 mm

Table 4.1: Measured dimensions of fabricated square

<table>
<thead>
<tr>
<th>Measurement No.</th>
<th>Horizontal Direction (µm)</th>
<th>Vertical Direction (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2410.86</td>
<td>2409.43</td>
</tr>
<tr>
<td>2.</td>
<td>2415.15</td>
<td>2409.45</td>
</tr>
<tr>
<td>3.</td>
<td>2415.15</td>
<td>2415.15</td>
</tr>
<tr>
<td>4.</td>
<td>2415.15</td>
<td>2415.15</td>
</tr>
<tr>
<td>5.</td>
<td>2410.86</td>
<td>2415.15</td>
</tr>
<tr>
<td>Average =</td>
<td>2413.434</td>
<td>2412.866</td>
</tr>
</tbody>
</table>

4.4 Measurement of viscosity of PPF/DEF and PPF/DEF/BSA prepolymer

The values of viscosities obtained from the rheometer for a temperature range of 25 °C to 100 °C for all the samples where plotted as shown in Figure 4.6 to Figure 4.13. Since the viscosity of PPF was very high to work with in SL as seen previously, therefore, DEF was added to PPF to reduce its viscosity. The ratio of PPF/DEF 70:30 and 50:50 (w/w) was considered. The viscosity of PPF/DEF (70:30) at room temperature was 240.2 cP, which was approximately 34 times lesser than the viscosity of PPF. Even though its
viscosity is much low compared to PPF still working with it in μSL would be tough as μSL works well with very low viscosities, at viscosities less than approximately 200 cP. The viscosity of PPF/DEF (50:50) at room temperature was 44.34 cP which was approximately 190 times lesser than the viscosity of PPF and 6 times lesser than the viscosity of PPF/DEF (70:70), which is a pretty reasonably low viscosity to work with in μSL. Figure 4.6 and Figure 4.7 shows the graph of variation of viscosity with temperature of PPF/DEF (70:30) and PPF/DEF (50:50) respectively.

It was observed that the viscosities of PPF/DEF (70:30) containing 1%, 2.5% and 5% (w/w) BSA was low compared to just PPF/DEF (70:30). The viscosity of PPF/DEF (70:30) with 1%, 2.5% and 5% BSA at room temperature was 164.6 cP, 183.6 cP and 224 cP respectively. The viscosity of PPF/DEF (70:30) with 1% BSA has the lowest viscosity when compared to PPF/DEF (70:30) with 2.5% and 5% BSA. Figure 4.8, Figure 4.9 and Figure 4.10 shows the graph of variation of viscosity with temperature of PPF/DEF (70:30) with 1%, 2.5% and 5% BSA, respectively.

Similarly, the viscosity of PPF/DEF (50:50) containing 1%, 2.5% and 5% (w/w) BSA was low compared to just PPF/DEF (50:50). The viscosity of PPF/DEF (70:30) with 1%, 2.5% and 5% BSA at room temperature was 39.27 cP, 40.22 cP and 42.1 cP respectively. The viscosity of PPF/DEF (70:30) with 1% BSA has the lowest viscosity when compared to PPF/DEF (70:30) with 2.5% and 5% BSA. Figure 4.11, Figure 4.12 and Figure 4.13 shows the graph of variation of viscosity with temperature of PPF/DEF (50:50) with 1%, 2.5% and 5% BSA, respectively.

It was observed that with addition of drug the viscosity of PPF/DEF (70:30) reduced to (50:50). This could possibly be due to the autoclaved water and DMSO that
were added with the drug to the PPF/DEF solution. As DMSO and water are liquid in nature so they might have reduced the viscosity as they are less viscous. Even though this reduced the viscosity, increasing the drug content in the solution increased the viscosity of PPF/DEF solution. Increasing the drug content, it was observed that the solution become more dense or thick in nature as the drug is in powder form and when added to DMSO and water it became a thick paste if the drug content was more when compared to the DMSO and liquid content as they were constant for all drug content ratios. Even though there is change in viscosities with addition of drug the change was very minor, this could probably be because of the ratio of DMSO, water and BSA content in the prepolymer solution being very small compared to PPD/DEF content.

It was observed that increasing the temperature decreased the viscosity of PPF/DEF (70:30) and (50:50) and PPF/DEF (70:30) and (50:50) with 1%, 2.5% and 5% BSA. Even though increasing temperature decreased the viscosity PPF/DEF containing BSA, but heating it above 50 °C or more, may cause it to form hydrophobic aggregates which do not revert to monomers upon cooling overtime and sometimes even at lower temperatures this may happen but at slower rate.

It was also observed that heating the material caused it to cure overtime as the BAPO in the PPF/DEF is a photoinitiator that can cure also to visible light and heating overtime. Therefore, for further experiments PPF/DEF (50:50) was chosen as it has lower viscosity at room temperature compared to PPF and PPF/DEF (70:30) which was easy to work with in μSL without heating. So it was advisable not to heat the material while using in SL.
Figure 4.6: Variation of viscosity with temperature of PPF/DEF (70:30)

Figure 4.7: Variation of viscosity with temperature of PPF/DEF (70:30)
Figure 4.8: Variation of viscosity with temperature of PPF/DEF (70:30) with 1% BSA

Figure 4.9: Variation of viscosity with temperature of PPF/DEF (70:30) with 2.5% BSA
Figure 4.10: Variation of viscosity with temperature of PPF/DEF (70:30) with 5% BSA

Figure 4.11: Variation of viscosity with temperature of PPF/DEF (50:50) with 1% BSA
Figure 4.12: Variation of viscosity with temperature of PPF/DEF (50:50) with 2.5% BSA.

Figure 4.13: Variation of viscosity with temperature of PPF/DEF (50:50) with 5% BSA.
4.5 Cure Depth Experiment

The cure model was fabricated as shown in Figure 4.14, using specified exposure times. The thickness of the crossbeams for the different exposure times were measured at the center and its mean value along with standard deviation was considered to plot the curing curve for PPF/DEF (50:50) with no BSA, 1% BSA, 2.5% BSA and 5% BSA.

The crossbeams for exposure times 1, 2, 3, 4 and 5 seconds for PPF/DEF (50:50) with no BSA are shown in Figure 4.15 and Figure 4.19 represents its cure depth versus exposure energy plot.

The crossbeams for exposure times 3, 4, 5, 6 and 7 seconds for PPF/DEF (50:50) with 1% BSA are shown in Figure 4.16 and Figure 4.20 represents its cure depth versus exposure energy plot.

The crossbeams for exposure times 3, 4, 5, 6 and 7 seconds for PPF/DEF (50:50) with 2.5% BSA are shown in Figure 4.17 and Figure 4.21 represents its cure depth versus exposure energy plot.

The crossbeams for exposure times 3, 4, 5, 6 and 7 seconds for PPF/DEF (50:50) with 5% BSA are shown in Figure 4.18 and Figure 4.22 represents its cure depth versus exposure energy plot. Table 4.2 shows all the critical energy (E_c) and penetration depth (D_p) values for PPF/DEF (50:50) with different BSA ratios.
Table 4.2: $E_c$ and $D_p$ values for PPF/DEF (50:50) with different BSA ratios

<table>
<thead>
<tr>
<th>PPF/DEF (50:50) + BSA</th>
<th>Critical Energy ($E_c$) (mJ/cm$^2$)</th>
<th>Penetration Depth ($D_p$) (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO BSA</td>
<td>19.5</td>
<td>146.51</td>
</tr>
<tr>
<td>1% BSA</td>
<td>56.63</td>
<td>247.66</td>
</tr>
<tr>
<td>2.5% BSA</td>
<td>63.61</td>
<td>260.96</td>
</tr>
<tr>
<td>5% BSA</td>
<td>65.66</td>
<td>264.86</td>
</tr>
</tbody>
</table>

Figure 4.14: 3D view of curing model built in the system
Figure 4.15: Front view of curing model for PPF/DEF (50:50) with no BSA built in the system showing the crossbeams for measurement of the curing depth that were the crossbeams were built with different exposure times (a) 1 second, (b) 2 seconds, (c) 3 seconds, (d) 4 seconds and (e) 5 seconds.
Figure 4.16: Front view of curing model for PPF/DEF (50:50) with 1% BSA built in the system showing the crossbeams for measurement of the curing depth that were the crossbeams were built with different exposure times (a) 3 second, (b) 4 seconds, (c) 5 seconds, (d) 6 seconds and (e) 7 seconds.
Figure 4.17: Front view of curing model for PPF/DEF (50:50) with 2.5% BSA built in the system showing the crossbeams for measurement of the curing depth that were the crossbeams were built with different exposure times (a) 3 second, (b) 4 seconds, (c) 5 seconds, (d) 6 seconds and (e) 7 seconds.
Figure 4.18: Front view of curing model for PPF/DEF (50:50) with 5% BSA built in the system showing the crossbeams for measurement of the curing depth that were the crossbeams were built with different exposure times (a) 3 second, (b) 4 seconds, (c) 5 seconds, (d) 6 seconds and (e) 7 seconds.
Figure 4.19: Cure depth versus exposure energy plot for PPF/DEF (50:50) – no BSA

Figure 4.20: Cure depth versus exposure energy plot for PPF/DEF (50:50) – 1% BSA
Figure 4.21: Cure depth versus exposure energy plot for PPF/DEF (50:50) – 2.5% BSA

Figure 4.22: Cure depth versus exposure energy plot for PPF/DEF (50:50) – 5% BSA
4.6 Verification of the microstereolithography system using HDDA prepolymer for fabrication of 2D and 3D microstructures

The 2D and 3D models that were designed have been built successfully. The 2D fabricated parts were observed under a microscope and measured. It was observed that the fabricated 2D parts meet the design requirements. Figure 4.23 shows the fabricated 2D circle and square. The fabricated 3D parts were observed by microscope and were captured using a photography camera (Nikon D5100, Tokyo, Japan). Figure 4.24 shows the fabricated 3D cylinder, cube and letters ‘UA’. It could be seen from the fabricated 2D and 3D parts that the developed microstereolithography system using the big vat and its platform can be used to build parts as per design requirements.

The microneedle arrays were fabricated successfully using the developed microstereolithography system with the small vat and its platform that were specifically designed for this work. It was observed that the microneedles that were built using HDDA prepolymer were the same as the designed models. The microneedle arrays were viewed under a microscope for capturing their features and measurements. Figure 4.25 and Figure 4.26 show the front view and 3D view of microneedle arrays that were built.

It could be observed from these built parts that the developed microstereolithography system that was changed for fabrication of parts using the small vat and its platform do fabricate parts as per design like the original microstereolithography system with the big vat and its platform.
Figure 4.23: 2D fabricated parts (a) circle and (b) square

Figure 4.24: 3D fabricated parts (a) cylinder, (b) cube and (c) letters ‘UA’
Figure 4.25: Front view of HDDA based microneedles (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
Figure 4.26: 3D view of HDDA based microneedles (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design (insert: the flower shape base design), (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design

4.7 Fabrication of PPF and PPF containing BSA microneedle arrays using microstereolithography system

The microneedle arrays that were designed have been fabricated using the developed microstereolithography system using the synthesized PPF/DEF and PPF/DEF along with 1%, 2.5% and 5% BSA. The fabricated microneedle arrays were viewed under the optical microscope to verify their feature and dimensions meet the design. For a clear 3D view these arrays were viewed in a scanning electron microscope (SEM). The scaling
shown in the SEM images is 500 µm and that in microscope images is 100 µm. It was observed that some of the microneedles edges in the arrays look shiny and wavy. This could possibly be due to either the uncured material that is still left on the surface of the microneedle seven after rinsing with isopropyl alcohol or the isopropyl alcohol that was used, which might not have completely dried out. It was observed that some of the features in the arrays like substrate or corner microneedles looked a bit eroded or broken, this could possibly be due to the handling of the array while detaching from platform and attaching to glass slides or metal discs for viewing in the optical microscope or in SEM. It was observed that the PPF/DEF microneedle arrays without drug had better features that were same as the design than PPF/DEF microneedles with drug (BSA) as shown in Figure 4.27 and Figure 4.28.

The microneedle arrays built from PPF/DEF containing 1%, 2.5% and 5% BSA were almost the same as the original design and microneedles built from PPF/DEF without drug as shown in Figure 4.29, Figure 4.30, Figure 4.31, Figure 4.32, Figure 4.33 and Figure 4.34 respectively. These microneedles could not meet the design requirements of the tip diameter mainly when compared to other dimensions like height of the needle and base diameter. It can be seen that the tip diameter was large compared to the given tip diameter by approximately 1.5 times. The reason for larger tip diameter could possibly be that the last few layers were not built properly or the layer thickness that was chosen might have been high to cure small sized features. When compared to HDDA microneedles, these microneedles tips were not sharp and even in some cases the flower shape base design was not clearly visible. The possible reason could be using of Tinuvin 327 in the HDDA prepolymer which controls the curing depth for the resin and therefore
we obtained better features even though they were very small and complex. Tinuvin 327 was not added to PPF/DEF and PPF/DEF with 1%, 2.5% and 5% BSA microneedles because these microneedles will be used for drug delivery in future, so they will be in contact with human body from outside and inside, so they shouldn’t be toxic, but Tinuvin 327 is toxic in nature, therefore it was not used. Possibly in future any material that is not toxic and that can control the cure depth can be used with the PPF/DEF and PPF/DEF with 1%, 2.5% and 5% BSA material to build better featured and perfect as designed microneedles. The % difference in overall height, cone height, tip diameter and base diameter of the microneedles were calculated as mentioned previously for all the four different microneedle arrays for PPF/DEF (50:50) with no BSA, 1%, 2.5% and 5% BSA as shown in Table 4.3.

Table 4.3: Difference in dimensions (%) of the microneedles

<table>
<thead>
<tr>
<th>PPF/DEF (50:50) + BSA</th>
<th>Type of microneedle array (circle/flower)</th>
<th>Difference in overall height (%)</th>
<th>Difference in cone height (%)</th>
<th>Difference in tip diameter (%)</th>
<th>Difference in base diameter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO BSA</td>
<td>3_3 circle base</td>
<td>6.52</td>
<td>13.12</td>
<td>(-) 8.25</td>
<td>(-) 10.48</td>
</tr>
<tr>
<td></td>
<td>3_3 flower base</td>
<td>8.13</td>
<td>16.53</td>
<td>(-) 62.8</td>
<td>(-) 9.43</td>
</tr>
<tr>
<td></td>
<td>5_5 circle base</td>
<td>6.37</td>
<td>14.41</td>
<td>(-) 52.95</td>
<td>(-) 15.88</td>
</tr>
<tr>
<td></td>
<td>5_5 flower base</td>
<td>9.09</td>
<td>18.57</td>
<td>(-) 60.12</td>
<td>(-) 14.23</td>
</tr>
</tbody>
</table>
Table 4.3: Difference in dimensions (%) of the microneedles (continued)

<table>
<thead>
<tr>
<th>PPF/DEF (50:50) + BSA</th>
<th>Type of microneedle array (circle/flower)</th>
<th>Difference in overall height (%)</th>
<th>Difference in cone height (%)</th>
<th>Difference in tip diameter (%)</th>
<th>Difference in base diameter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% BSA</td>
<td>3_3 circle base</td>
<td>3.84</td>
<td>7.59</td>
<td>(-) 24.65</td>
<td>(-) 11.46</td>
</tr>
<tr>
<td></td>
<td>3_3 flower base</td>
<td>3.84</td>
<td>13.63</td>
<td>(-) 65.35</td>
<td>(-) 9.34</td>
</tr>
<tr>
<td></td>
<td>5_5 circle base</td>
<td>5.861</td>
<td>13.63</td>
<td>(-) 86.05</td>
<td>(-) 14.88</td>
</tr>
<tr>
<td></td>
<td>5_5 flower base</td>
<td>9.49</td>
<td>15.42</td>
<td>(-) 86.45</td>
<td>(-) 9.96154</td>
</tr>
<tr>
<td>2.5% BSA</td>
<td>3_3 circle base</td>
<td>1.74</td>
<td>6.02</td>
<td>(-) 43.75</td>
<td>(-) 21.465</td>
</tr>
<tr>
<td></td>
<td>3_3 flower base</td>
<td>6.068</td>
<td>14.45</td>
<td>(-) 136.25</td>
<td>(-) 10.55</td>
</tr>
<tr>
<td></td>
<td>5_5 circle base</td>
<td>9.04</td>
<td>16.17</td>
<td>(-) 106.55</td>
<td>(-) 23.49</td>
</tr>
<tr>
<td></td>
<td>5_5 flower base</td>
<td>9.65</td>
<td>17.47</td>
<td>(-) 130.55</td>
<td>(-) 7.95</td>
</tr>
<tr>
<td>5% BSA</td>
<td>3_3 circle base</td>
<td>3.45</td>
<td>12.32</td>
<td>(-) 53.55</td>
<td>(-) 22.48</td>
</tr>
<tr>
<td></td>
<td>3_3 flower base</td>
<td>6.064</td>
<td>14.01</td>
<td>(-) 147.4</td>
<td>(-) 8.11</td>
</tr>
<tr>
<td></td>
<td>5_5 circle base</td>
<td>8.64</td>
<td>14.45</td>
<td>(-) 130.55</td>
<td>(-) 7.95</td>
</tr>
<tr>
<td></td>
<td>5_5 flower base</td>
<td>11.22</td>
<td>18.01</td>
<td>(-) 150.77</td>
<td>(-) 25.72</td>
</tr>
</tbody>
</table>
It could be observed from the % differences that all the prepolymer combinations used for fabrication the % change in overall height and cone height is positive which means that the fabricated microneedles were smaller in height when compared to the designed microneedles. The % change in tip diameter and base diameter is negative (-) which means that the fabricated microneedles diameters were bigger in size when compared to the designed microneedles. The reason for the change in based diameter of the microneedles fabricated from that of designed microneedles could be the uncured material that is left on the surface that was not completely removed by spraying isopropyl alcohol or the isopropyl alcohol that is left on the surface which was not dried out completely. The reason for change in tip diameter, cone height and overall height might be because of the last layer (top layers of the microneedles) not being built, as it can be seen that the % change of cone height is more than % change in overall height, which in turn could be because of the exposure time and layer thickness which is either too low or high. This could not be controlled because the LabVIEW software used can just take one exposure time and layer thickness which is same for all the layers, but during fabrication smaller features like tip may need different exposure time and layer thickness. Therefore modifications are needed to be made to the software where it has more options with regards to controlling the exposure time and layer thickness for different layers. The other possible reason could be the thickness of first layer built which cannot be controlled, because the first layer thickness is just adjusted by observation rather than giving some value. So this first layer thickness might have affected the distance between the resin surface and the image from objective lens leading to a blunt tip.
Figure 4.27: Front view of PPF based microneedles (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
Figure 4.28: 3D view of PPF based microneedles (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
Figure 4.29: Front view of PPF based microneedles containing 1% BSA (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
Figure 4.30: 3D view of PPF based microneedles containing 1% BSA (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
Figure 4.31: Front view of PPF based microneedles containing 2.5% BSA (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
Figure 4.32: 3D view of PPF based microneedles containing 2.5% BSA (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
Figure 4.33: Front view of PPF based microneedles containing 5% BSA (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
Figure 4.34: 3D view of PPF based microneedles containing 5% BSA (a) 3 x 3 array circle base design (b) 3 x 3 array flower base design, (c) 5 x 5 array circle base design and (d) 5 x 5 array flower base design
4.8 Mechanical testing

The compressive load applied and compressive extension values were obtained from the compression test using which depending on the specimen cross sectional area and length, the compressive and compressive strain values were calculated. Compressive stress - strain curves were plotted using all the samples from the compression testing. The elastic modulus and ultimate stress were measured from the plotted compressive stress – strain curves for every sample, as mentioned previously. The values obtained for each sample for each case were then used to calculate mean along with standard deviations for each prepolymer solution that was used.

The young’s modulus and ultimate compressive strength of PPF/DEF (50:50) are 0.173 ± 0.0327 GPa and 21.4392 ± 5.1291 MPa, respectively. Figure 4.35 shows the stress strain curve of one of the samples of PPF/DEF (50:50).

The elastic modulus and ultimate compressive strength of PPF/DEF (50:50) containing 1% BSA are 0.0694 ± 0.0307 GPa and 4.5956 ± 1.2136 MPa, respectively. Figure 4.36 shows the stress strain curve of one of the samples of PPF/DEF (50:50) containing 1% BSA.

The elastic modulus and ultimate compressive strength of PPF/DEF (50:50) containing 2.5% BSA are 0.0556 ± 0.0243 GPa and 4.041 ± 0.9637 MPa, respectively. Figure 4.37 shows the stress strain curve of one of the samples of PPF/DEF (50:50) containing 2.5% BSA.

The elastic modulus and ultimate compressive strength of PPF/DEF (50:50) containing 5% BSA are 0.029 ± 0.0112 GPa and 2.9667 ± 1.0262 MPa, respectively.
Figure 4.38 shows the stress strain curve of one of the samples of PPF/DEF (50:50) containing 5% BSA.

PPF/DEF (50:50) had higher elastic modulus and ultimate compressive strength when compared to PPF/DEF (50:50) containing 1%, 2.5% and 5% BSA. It was observed that increasing the percentage of drug (BSA), the elastic modulus and ultimate compressive strength of PPF/DEF (50:50) decreased.

The elastic modulus and ultimate compressive strength of PPF/DEF (50:50) with 1% BSA decreased approximately 2.5 times and 5 times when compared to PPF/DEF (50:50) (i.e. no BSA), respectively.

The elastic modulus and ultimate compressive strength of PPF/DEF (50:50) with 2.5% BSA decreased approximately 3 times and 5 times when compared to PPF/DEF (50:50) (i.e. no BSA), respectively. It was observed that the elastic modulus and ultimate compressive strength of PPF/DEF (50:50) with 1% BSA and PPF/DEF (50:50) with 2.5% BSA were almost nearby. The elastic modulus and ultimate compressive strength of PPF/DEF (50:50) with 2.5% BSA decreased approximately 1.1 times and 1.1 times when compared to PPF/DEF (50:50) with 1% BSA, respectively.

The elastic modulus and ultimate compressive strength of PPF/DEF (50:50) with 5% BSA decreased approximately 6 times and 7 times when compared to PPF/DEF (50:50) (i.e. no BSA), respectively. It could be observed that the elastic modulus and ultimate compressive strength of PPF/DEF (50:50) decreased drastically with addition of 5% BSA when compared to 1% and 2.5% BSA. The elastic modulus of PPF/DEF (50:50) with 5% BSA decreased approximately 2.4 times and 1.5 times when compared to PPF/DEF (50:50) with 1% BSA, respectively. The elastic modulus and ultimate
compressive strength of PPF/DEF (50:50) with 5% BSA decreased approximately 2 times and 1.4 times when compared to PPF/DEF (50:50) with 2.5% BSA, respectively.

Graphs were plotted to show variation of elastic modulus and ultimate compressive strength for PPF/DEF (50:50) with no drug, 1%, 2.5% and 5% BSA as shown in Figure 4.39 and Figure 4.40, respectively.

![Stress vs Strain - PPF/DEF (50:50)](image)

**Figure 4.35:** Variation of compressive stress with compressive strain for PPF/DEF (50:50)
Figure 4.36: Variation of compressive stress with compressive strain for PPF/DEF (50:50) with 1% BSA

Figure 4.37: Variation of compressive stress with compressive strain for PPF/DEF (50:50) with 2.5% BSA
Figure 4.38: Variation of compressive stress with compressive strain for PPF/DEF (50:50) with 5% BSA

Figure 4.39: Variation of elastic modulus with % BSA for PPF/DEF (50:50)
Figure 4.40: Variation of ultimate compressive strength with % BSA for PPF/DEF (50:50)
CHAPTER V
CONCLUSION AND FUTURE WORK

The fabrication of PPF/DEF and PPF/DEF with drug (BSA) was successfully executed using the developed microstereolithography system. Firstly, pure PPF was successfully synthesized and its molecular weights were obtained. The viscosity of PPF was measured and its value was too high to work with, in µSL system. So PPF/DEF (70:30) and (50:50) (w/w) were considered. Then the viscosities of prepolymer of PPF/DEF (70:30) and (50:50) with no drug, 1%, 2.5% and 5% (w/w) were found out successfully, from these results it was observed that the viscosity of PPF/DEF (70:30) was very high when compared to PPF/DEF (50:50), hence the fabrication of the microneedles was done using PPF/DEF (50:50) as resins with low viscosity work better in µSL system. The microstereolithography system was successfully developed and calibrated. The curing characteristics of PPF/DEF (50:50) were found by conducting cure depth experiments which provided the exposure energy and penetration depth. The developed system was then verified using a HDDA prepolymer to know the manufacturability of the system. The HDDA microneedle arrays were fabricated as they were designed which proved that the developed system can be used for further fabrications. Then the PPF/DEF (50:50) with no BSA, 1%, 2.5% and 5% BSA microneedle arrays were fabricated and where viewed under the microscope and SEM which were nearby to the original design. The percentage change in dimensions was
measured for the fabricated microneedle arrays and the reason for those errors was also discussed. Finally, the mechanical properties such as elastic modulus and ultimate compressive strength of the PPF/DEF (50:50) with no drug, 1%, 2.5% and 5% (w/w) were found out by conducting compression test on the specimens made out of the samples. The reason for finding out the mechanical properties was as these are new materials and they will be used as microneedles on which different forces will be acting during their application so its properties are needed to be known, which could be used as reference for future works.

Since this is an ongoing work, the future work is to test these microneedle arrays for drug delivery, to see the degradation rate of these microneedles and the rate at which they deliver the drug and the amount of drug that will be delivered over time. Also these microneedle arrays should be tested for their insertion and failure mechanism when they are applied on human skin. Once these in vitro works provide successful results then in vivo experiments have to be conducted, which is to test these microneedle arrays on human skin.
REFERENCES


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33. Dimethyl sulfoxide, Wikipedia.


APPENDICES
APPENDIX A

PROCESS FOR PPF SYNTHESIS

Step 1: Production of Bis(hydroxypropyl) Fumarate Diester Intermediate (The following steps should take approximately 12 h to complete)

1. Add diethyl fumarate (196.6 g), propylene glycol (259.4 g), hydroquinone (0.25 g) and ZnCl2 (1.55 g,) into the three-neck round-bottomed reaction flask.

2. Remove moisture from the system by establishing a purging flow of ultra-high purity nitrogen gas (no drying required). The exclusion of water from the reaction system is essential to ensure the efficiency of the reaction. One may monitor the flow rate of nitrogen through the system by observing the nitrogen bubbles created in a water filled beaker. A moderate flow rate (3 bubbles per second) of nitrogen increases the rate of ethanol removal from the system. However, an excessively high nitrogen flow rate (> 10 bubbles per second) may result in undesired removal of volatile reactants, such as the diethyl fumarate.

3. Initiate stirring at approximately 150 rpm. Ensure that the stirrer is set at 0 rpm before switching on the unit, and then increase the speed slowly to approximately 150 rpm.

4. Establish flow of chilled water through the condenser and increase the stirring rate to 300 rpm.
5. Heat the flask in a silicone oil bath at 110°C for 30 min.

6. Increase the temperature of the oil bath to 120°C for 30 min then to 130°C.

7. Increase the temperature of the oil bath to 120°C for 30 min then to 130°C.

8. Continue the reaction at 140°C until approximately 90% of the theoretical yield of ethanol has been collected in the receiving flask (approximately 95 g of the theoretical 105 g). The reaction generally requires 6 to 8 h. The time required for the reaction may be decreased by increasing the temperature of the oil bath, increasing the stirring rate, and/or increasing the flow rate of the nitrogen gas. However, one should avoid temperatures above 150°C to mitigate potential side reactions. High flow rates of nitrogen gas should likewise be avoided as previously mentioned.

9. Once the desired amount of ethanol has been collected, cool the reaction flask to room temperature (25°C) with a continued flow of nitrogen gas through the system. Once cooled, the reaction products may be sealed and stored overnight in the fume hood, if needed.

**Step 2: Transesterification Reaction** (These steps should take approximately 9 h to complete)

1. Reassemble the apparatus as in the first reaction using the three-neck round bottom flask containing the bis(hydroxypropyl) fumarate diester intermediate.

2. Initiate flow of the purging nitrogen gas through the apparatus and of the chilled water through the condenser. Initiate stirring of the diester intermediate at a rate of 300 rpm.

3. Heat the flask in a silicone oil bath at 100°C for 30 min.
4. Stop the nitrogen gas purge and replace the gas outlet house attached to the vacuum take-off adapter with a hose connected to a high vacuum apparatus. The vacuum apparatus should be equipped with a liquid nitrogen cooled vacuum trap to collect potentially harmful volatile compounds.

5. Increase the temperature of the oil bath to 110°C for 30 min.

6. Increase the temperature of the oil bath to 120°C for 30 min.

7. Increase the temperature of the oil bath to 130°C for 30 min.

8. Increase the temperature of the oil bath to 140°C. The extent of transesterification and the molecular weight of the resulting PPF increase with reaction time.

9. Terminate the reaction and allow the system to cool to room temperature under a purge of nitrogen gas when the desired molecular weight has been achieved (accounting for the change in average molecular weight in the purification procedure).

10. Add methylene chloride (approximately 300 ml) to the polymer product in the reaction flask and purge the system with nitrogen gas. The cooled reaction products may be sealed and stored overnight at 21°C in the fume hood, if needed.

Step 3: Purification of Poly(propylene fumarate): Aqueous Washes (Steps listed below should take approximately 6 h to complete)

1. Add additional methylene chloride (approximately 300 ml) to the polymer solution to bring the total solution volume to approximately 800 ml.

2. Transfer the polymer solution into a 2-L separatory funnel positioned in a ring clamp above a 2-L Ernlenmeyer flask.
3. Add a volume of 1.85 % (vol/vol) HCl solution approximately equal to the volume of the polymer solution (approximately 800 ml) to the separatory funnel, such that the acid and polymer solutions are at a volume ratio of 1: 1. Mark the interface between the solutions on the outside of the separatory funnel.

4. Cap and invert the separatory funnel then open the stopcock to vent gas while the funnel is inverted.

5. Close the stopcock, briefly shake the funnel to agitate the solution, invert the funnel, and open the stopcock to vent liberated gas.

6. Repeat the shaking/venting step (in Step 3 – no. 5) several times, increasing the amount of shaking each time.

7. Close the stopcock, shake the solution vigorously, then place the separatory funnel on the ring stand and open the cap of the funnel.

8. Allow the phases to separate for approximately 10 min. One may use the mark made at the interface between the phases in (Step 3 – no. 3) to facilitate visualization of the two separate phases.

9. Collect the polymer phase (the bottom phase) into a 2-L Erlenmeyer flask and appropriately discard the aqueous phase. The polymer solution should appear cloudy at this point.

10. Repeat the wash procedure (Step 3 – no. 2 through no.9) twice using double-distilled H₂O instead of the 1.85 % (vol/vol) HCl solution. Rinse the separatory funnel and the 2-L collection flask with acetone between each wash cycle. In the event that the separation of the phases becomes too difficult to discern, then add a
small amount of brine to the aqueous phase after shaking. The polymer solution should appear milky white following the 1st wash with $\text{H}_2\text{O}$.

11. Repeat the wash procedure (Step 3 – no. 2 through no.9) twice using brine solution instead of the 1.85 % (vol/vol) $\text{HCl}$ solution. The collected polymer solution should be turbid with a light yellow color following the brine washes.

**Step 4: Purification of Poly(propylene fumarate): Drying with Sodium Sulfate** (The following steps should take approximately 6 h to complete)

1. Stir the polymer solution using a magnetic stir bar.

2. Slowly add sodium sulfate to the polymer solution under stirring until small aggregates of sodium sulfate are apparent and the polymer solution becomes transparent with a light yellow color.

3. Cap or cover the flask and allow the solution to stir for 30 min.

4. Vacuum filter the solution using a Buchner funnel with filter paper (Whatman #40 Ashless Circles) to remove the sodium sulfate.

5. Transfer the polymer solution filtrate into a 1-L round-bottom flask.

6. Remove methylene chloride through reduced pressure and rotation using a rotary evaporator with an associated heated water bath (40°C). When the volume of solution in the flask has been reduced through evaporation of the methylene chloride, additional polymer solution (if any) may be added and the procedure continued.

**Step 5: Purification of Poly(propylene fumarate): Ether Washes** (These steps should take approximately 3 h to complete)

1. Prepare 1-L of ethyl ether in a 2-L Erlenmeyer flask cooled with an ice bath.
2. While stirring the ethyl ether with a magnetic stirrer, slowly pour the polymer solution from (Step 4 – no. 6) into the ethyl ether. The ether solution will become white in appearance and the polymer will appear as a yellow precipitate.

3. Upon complete addition of the polymer solution, stop the stirring and decant the ether phase into an appropriate container for disposal.

**Step 6: Purification of Poly(propylene fumarate): Solvent Removal** (the following steps should take approximately 3 h to complete)

1. Dissolve the polymer product from (Step 5- no. 3) in methylene chloride (300 ml).

2. Remove methylene chloride from the polymer solution through rotary evaporation at reduced pressure using a rotary evaporator with an associated heated water bath (40°C).

3. Complete drying of the polymer product under high vacuum at room temperature for at least 8 h. The vacuum apparatus should be equipped with a liquid nitrogen cooled vacuum trap to collect potentially harmful volatile compounds.

4. Vacuum-evacuate the flask and purge with nitrogen gas. Store the polymer product at 4°C for up to 3 months.

**Step 7: Determination of Molecular Weight of Poly(propylene fumarate)** (This Step should take approximately 6 h to complete)

1. Determine the molecular weight of the PPF product using gel permeation chromatography (GPC) at 25°C using a GPC column (Waters, Styrage HR 4E, 7.8 x 300 mm column (50 – 100000 Da range)) at a flow rate of 1.0 ml/min with degassed chloroform as the eluent. For systems using a refractive index detector,
the molecular weight distributions should be determined relative to a calibration curve generated from polystyrene standards ($M_n = 500, 2630, 5970, \text{ and } 9100 \text{ Da}$).

**Timing**—Synthesis of PPF: Steps 1, 12 h; Steps 2, 9 h.

Purification of PPF: Steps 3, 6 h; Steps 4, 6 h; Steps 5, 3 h; Steps 6, 3 h.

Determination of molecular weight: Step 7, 6 h.
APPENDIX B

FABRICATION PARAMETERS AND PROCEDURE FOR HDDA PREPOLYMER BASED MICRONEEDLE ARRAYS

The procedure for fabrication of HDDA microneedle arrays starts with first build the substrate for which the below steps are followed.

**Step 1: Positioning the stage at Z = 1.511 mm**

1. The power of the Z-stage is switched on.

2. The software that controls the Z-stage given by the company is opened and the Z-stage is enabled and moved to home position first which is considered as Z = 0.

3. Then the Z-stage is moved 1.511 mm in downward direction manually by using the interface by entering this value.

**Step 2: Setting the exposure time and energy**

1. The power of the lamp is switched on.

2. Then the exposure time and intensity level of the lamp are set to 12 seconds and 100%.

**Step 3: Setting the required substrate image on DMD**

1. The power of the DMD is switched on.
2. Then the software given for the DMD ALP basic is opened and then script DMD format is selected from the file menu, which prompts to select the DMD type, in which 1080p is selected.

3. Then in the device menu in the software is selected which shows a prompt saying which device to attach, in that ALP-4.12 is selected, which makes the device attach to the system.

4. Then the image that has to build is selected by selecting the load menu tab and then clicking the import picture option and selecting the image, which is the square base of 2.6 x 2.6 mm on which the microneedles are built, in this particular case. The insert option is then selected, then the reset tab is selected and then the program is made to run by selecting a green arrow symbol that in the top tab of the interface, to display the image on DMD.

**Step 4: Building of the rest of the layers**

Once the first substrate layer is built then the developed Labview software was used to fabricate then next layers. The DMD software, manual Z stage software and Lamp are no more manually used. The DMD software is turned off by just selecting the quit option in the file tab. The parameters given in Table B were considered as inputs to the software. The fabrication process started and the whole fabrication is controlled by the developed LabVIEW software which was mentioned previously.

Brief details of each parameter are:

1. axis is selected as Z because the platform is fixed to Z stage
2. speed is the speed of Z stage at which the Z stage moves during the upward and downward movement for refreshing the resin surface

3. immersing depth is the distance the Z stage should move in order to refresh the resin surface

4. Once the platform is immersed it stay there for some time which is the dwell time

5. Settling time is the time required for the resin surface to become flat when it moves upward (i.e. comes back to a position after immersing down)

6. Layer thickness is the thickness of each layer that needs to be built.

7. Number of layers is the number of bitmap images that are obtained once the STL file is sliced depending on the required layer thickness.

8. Exposure time is the time the resin surface is exposed to the UV light.

9. Iris level is the intensity of light in %.

Table B. Fabrication parameters a for HDDA prepolymer based microneedle array

<table>
<thead>
<tr>
<th>Axis</th>
<th>Speed (mm/sec)</th>
<th>Immersing depth (mm)</th>
<th>Settling time (sec)</th>
<th>Dwell Time (sec)</th>
<th>Layer thickness (mm)</th>
<th>Number of Layers</th>
<th>Exposure time (sec)</th>
<th>Iris level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>1</td>
<td>2</td>
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<td>1</td>
<td>0.005</td>
<td>220</td>
<td>2</td>
<td>100</td>
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APPENDIX C

FABRICATION PARAMETERS AND PROCEDURE FOR PPF/DEF WITH BSA (NO BSA, 1%, 2.5% AND 5%) PREPOLYMER BASED MICRONEEDLE ARRAYS

The procedure for fabrication of PPF/DEF WITH BSA (NO BSA, 1%, 2.5% AND 5%) microneedle arrays starts with first build the substrate for which the below steps are followed.

**Step 1: Positioning the stage at Z = 1.511 mm**

1. The power of the Z-stage is switched on.
2. The software that controls the Z-stage given by the company is opened and the Z-stage is enabled and moved to home position first which is considered as Z = 0.
3. Then the Z-stage is moved 1.511 mm in downward direction manually by using the interface by entering this value.

**Step 2: Setting the exposure time and energy**

1. The power of the lamp is switched on.
2. Then the exposure time and intensity level of the lamp are set to 20 seconds and 100%.

**Step 3: Setting the required substrate image on DMD**
1. The power of the DMD is switched on.

2. Then the software given for the DMD ALP basic is opened and then script DMD format is selected from the file menu, which prompts to select the DMD type, in which 1080p is selected.

3. Then in the device menu in the software is selected which shows a prompt saying which device to attach, in that ALP-4.12 is selected, which makes the device attach to the system.

4. Then the image that has to build is selected by selecting the load menu tab and then clicking the import picture option and selecting the image, which is the square base of 2.6 x 2.6 mm on which the microneedles are built, in this particular case. The insert option is then selected, then the reset tab is selected and then the program is made to run by selecting a green arrow symbol that in the top tab of the interface, to display the image on DMD.

**Step 4: Building of the next substrate layer**

1. Once the first layer is built then the same exposure time and image are used to build the second layer, but first moving the Z-stage 3mm down and wait for 2 seconds and then moving it back 0.02 mm back, this is done to refresh the resin surface for the next layer and get a layer thickness of 5 µm. At this position, the resin surface is left to settle for about 60 seconds and then the resin is exposed to the as substrate pattern previously used for 20 seconds
Step 5: Building of the rest of the layers

Once the first substrate layer is built then the developed Labview software was used to fabricate then next layers. The DMD software, manual Z stage software and Lamp are no more manually used. The parameters given in the Table C were considered as inputs to the software and the fabrication process started and the whole fabrication is controlled by the developed Labview software.

Brief details of each parameter are:

1. axis is selected as Z because the platform is fixed to Z stage
2. speed is the speed of Z stage at which the Z stage moves during the upward and downward movement for refreshing the resin surface
3. immersing depth is the distance the Z stage should move in order to refresh the resin surface
4. Once the platform is immersed it stay there for some time which is the dwell time
5. Settling time is the time required for the resin surface to become flat when it moves upward (i.e. comes back to a position after immersing down)
6. Layer thickness is the thickness of each layer that needs to be built.
7. Number of layers is the number of bitmap images that are obtained once the STL file is sliced depending on the required layer thickness.
8. Exposure time is the time the resin surface is exposed to the UV light.
9. Iris level is the intensity of light in %.
Table C. Fabrication parameters a for PPF/DEF with BSA (no BSA, 1%, 2.5% and 5%) prepolymer based microneedle arrays

<table>
<thead>
<tr>
<th>Axis</th>
<th>Speed (mm/sec)</th>
<th>Immersing depth (mm)</th>
<th>Settling time (sec)</th>
<th>Dwell Time (sec)</th>
<th>Layer thickness (mm)</th>
<th>Number of Layers</th>
<th>Exposure time (sec)</th>
<th>Iris level (%)</th>
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<td>2</td>
<td>0.02</td>
<td>52</td>
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