Lasers in Tissue Engineering

S Venkat Bharadwaj

Contents

1 Introduction **2**

2 Theory **2**

2.1 3D Structure Fabrication 2

2.1.1 Stereolithography 2

2.1.2 Multi-photon Polymerization 3

2.2 Laser-Induced Forward Transfer 4

2.3 Matrix Assisted Pulsed Laser Evaporation 6

2.4 Laser Ablation 7

2.5 Laser Tweezers 8

3 Discussion **9**

3.1 Short Pulsed Lasers 9

3.2 Non-Linear Optics 9

3.3 Ablation 9

4 Conclusion **9**

5 References **10**

Acknowledgement

This academic project was supported by the Indian Institute of Technology Guwahati. I extend my thanks to Professor Ashwini Kumar Sharma, who provided insight and expertise that greatly assisted the study and motivated me to learn about the use of lasers in tissue engineering and compile my knowledge and passion for the subject into a semester term paper.

Main Reference

Koo, S., Santoni, S.M., Gao, B.Z. et al. Laser-assisted biofabrication in tissue engineering and regenerative medicine. Journal of Materials Research 32, 128–142 (2017)

1 Introduction

Tissue Engineering is a field of biomaterial development that deals with construction of scaffolds/-models using biological molecules. It combines the field of engineering and medicine to help improve current medical capabilities. The aim of this is to create artificial 3D substitutes for organs/tissues that can survive/function in the biological micro-environment in a human. To achieve this, the structural geometry and morphology should be constructed very precisely at various length scales to mimic the actual tissue complexity of the original organ. For such precise constructions at small length scales, microtechnology based tissue engineering focuses on printing 3D biomaterial with high spacial resolution.

The issue in achieving the goals are that the cells in the tissue need to be responsive to the micro-environment, and be physically similar to the physical structure. Several methods such as nanoimprinting, electron beam lithography have been used to construct precise 2D structures. But these techniques are very expensive as they require special apparatus and time consuming. Moreover, the surface topology alone is not enough for the building of artificial tissues. 3D techniques such as electrospinning, solvent-casting have been experimented with , however, it is very difficult to alter the physical structures below 10μm.

A solution to these problems come in the form of laser assisted techniques. Due to the sharp nature of lasers, these can be used for precise modifications in tissue engineering in sub micro length scales. As they are optical effects, it provides as with contact free methods which leads to less contamination. Laser-assisted biofabrication are versatile techniques that can operate in various conditions, and can deposit cells at density of upto 10^8 cells/ml at speeds of 1600 mm/s.

In this paper we review current technologies and recent developments in the field of Laser assisted tissue engineering. In specific, we focus on techniques such as LIFT, MAPLE, Laser ablation techniques, use of laser tweezers, and laser induced polymerization techniques.

2 Theory

2.1 3D Structure Fabrication

3D lithography is a process in which lasers are used to induce linear or non-linear excitations to promote polymerization to create 3D structures. Such photo-excitations can be induced using one or many photon absorption.

2.1.1 Stereolithography

Stereolithography (SLA) is one such technique that is used to manufacture 3D objects. Here, UV lasers are irradiated on photosensitive resin to polymerize them allowing layer-by-layer processing of the material. In this technique, a scanning system is used to expose the photo-curable resin (resin containing photo-initiators) in a line-by-line/point-by-point style while a projection system uses a digital micro-mirror device to cure a whole layer in one go. Once the layer is cured, the stage moves in z-axis for the next layer to be cured. SLA is a relatively fast technique that can be use to fabricate high-resolution structures in the micron regime. It is also low cost as only the cured resin is consumed. This technique has been used to manufacture scaffolds for tissue engineering. It is also used in the

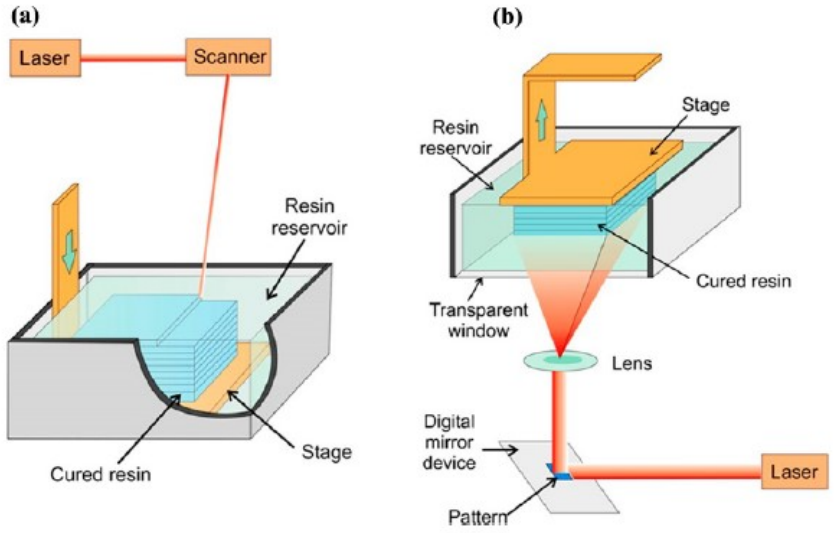

Figure 1: Schematic Diagram for a typical SLA in (a) Scanning (b) Projection

fabrication of micro-vascular stamps and examination of cell-to-cell interaction. However, the UV range is harmful to the human tissues and researchers are still looking into reproducing this technique using less harmful wavelengths. As such, its use has been limited and Multi-photon polymerization is a more widely used technique.

2.1.2 Multi-photon Polymerization

LDW is a technique that focuses laser beam on the material in a specific pattern to create 3D structures. A typical system capable of LDW works based on Multi-photon polymerization. This system usually contains:

- Laser Source: Ultra-short pulsed lasers are used to employ non-linear process such as Multi-photon absorption.
- Motion System: Galvanometric scanners for moving the sample on high-resolution XYZ stages.
- Focusing Optics: This is required for maintaining a consistent high-intensity laser beam.
- Beam Intensity control: Consists of electro-optic modulators for Beam On-Off and a combination of polarizers and waveplate for Intensity control.
- Control/Monitoring software: A central computer that is used to synchronize all the optical and mechanical components of the system.

The key-difference between a SLA and Two-photon Polymerization uses the two-photon absorption phenomenon for the excitation of the photo-initiator phenomenon. In this, a virtual state is created when 2 photon of same or different frequency are absorbed by the photo-initiator. As such, photons of lower energy can be used to imitate an electronic excitation using a single high energy photon. This leads to higher resolution writing (up to 100nm). For this, Femto second lasers are generally used as they are based on second or third order optical effects which are more efficient at higher temperature. However, it requires the laser beam to pass through the entire resin, which limits its use to transparent materials.

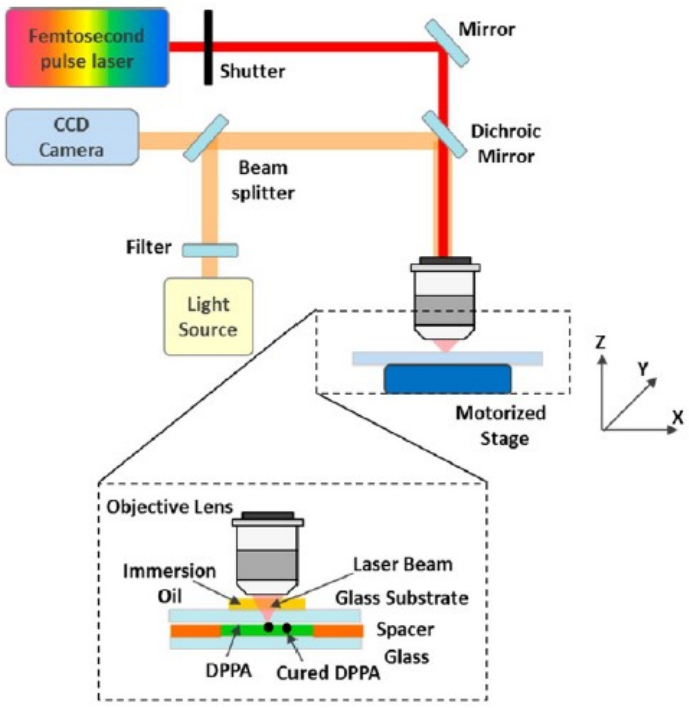

Figure 2: Schematic Diagram for a laser setup for TPP

For the purpose of tissue engineering, bio-compatible hydrogels are used for development of scaffolds for encapsulation of living cells. This has been used widely used for highly controllable structures with defined geometry and porosity. Thus, it has breakthrough applicability to creation of human in vitro cardiac tissue. Using such models, researchers are able to properly study cardio-vascular diseases (study by Ma et al.). Researchers have also fabricated other models such as a microstructure for the study of trabecular bone (Marino et al.). This technique is limited by its slow processing speeds and poor mechanical properties of the hydrogels used as scaffolds.

2.2 Laser-Induced Forward Transfer

Laser Induced Forward Transfer (LIFT) is a printing technique that transfers materials from a donor layer onto a substrate layer. In LIFT, a laser beam is focused on a thin layer of absorbing material, and light matter interaction takes place. Due to the action of the laser, a strong pressure is observed at the area of irradiation. Due to this pressure, part of the donor layer is ejected and is placed on a receiving substrate. By employing the laser on specific positions, one can deposit materials in any required pattern.

As the laser needs to go through the donor substrate to pickup the donor, it is necessary that the laser wavelength is transparent to the donor substrate. Other than this, LIFT does not have any conditions on the laser wavelength. Generally, pulsed laser systems with pulses of several nanoseconds is used. It can also be implemented using ultrafast laser system such as a femto/pico second laser.

Printing of cells has been an active field of research. By optimizing multiple parameters in a LIFT

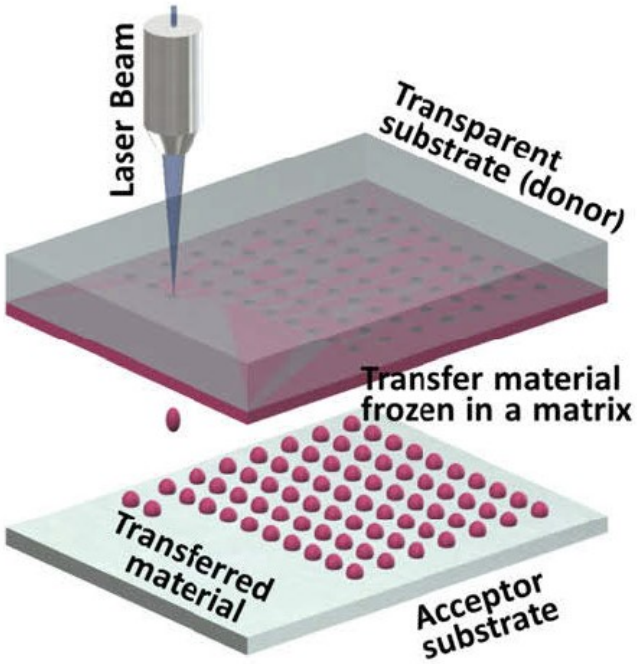

Figure 3: Schematic Diagram for LIFT

system to avoid damage to cells, it can be used as a successful technique tissue engineering. As such, the laser energy, ink viscosity and processing time of the process need to be taken into account. It is also necessary to limit laser fluences, as high heat diffusion will lead to molten debris, or generate shockwaves that may reflect back the donor material. The absence of nozzles eliminates clogging issues that can be seen in other techniques such as inkjet and extrusion printers.

Ultimately, the most important objective of tissue engineering is construction of fully developed 3D organs that can mimic the biological tissue architecture and function. Using LIFT, researchers are one step closer to achieving this objective. Although fully functioning organs have not been printed, small scale tissue constructs have been achieved. Through precise deposition of bio-materials, researchers are able to create defined co-culture/multi cultured models. This allows them to study cell-cell communication. In specific, they can use these models to simulate different micro environments to study the differentiation pattern of cells. Moreover, making 3 dimensional constructs of tissues/organs will enable researchers to study the effects of drugs and introduces a safe and ethical way of testing new medicine in different conditions and doses.

For investigating the application of LIFT in construction of fully functioning organs, Micheal et al. printed a fully cellularized auxiliary skin. These printed skins were tested on animal models. These substitutes completely integrated with neighbouring tissues and differentiation was observed. They also developed collagen and other adherants, and blood vessels also appeared to grow from the bottom of the wound. Apart from skin tissue, blood vessels, cardiac tissue, nerve tissue have also been printed.

2.3 Matrix Assisted Pulsed Laser Evaporation

Matrix Assisted Pulsed Laser Evaporation (MAPLE) is technique that is an extension of the Pulsed Laser Deposition (LIFT). This technique uses low powered UV laser for the deposition of the material, which is a safer technique when it comes to heat sensitive organic tissue. In MAPLE, the biomaterials that are to be deposited are dissolved in a solvent placed on a cryogenic substrate. This solvent is then irradiates with the laser, thus, most of the laser energy is absorbed by the solvent and dissipates which reduces laser-matter interaction. This dissipation causes a natural rise in temperature because of which volatile solvents evaporates. This leads to a uniform thin film deposition on a receiving substrate. The receiving substrate is kept at low temperatures (around 200K) to prevent degradation of solute molecules.

For proper implementation of MAPLE, there are 3 criteria that need to be satisfied:

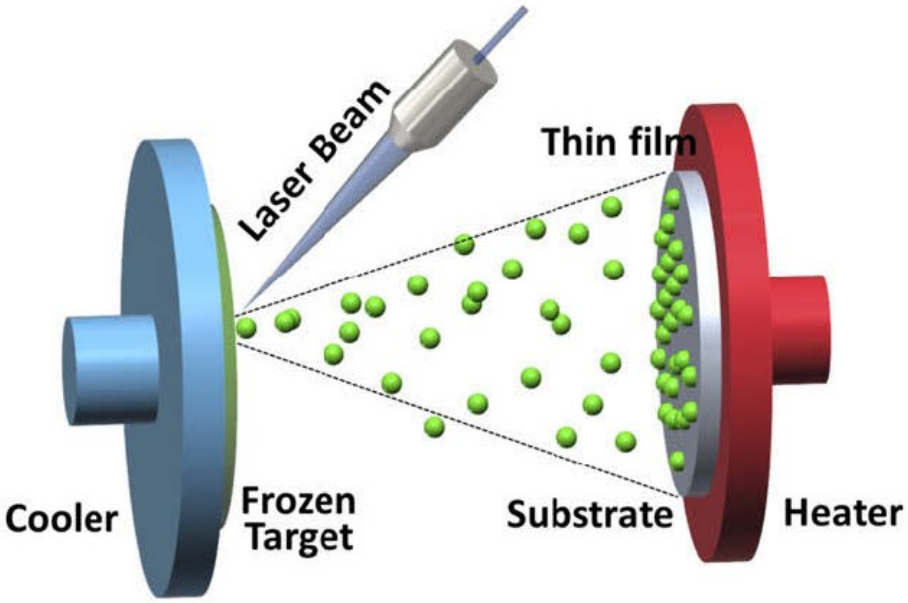

Figure 4: Schematic Diagram for MAPLE Technology

- The solvent must be volatile at room temperature. Otherwise the dissolved biomaterials do not have an easy release.
- The biomaterial should be highly soluble in the solvent at cryogenic temperatures.
- The laser energy should be mostly absorbed by the solvent. Without this, there wont be an even temperature rise, thus creating non uniform streams of biomaterial. It could also lead to damage of the biomaterial due to laser-matter interaction.

In MAPLE, different heat sensitive material that are damaged upon direct laser irradiation (such as in LIFT) can be utilized for deposition. The choice of these materials is only limited by the above specified conditions. This also allows for better thickness and surface morphology control. This technique does not require ultra high vacuum from contaminant free deposition which is another one of its virtue. However, for the application in tissue engineering, parameters such as laser fluence, pulse repetition rate, distance between target and receiver needs to be optimized.

Biomaterials range from simple polylactic acids to very complex proteins. As they have inherent activity, it is mandatory that the functioning of these are not affected while deposition. Moreover, they can be denatured in high temperature. Therefore, MAPLE being a low energy technique, can be widely used for this purpose. It has been shown that (Popescu et al.) protein like Ribonuclease that can inhibit growth of cancer cells can be deposited on a substrate while maintaining its activity. With this technology, Paun et al. created a two layer polymer substrate for potential use in tissue engineering. Using laser ablation techniques, they fabricated microchannels in this substrate that allowed for selective attachment of cells.

In bone tissue engineering, use of this technique has allowed for one to deposit nanocrystalline apatite preserving its structural and chemical nature. Magnesium or Stroncium doped Octocalcium phosphate has been deposited on titanium substrates showing better proliferation and differentiation of human osteo-blast like cells on the surface. Using MAPLE has also led to improvements in bioceramic scaffolds. By Extracellular Matrix deposition using MAPLE. it has been shown that MG 63 (osteoblastic seel line) has higher cell viability and proliferation that suggest MG 63-Hypoxiapatite composites for cell scaffold structures.

2.4 Laser Ablation

Ablation is generally defined as the phase transition of a material into the vapour phase. Laser Ablation is a technique that removes material from a substrate due to the absorption of laser energy. Due to the Gaussian nature of laser, energy at the center of the beam is high enough to induce non-linear optical phenomenon and cause ablation. The use of femto-second laser has allowed for removal of material in a more precise manner with very little debris. Another reason for using pulsed laser is to reduce the thermal effects on neighbouring cells which may cause cell damage.

For ablation of biomaterials, the laser is physicaaly defined by the four parameters: Ablation

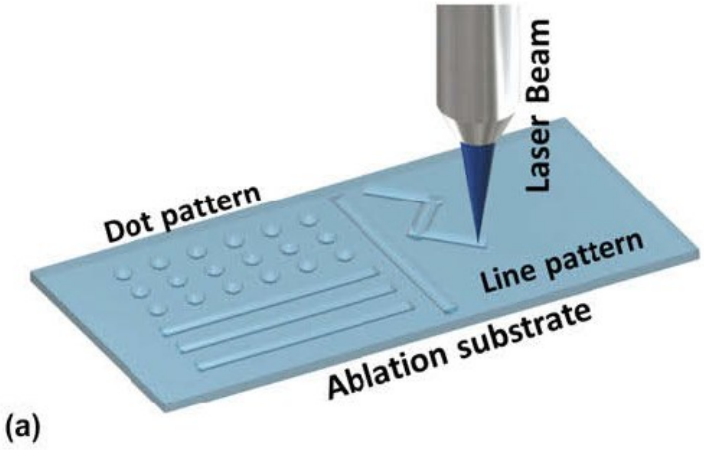

Figure 5: Schematic Diagram for Biomaterial Removal using Laser AbLation

rate per pulse(d(F)), Effective absorption coefficient(α_{eff}), irradiation fluence(F), ablation threshold fluence(F_{th}). F_{th} is given by minimal energy for the plasma ignition. These parameters are related

as

$$d(F) = \frac{1}{\alpha_{eff}} \ln \frac{F}{F_{th}}$$

In tissue engineering, Laser ablation has been used to fine tune the micro/nano scale geometries of scaffolds and other biomaterial constructs due to its precise and debris-less nature. This technique has also been used to create micro-grooves in biomaterial surface. Sch substrates can be used as templates for cell culturing in required patters. Furthermore, it can also be used to modify structure underneath the surface. In a study by Applegate et al., 3D microchannels that supported cell growth and mitigation in specific direction were constructed upto a depth of 1cm.

Similarly, in different studies by De Marie et al., Li et al., ablation based technique was used to culture cells of different types in 3D micro-topologies similar to nature cells. It was noted that cells did not show any signs of mechanical or thermal stress. It was also seen that as time increased, the adhesion of molecules increased due to serum protein adsorption. As a result, it was concluded that laser ablation can be used for adjustment of surface topography and different cell patterns to study the cellular interactions and create tissue equivalents.

2.5 Laser Tweezers

When light is incident on a particle, the momentum of the photon changes due to refraction and reflection. Due to principle of conservation of momentum, the particle's momentum changes, i.e. it is acted upon by a force termed as optical force. These forces are used to trap individual particles and manipulate them. This is known as Laser Tweezer. With its high accuracy and precission due to manipulation of single cell, this techique has had many contributions cell biofabrication which has led to development in tissue engineering. Using this technique, researchers achieve accurate cell arrangements that can be used for study of cell-cell, cell-ECM interaction.

Using this technique, Gao's lab were able to micropattern rat stem cells with rat cardyomyocytes

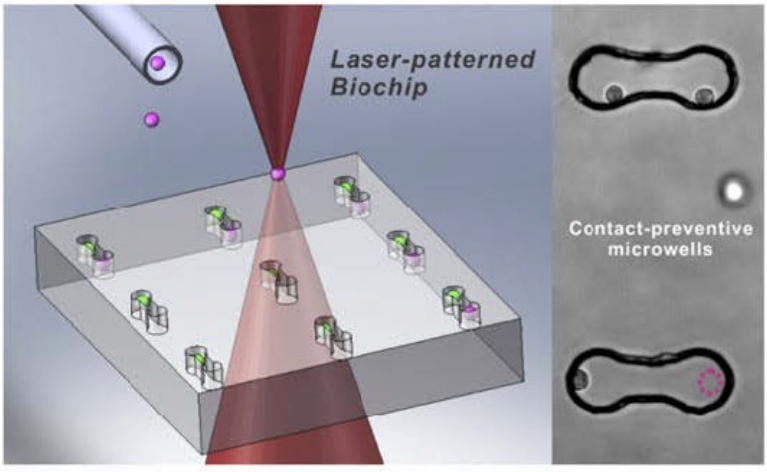

Figure 6: Schematic Diagram for Laser Tweezer for cell micropatterning

on a substrate. Studying the electrical interactions of these cells led to the discovery that stem cells were more electrically compatible with native cardiac than other cells and thus a promising source for cardiac therapies. In a study by Pirlo et al., laser tweezers were used to pattern neuron and neuron-glial

cell pairs onto a microchip. This study was used to demonstrate different microchannel geometries influencing axon growth and polarity.

3 Discussion

The concepts discussed in class that relates to this paper are as follows:

3.1 Short Pulsed Lasers

As discussed in class, short pulsed lasers can be generated by using various crystals as shutters to increase population above some threshold and opening the shutter leads to a high intensity laser pulse. Unlike CW lasers, due to their pulsed nature, they can deposit materials at a much faster rate. Since the interaction time is also very little, there is very minimal heat dissipation in the system which leads to less damage of cells.

3.2 Non-Linear Optics

In the class, we discussed about non-linear optical effects and how they are only shown at specific intensity regions. We also saw the generation of 2nd Harmonics as an application of this non-linearity. Similarly, another such application of the second/third order optical effects, is the multi-photon polymerization which is used for laser writing.

3.3 Ablation

During last few lectures of the class, we saw the applications of laser in production of plasma, where we learnt about laser-matter interaction that leads vaporization/explosion termed as laser ablation. We also saw the variation of molten debris wrt to the pulse width of the laser used. For ultra-short pulsed laser such as the femto-second laser, the debris is very minimal. This is one major principle beacause of which of lasers in tissue engineering is feasible.

4 Conclusion

In this paper, we reviewed laser assisted biofabrication techniques such as the use of laser tweezers to trap individual cells, laser initiated polymerization techniques that uses non-linear optical effects to generate chemical bonds, LIFT and MAPLE that is used to deposit polymeric material onto substrate, and finally laser ablation techniques for biomaterial removal. We have seen that these techniques prove to be very useful techniques in tissue engineering due to their noninvasive, material independent, and highly precise nature. These systems, albeit not at the level of organ construction, can be used to mimic various biological system in laboratory conditions and can be used to study their properties.

Despite the use of these techniques, some limitations researcher face in using it are - i) Due to the micro-scale architecture, these cannot be used for manufacturing large scale scaffolds and are limited to millimeter dimensions. ii) Improving the functioning of the tissues/ integrating these systems with a larger network where inter transportation of medium does not seem feasible. For overcoming these

limitations, researchers are studying integrating laser assisted methods with other 3D printing methods. They are also looking at various other laser sources and studying their intensity distribution for faster and highly efficient 3D fabrication.

5 References

- Laser-assisted biofabrication in tissue engineering and regenerative medicine
- Femtosecond-Laser-Based 3D Printing for Tissue Engineering and Cell Biology Applications
- Laser-Induced Forward Transfer
- Matrix-Assisted Pulsed Laser Evaporation (MAPLE) technique for deposition of hybrid nanostructures
- Laser Ablation of Biomaterials