A brief review on laser surface texturing of biomaterials for cell culture applications

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ABSTRACT

Cell culture is the process of removing cells from the organisms and growing in vitro conditions. In contrast to cell culture on a petri dish, cell culture on textured surfaces provides an environment similar to in vivo conditions for testing pharmacokinetics and pharmacodynamics effects of drugs. Here, the effect of laser texturing on the cell culture of different cells is studied. Laser induced periodic surface structures (LIPSS) are produced on biomaterials using laser micro processing, which improves the adhesion of cells on the surface of the biomaterial, shows cells align to the textures and get oriented in a particular direction. Ultrashort pulse lasers are used to produce these LIPSS in orders of several nanometers to a few micrometers.

KEYWORDS

Contact Angle,
Micropatterns,
Cell Adhesion,
Cell Growth.

1. Introduction

Cell culture is the process where cells are grown in an external environment from the organism under controlled conditions. It is one of the oldest techniques in medical history, which dates back to the nineteenth century, when an English physiologist, Sydney Ringer maintained the beating of an animal heart in a salt solution consisting chlorides of sodium, potassium, calcium and magnesium. In the mid nineteenth century, cell culture took a steep advancement to support research in virology. Viruses were grown in cell culture samples for the purpose of vaccine manufacturing, injectable polio vaccine became one of the first mass produced product by cell culture techniques. Earlier cell cultures were done on a petri dish which is also known as 2D cell culture, but to provide cell conditions close to its natural environment, 3D cell culture was introduced. In 3D cell culture, cells are grown in the extracellular matrix with a three-dimensional high aspect ratio surface textures [1,2]. Since the 3D structure is designed in such a way to mimic the natural environment of the cells, better biocompatibility is observed.

Surface textures ranging from several nano to micrometers can be produced by laser surface texturing. A wide range of machinability of different types of materials and structures from nano to microscale level, high resolution, fast, repeatable and contactless process make femto second laser one of the prime options for making surface textures on biomaterials for cell culture applications [3,4]. These textures are produced on biomaterials which can be defined as “a nonviable material used in a medical device, intended to interact with biological systems” [5]. High strength, low density and high corrosion resistance make TI-6Al-4V one of the most popular materials for cell culture applications. A femtosecond laser is used to scan the surface and Laser Induced Periodic Surface Structures (LIPSS) are produced which increases the adhesion and biocompatibility of cells. In this paper, the effect of different biomaterials and surface textures on the cell cultured are studied.

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2. Materials and Methods

2.1 Sample preparation

Biomaterials are those materials which can interact with a biological system without producing any adverse effect and are generally employed in medical applications such as scaffolds etc. In cell culture on laser surface textured samples, experiments have been conducted on various materials such as PMMA, PDMS, Polystyrene, Ti-6Al-4V, Stainless steel, NiTi, Mg-6Gd-Ca, etc. using cells such as Astrocyte cells, Murine calvarial preosteoblasts cells

(MC3T3-E1), L929 murine fibroblast cells, human mesenchymal stem cells, NIH/3T3 fibroblasts cells, PC12 cells, DRG/SCG nerve cells, etc.

An ultrashort laser setup is used to create texture on these materials which may range from several nanometers to a few micrometers as shown in Fig.1. [6]. A scanner is used for steering the laser beam with increased accuracy and precision. In the experiment performed on Ti-6Al-4V to study the cell growth of C3H10 T1/2 murine mesenchymal stem cells, Ophélie Raimbault et al., (2016), produced grooves of width 25 µm, 50 µm or 75 µm, depth of 1 µm or 5 µm, and 10 µm for 25 µm width and a constant pitch of 10 µm [8]. In the experiment performed by Rui Zhou et al., (2017), the groove width of 20, 50 and 100 µm and constant pitch of 10 µm were produced on NiTi alloy using a pulsed laser of wavelength 1064 nm to study Human mesenchymal stem cells morphology and its interaction with the surface [9].

In the experiment performed by Guoqing Hu et al., (2018), Mg-6Gd-Ca surface was first ablated using Ti-sapphire chirped-pulse regenerative amplification laser system of wave length 1064 nm and then LIPSS of depth 250 nm and pitch 900 nm were produced on the ablated surface to study the Murine calvarial preosteo blasts cell biocompatibility, Fig.2. [7]. In the experiment performed by Ajay V. Singh et al., (2015), on PDMS, grooves of width 250 nm, depth 500 nm and pitch 1 µm were produced to study cell and nuclear elongation and alignment on patterned surfaces, compared to flat surfaces [10]. In the experiment performed by Evon S. Ereifej et al., (2012) on PMMA, grooves of width 277 nm, 500 nm and depth 200 nm were produced to study the effect of nano patterning on astrocyte cell reactivity [11].

2.2 Characterization

Characterization of samples is primarily done to check the morphology and surface chemistry of the grooves produced which includes groove width, depth, pitch and surface roughness, presence of different chemical composition on surface, etc. Different instruments such as confocal microscopy, AFM, SEM, FTIR, etc. are used for the purpose.

In the experiment performed by Guoqing Hu et al., (2018), characterization of Mg-6Gd-Ca textured surface was done by SEM and AFM

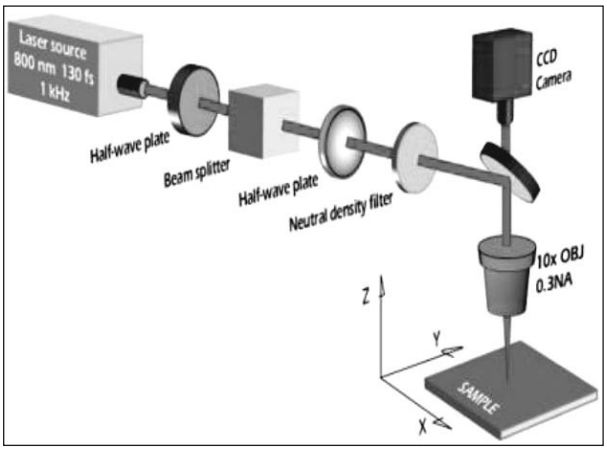

Fig. 1. Laser surface texturing setup.

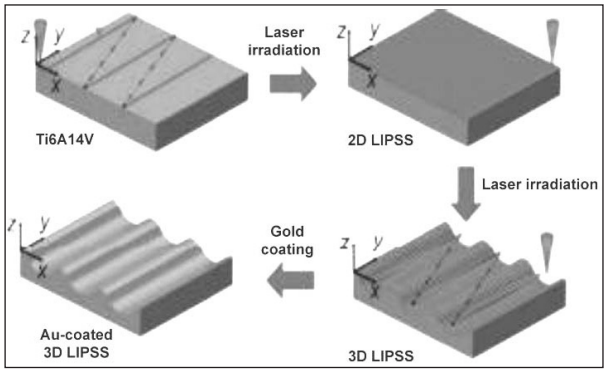

Fig. 2. Laser surface texturing of Mg-6Gd-Ca using Ti-sapphire chirped-pulse regenerative amplification laser system.

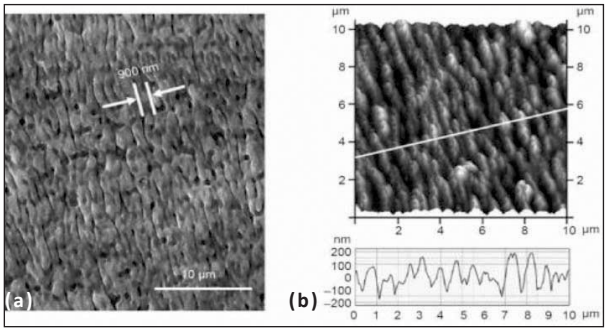

Fig. 3. (a) SEM (b) AFM characterization of laser melted Mg alloy surface.

and morphology of the surface was studied, Fig.3. [7].

2.3 Contact angle measurement

Contact angle measurement is done before and after texturing to check wettability of surface which plays a crucial role in cell adhesion. `

In the experiment performed by Ophélie Raimbault et al., (2016), 2.5 ± 0.2 µl water droplet was used to measure the static contact angle of

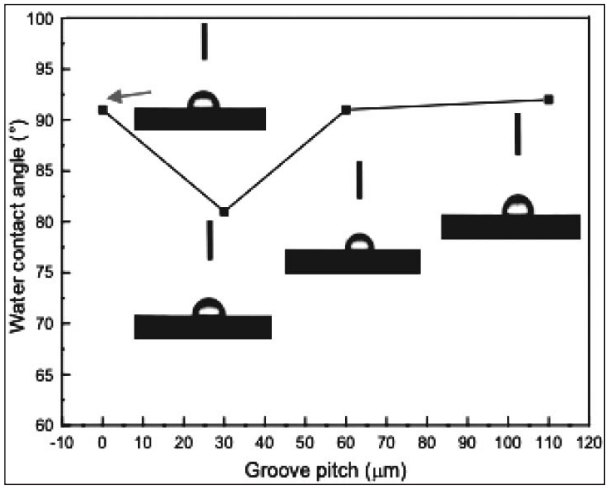

Fig. 4. Contact angle measurement of NiTi alloy.

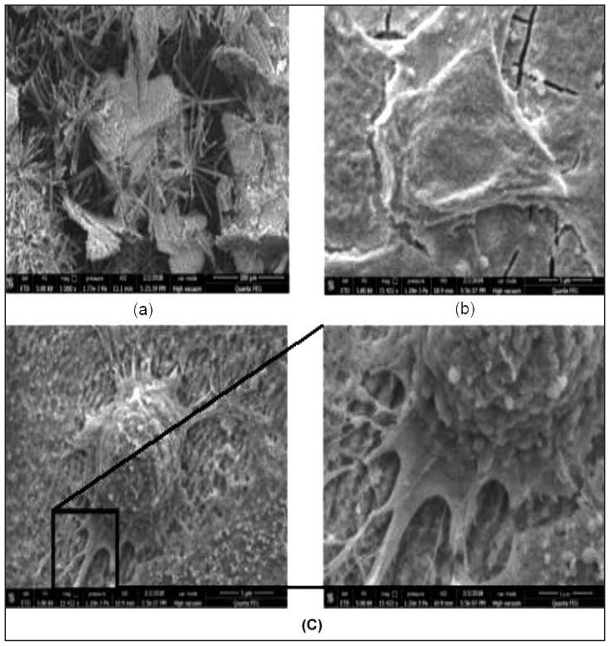

Fig. 5. SEM imaging of (a) non-textured (b) laser melted (c) laser melted and laser textured samples.

samples dipped in boiling water and samples kept in ambient air after texturing [8]. In the experiment performed by Rui Zhou et al ., (2017), 2 µl water droplet was used to measure the contact angles Fig. 4. [9]. In the experiment conducted by Wilhelm Pfleging et al., (2007), distilled water was used to find the contact angle of the textured and non-textured surfaces [12].

2.4 Cell culture

Textured samples are first cleaned using reagents such as chloroform, ethanol, isopropanol, etc. to remove biological and dust particles present on the surface. These samples are then sterilized at

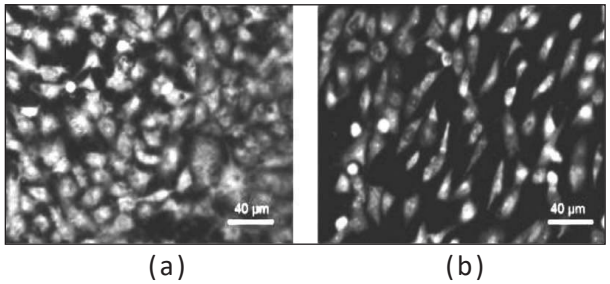

Fig. 6. Fluorescence imaging of (a) laser melted (b) laser melted and textured samples.

a suitable temperature for a certain time period. The sterilized samples are coated with ECM matrix such as collagen, laminin, etc. and are incubated for a few hours in order to prepare them for cell cultures. The cells are then seeded on these prepared samples with other mediums and serum such as DMEM, FBS, etc. at the appropriate concentration and then are incubated at the appropriate temperature to allow cell growth [13]. The growth and proliferation of cells on these samples are checked at periodic time intervals.

After cell culture, cell spreading, proliferation, migration, morphology, viability, etc. are tested using equipment such as SEM, Confocal Microscopy, AFM, etc. In the experiment performed by Guoqing Hu et al., (2018), the cell spreading was studied under SEM for all three samples Fig. 5. [6] (non-texture, laser melted and laser melted and textured). The adhesion of the cells to the surface was studied using fluorescence imaging of laser melted and laser melted and textured surface, which showed varying morphology of the cells as shown in Fig.6. [7].

3. Conclusion

Cell culture mainly depends on the surface chemistry, wettability, surface roughness, and rigidity of the samples on which the cells are being cultured. It can be concluded from the experiments performed on cell culture that textured surfaces have several significant advantages over non-textured surfaces. The cells adhesion is improved and cells stretch heavily on textured surface with long filopodia distribution on their outer edges. The use of pulsed laser for producing these textures has many benefits such as limited heat affected zone, small material distortion, environmentally friendly properties, cost-effective, etc. over other processes such as hot embossing, electrospinning, etc.

Cell adhesion to the substrate surface is one of the essential factors to be noted in any cell culture experiments. They play a significant role in cell communication and regulation, in turn, control cell behavior and function. It can be noted from the experiments conducted that the cells exhibit good surface adhesion on textured samples as compared to non-textured samples. Versatile topographies such as grooves, pores, ridges improve the binding strength of the cells to the surface of the substrates. On texturing with lasers of different wave lengths, 193 nm and 247 nm, on polystyrene samples, the cells showed adhesion on surfaces textured with 247 nm wave length laser at lower fluence as compared to that of the 193 nm laser. The cell adhesion improved significantly at 9 mJ/cm2 and dropped to zero after 19 mJ/cm2. This increase in adhesion can be attributed to the removal of -COOH groups from the surface, which in turn increased the wettability of the surface. The increase in laser fluence causes the increase in surface roughness of the textured surface, which has a negative effect on the hydrophilic nature of the surface, thus affecting the adhesion capability of the cells to the surface.

Wettability of the surface plays a significant role in the adhesion and spreading of cells on the sample surfaces. Cells growing on less hydrophobic surfaces shows a wide area of dense filopodia extension in comparison to cells growing on more hydrophobic surfaces. The wettability of these materials can be modified by laser surface texturing, due to the change in the surface chemistry and morphology. The surface chemistry of the samples can be modified by changing the laser fluence, which in turn causes chemical bond modification. Higher fluence produces groups such as O-C, O=C, etc. which affects the wettability of the surface. The presence of polar groups such as O-C, O=C, O-C=O increases the hydrophilic nature of the surface and whereas non-polar groups such as C-C(H) decrease their hydrophilic nature. As the samples are being ablated formation of oxides takes place which increases the hydrophilic nature whereas a steep change in contact angle can be observed from hydrophilic to hydrophobic when the samples are exposed to air, since the ambient air has various organic matters with non-polar short chain, which due to carboxylation gets chemisorbed into the surface, reducing their hydrophilicity.

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