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# Short Pulsed Laser Surface Texturing of Metallic Implants for Biomedical Applications

**Ahmad W. Alshaer[1*], PhD**

**Fatema H. Rajab[2,3]**

**and Abubaker H. Hamad[3], PhD**

1. School of Engineering, University of Central Lancashire, Preston, Lancashire, UK.
2. School of MACE, The University of Manchester, Manchester, Lancashire, UK.
3. Laser and Optoelectronic Engineering Department, University of Nahrain, Baghdad, Iraq.
4. General Science Department, Faculty of Education, Soran University, Soran City, Erbil, Kurdistan Region, Iraq.

**Abstract:**

Surface texturing has been paid more attention by researchers for bio-applications due to its major role in controlling the integration of the implanted biomaterials and the surface fouling behaviour. Accurate control over the surface chemistry and physical characteristics significantly influence the interaction between the cells and the material's surface regarding adhesion and migration. Short pulsed lasers have been widely used in modifying the surface topography and in generating structures ranging from micro-patterns to nanostructures. So far, bacterial and fouling activities and the biocompatibility of the implants' laser-treated surfaces are not entirely understood. In this chapter, a brief overview of the lasers and techniques utilised in micro- and nano-surface modifications is presented, followed by a detailed discussion of the surface chemistry and topography effect on bacteria aggregation

* Corresponding author: awalshaer@uclan.ac.uk or ahmad_sh1986@yahoo.com

and adhesion. Also, the role of the laser-induced superficial patterns on the response and sensitivity of bio-implants will be explored in depth

## Keywords:

Short pulse lasers, surface texturing, implant, human cell, bacteria.

## 1. Introduction

Biomaterials have received increasing interest by researchers in the recent years in different applications including biology, physics, implant design and cell and drug research (Caruso, 2001, Cheng et al., 2004, Katsikogianni and Missirlis, 2010, Bazaka et al., 2011). Due to the complex response of living organisms to the bio-surfaces, many factors play a crucial role in the interaction between cells and the bio-devices or implants. It is essential, for designing the biochips (Lamolle et al., 2009, Wu et al., 2014), bio-detectors (Liu et al., 2004), orthopaedic and dental prostheses (Chu et al., 2002, Anselme et al., 2010), to understand the mechanism behind the cell-biomaterials interaction in order to tailor each product to the suitable medical or engineering applications. For example, the growth of the living cells can be promoted in a microchip matrix (El-Ali et al., 2006) by controlling the cell culture in the microchannels inside the microfluidic system (Zare and Kim, 2010, Sugioka et al., 2010, Sugioka and Cheng, 2012). Also, controlling the cancer cells' migration through a confined microchannels was used as a method for preventing cancer cells from sneaking into the blood stream or lymphatic system (Malboubi et al., 2015). Therefore, methods for fabricating microchips and modifying implants' surfaces have been developed to offer full control over the cells' response at the interface. The topography and chemistry of the bio-surfaces not only influence the cell's morphology but also affect the cellular behaviour regarding spread/elongation, migration, proliferation, orientation and protein synthesis (Murugan et al., 2009, Liu et al., 2005). Before reviewing these aspects, it is crucial to differentiate in the behaviour of the human cells and bacteria due to the differences in their cell's shape, biological structure, functions, reproduction mechanism and their movement. Human cells are eukaryotic and static with either spherical or oval shape and depend greatly on other cells for their survival. However, bacteria are independent organisms which have different shapes and functions and can survive independently even in harsh environments. Moreover, bacteria can move and rotate

using their built-in flagella and can, therefore, easily migrate between locations unlike human cells (Ranganr, 2017).

The significance of the differentiation between these types of cells is vital for the design of the scaffolds' or implants' surfaces since each type of these surfaces has a unique interaction mechanism with the cells in contact. Human cells are usually needed to adhere and grow on the prostheses surfaces or migrate through miniaturised laboratories that perform analysis, processing or separation (Huebsch and Mooney, 2009, Sugioka and Cheng, 2011). Therefore, the surfaces of the medical devices and engineered tissues should promote cells attachment, provide a stimulating microenvironment for their proliferation and function as if they are in vivo (Koufaki et al., 2011). On the other hand, bacteria have detrimental effects on tissues causing diseases and infections. Hence, their presence can be fought by preventing their adhesion on the surface of the biomaterial or by killing them. Three types of bacteria-biomaterial interactions, namely: bactericidal, anti-fouling and bacteriostasis, should be understood to modulate the surface of interest using the correct fabrication methods. Bactericidal effects embrace the engineered surfaces' ability to kill the bacteria by penetrating the cell or destroying the protective cell wall as occurs in the case of nano-pillars which rupture the bacteria's cell wall (Pogodin et al., 2013). Anti-fouling surfaces, however, prevent the bacterial adhesion by introducing periodic micro- or nano- structures smaller than the bacteria size. These structures reduce the contact area used by the bacteria to attach to the bio-surface and hence prevents their colonisation. Lastly, bacteriostatic effects of antibacterial surfaces or agents mean that the bacteria are prevented from reproduction without being killed or prevented from adhesion to the surface (Price, 2006). Figure 1 provides a representation of the mechanism whom micro/nanostructure use for killing bacteria.

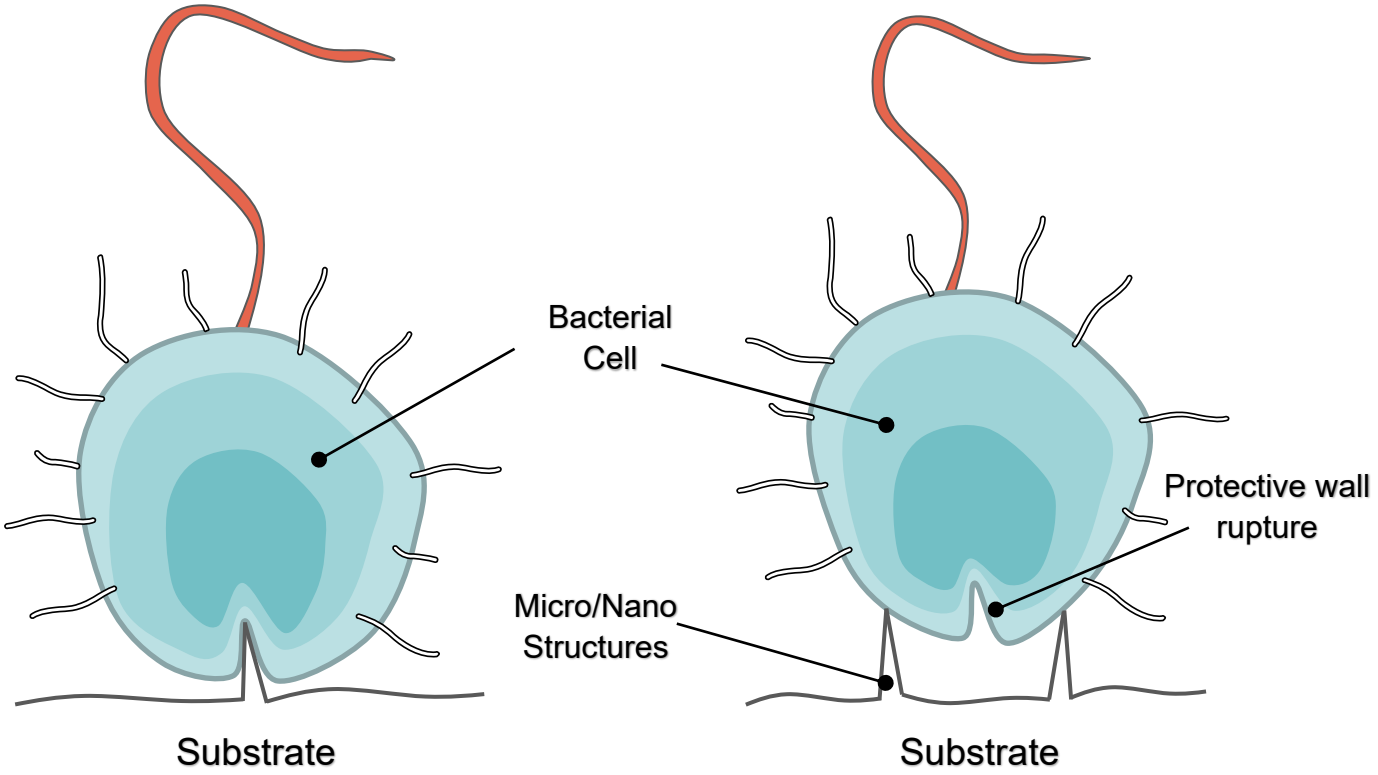


**Figure 1.** Micro-/nano-structures are killing the bacteria by penetrating their internal components **(left)** or by stretching the bacteria's wall causing its rupture **(right)**.

## 2. Surface Modification Methods

Over the last two decades, natural surfaces have been engineered for cell adhesion enhancement using two main approaches: substrates coating with bioactive substances and direct modification of the superficial chemistry or/and topography (Price, 2006).

The former embraces the introduction of a bioactive agent to the surface using different techniques such as polymerisation, functionalisation and derivatisation (Hasan et al., 2013, Cooke et al., 2008, Alves et al., 2009). However, these methods are usually carried out in multiple steps using various materials which, in some cases, undergo residual stresses and/or delamination (Maruo et al., 1997, Davis et al., 1996, Ching et al., 2014).

Micro- and nanostructures such as grooves, pillars and pores have been created on the surface to modify its topography using various techniques such as lithography (Loesberg et al., 2007, Qin et al., 2010, Zheng et al., 2013), direct electron beam vaporisation (Puckett et al., 2008), imprinting (Truskett and Watts, 2006, Plasschaert and Bartolomei, 2014), polymer demixing (Dalby et al., 2002a, Dalby et al., 2002b, Lim et al., 2005), plasma micromachining

(Chu et al., 2002, Avram et al., 2007, Oehr, 2003) and microscale chemical patterning (Oliveira et al., 2014, Liu and Wang, 2014) etc. Figure 2 shows different traditional methods for surface modification of the biocompatible materials.

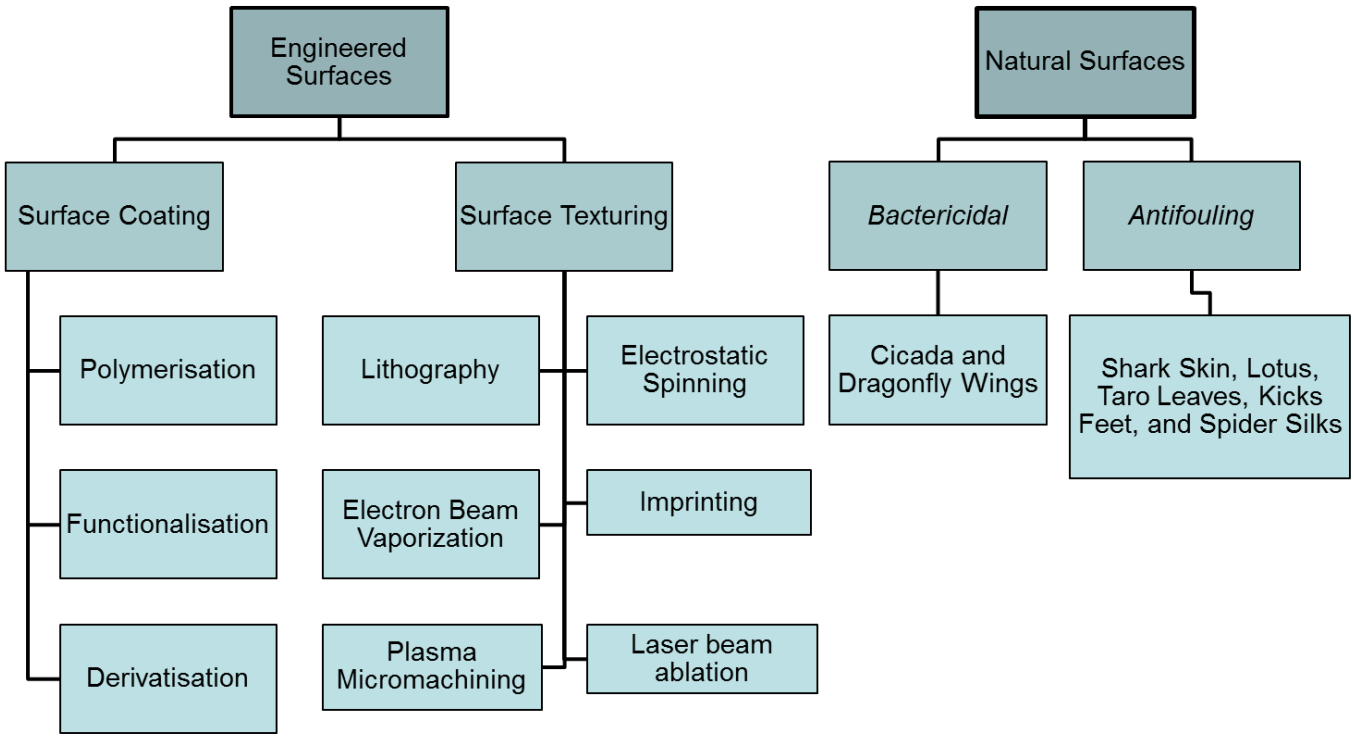


**Figure 2.** Different types of bio-surfaces and the techniques used for surface modification

Short pulsed lasers were used to replace the long and expensive processes, used for creating multi-level structures using masks, special materials and environments, by a single and a direct step for creating uniform micro- or nanostructures on various material in ambient atmosphere (Nuutinen et al., 2012). This process is usually referred to as Light Induced Periodic Surface Structures (LIPSS) (Qi et al., 2009, Zhao et al., 2007). Short laser pulses are versatile in their output and can be easily produced with multiple spatial and temporal distributions and polarisation needed for the production of periodic surface structures. Among the commercially available lasers, ultrashort lasers (such as femto- and picosecond lasers) have an additional privilege over longer pulse lasers (nano- and microsecond lasers) due to the localised thermal effect and the limited heat affected zone generated in the substrate (Chichkov et al., 1996). Figure 3 shows an example of picosecond and nanosecond laser-induced surface structures on a stainless steel substrate.

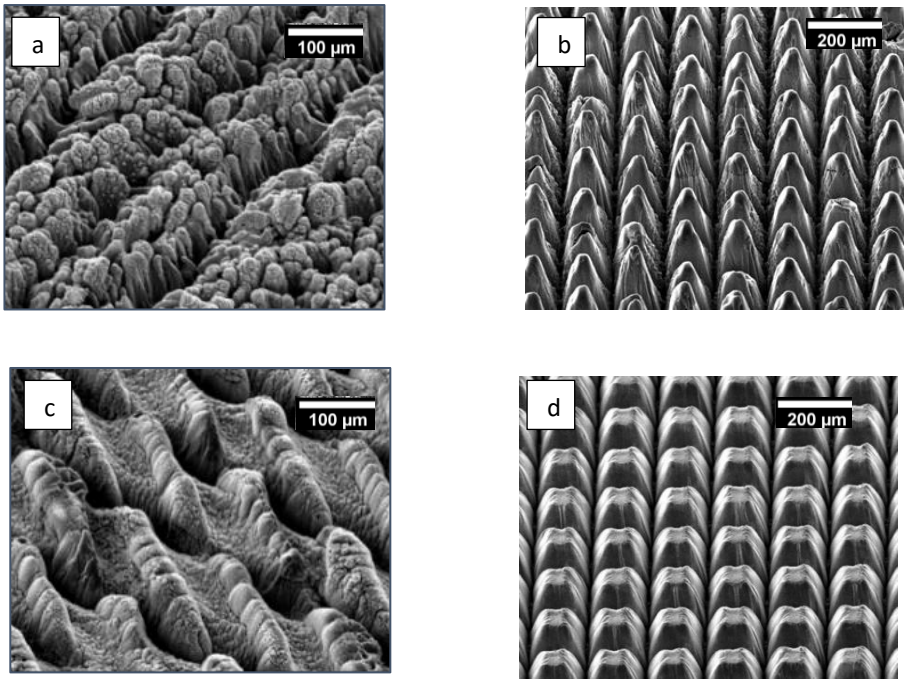


**Figure 3.** Textured surfaces using a 125 µm hatching distance and different scanning direction and laser parameters **(a)** 30 deg and 60 deg, nanosecond pulses of 9.2 J/cm$^2$, 1 mm/s, single pass **(b)** 0 deg and 90 deg, nanosecond pulses of 9.2 J/cm$^2$, 50 mm/s, 100 passes **(c)** 30°& 60°, picosecond pulses of 2.6 J/cm$^2$ 1 mm/s, single pass **(d)** 0 deg and 90 deg, nanosecond pulses of 2.6 J/cm$^2$, 50 mm/s, 100 passes.

In the following sections, the influence of the laser pulses on surface topography and chemistry is thoroughly reviewed for controlling human and bacterial cells' behaviours.

## 3. Effect of Laser-fabricated Structures on Cell Behaviour

The number of hip and knee endoprostheses recorded in 2015 reached more than 160,000 and 375,000 cases in the UK and Germany respectively (National Joint Registry, 2015, Statistisches Bundesamt (Destatis), 2015). This number is ever increasing each year with an urgent need for biomaterials that can meet the biological compatibility of the inserted devices, chips and implants in the human bodies' tissues which are very diverse in their functionality and composition (Anderson, 2001, Babensee et al., 1998). The biocompatibility of the material may reduce over time because of the inflammatory reactions resulted during the formation of fibroblast scar tissues, causing harmful influence to the implant and hence the patient. Due to the detrimental effects of the fibroblast proliferation, biomaterials have to selectively inhibit the fibroblast reproduction yet to simultaneously enhance the neuronal

attachment and differentiation in the area of interest. In this case, inflammation will be hindered, and the neuronal cells can easily adhere to the biomedical surfaces (Fadeeva et al., 2013).

Manipulating the biomaterial's surfaces is an effective approach for stimulating the biocompatibility. It has been approved that the surface morphology (structure and roughness) and chemistry (wettability) influence the cell orientation, morphology, proliferation and differentiation (Flemming et al., 1999, Wilkinson et al., 2002, Fadeeva et al., 2009a). However, laser-induced surface structures are preferable because they are simple to manufacture, environmentally clean and can be fabricated on different substrates and in ambient and non-standard atmospheres (Gaggl et al., 2000, Pető et al., 2002). Table 1 lists some of the biomaterials used for cell response control.

**Table 1.** Metallic and non-metallic materials used in cellular behaviour control

| Material | Application(s) | Ref. |
|---|---|---|
| Silicon | Cell Proliferation | (Fadeeva et al., 2009b, Fadeeva et al., 2013) |
| Stainless Steel | Controlling Cell Spreading/Elongation, Localization and Orientation. | (Nuutinen et al., 2012) |
| Polycarbonate | | |
| Polydimethylsiloxane (PDMS) | Selective Cell Adhesion | (Alshehri et al., 2016) |
| Chitosan | Bacterial Cell Growth Control | (Estevam-Alves et al., 2016) |
| Chitosan (Positively charged) | Promoting Erythrocyte adhesion, Fibrinogen adsorption and Platelet adhesion and activation | (He et al., 2011) |
| Titanium | Orthopedic and Dental Applications | (Chai et al., 2010, Cei et al., 2011, Orsini et al., 2012, Ota-Tsuzuki et al., 2011) |
| Platinium | | (Fadeeva et al., 2013) |

## *3.1. Effect of Surface Geometry*

Due to the critical role of the fibroblast in tissue regeneration and wound healing, research has been carried out to study the effect of laser induced surface topography on fibrotic

encapsulation within different environments. The surface of various substrates including silicon, titanium, stainless steel, polymers and platinum has been processed using femtosecond pulsed laser which produces high-quality structures and small heat affected zones.

### *3.1.1. Effect of surface roughness*

It was noted that surfaces with low roughness values stimulate fibroblasts NIH/3T3 cell's attachment (Ranella et al., 2010). The silicon substrate was modified by producing conical structures (spikes) of different dimensions and aspect ratio, and their attachment and proliferation were studied in comparison with other cell types such as Neuroblastoma and Osteoblasts (Schlie et al., 2011, Schlie et al., 2012, Ranella et al., 2010, Fadeeva et al., 2014, Fadeeva et al., 2009b). Both infrared (800 and 1064 nm wavelength) femtosecond (30-150 fs) and picosecond (12 ps) laser pulses were utilised to produce these spikes in a Sulfur Hexafluoride (SF6) gas atmosphere.

The fabricated spikes not only reduced the number of the fibroblast on the textured areas but also changed the fibroblast cell morphology from elongated to rounded cells on the nano- and micro-structured silicon and platinium substrates. According to the measured cells numbers, fibroblasts proliferated at much lower rates in comparison with Neuroblastoma and Osteoblasts whom numbers were not negatively influenced (Fadeeva et al., 2014, Schlie et al., 2011). It was noted that fibroblasts prefer to settle on top of the spikes whereas other cells attach and spread on both the top and the bottom (the grooves) of the spikes. This trend in settlement indicates that the conical microstructures increased the surface-cells contact area and hence promoted their proliferation after achieving the adhesion (Fadeeva et al., 2014, Schlie et al., 2011).

Fadeeva et al. (2009b) used femtosecond laser pulses to generate quasi-regular spiky structures on silicon and silicone elastomer. The fabricated structures enhanced the hydrophobic character of the surfaces by increasing the contact angle for water drops in the sessile drop tests by about 47 degrees. The increase in hydrophobicity can be explained by the air molecules trapped within the micro- and nano-structures which form an "air bag" to separate particular type of cells from the surface. The response of a particular type of cells to the surface wettability varied depending on the substrate material and the surface condition (the roughness produced by the microstructures). For instance, fibroblasts can easily adhere

to the silicon and glass surfaces, which are hydrophilic in nature, and can subsequently spread and grow to large numbers. However, a significant reduction in fibroblast's adhesion was observed once silicon and silicone elastomer surfaces were textured using femtosecond lasers. The laser-induced reduction in the surface wettability made it difficult for such cells to bond and culture on the surface. On the contrary to the fibroblast's behaviour, SH-SY5Y Neuroblastoma cells did not show any significant change in their adhesion response to the textured and non-textured surfaces and continued to adhere well to the surfaces regardless of their type and condition. This selective response can be attributed to the different mechanisms each type of cells use for their adhesion and proliferation. The cell attachment is usually governed by the intracellular signaling pathways via Ras, Rho and MAPK cascades in the cytoskeleton (Clark and Hynes, 1996, Giancotti and Ruoslahti, 1999).

This selective adhesion of the cells was also noted when PDMS (Fadeeva et al., 2009b, Koufaki et al., 2011), positive photocurable and poly (lactide-coglycolide) polymers (Koufaki et al., 2011) were textured using 150 fs laser pulses. The results showed that the fibroblasts NIH/3T3 cells preferred the adhesion on surfaces with low roughness regardless the polymer type and its wettability. Similarly, PC12 cells tend to adhere to the textured surfaces irrespective of its roughness and wettability (Koufaki et al., 2011).

Paul et al. (2008) discussed the influence of the laser-ablated surface patterns on the macrophage function of Polyvinylidene fluoride (PVDF) using an ArF Excimer laser pulses of 1.08 J/cm$^2$ fluence. The created surface features were dome-like microstructure of 1.8 µm in height and about 10 µm in diameter with a spacing of 30 µm from centre-to-centre. It was found that the cell polarisation (by monitoring CD163 and 27E10 expressions) was increased in the case of micro-scale features compared to the nanoscale structures. On the other hand, C2C12 cells and the rabbit anti-mouse protein were found to bond to the surrounding of the patterned regions due to the presence of nanoscale features (the debris of ablation process which generates nanoparticles) (Alshehri et al., 2016).

### *3.1.2. Effect of structure size and replication*

The size of the spikes also played an active role in altering the fibroblasts adsorption when it was promoted using small spikes with a spike-to-spike distance of about 2 µm and 4 µm, while it was reduced using bigger spikes of size 6 µm (Schlie et al., 2012). The fibroblasts shape was also modified according to the structures size. Elongated morphology, which is usually the

common shape for fibroblast cells, were observed on surfaces with small spikes, while rounded cells were dominant when large spikes exist. The elongated shape helps the cell to spread and proliferate (Schlie et al., 2012).

In a relevant research (Marticorena et al., 2007), titanium foils were laser patterned using a pulsed Nd:YAG laser with a UV light of 355 nm wavelength and 2.5 J/cm$^2$ fluence. It was proved that the titanium nitride TiN layer formed above the laser-treated surface along with the high surface roughness significantly improve the bone response to the introduced implant comparing to the untreated Ti. The generation of spikes on the Ti substrate resulted in a significant reduction in the fibroblast proliferation to about 91% and a rapid Osteoblasts growth from 163% (control) to 203% (laser-textured Ti) (Schlie et al., 2011).

Regular groove structures and self-organized hierarchical micro features with nanostructures were generated on Ti substrate and its effect on human fibroblast and MG-63 osteoblasts were investigated. Different groove dimensions were fabricated, and it was observed that these grooves play a major role in forming a contact guidance for the cultivated cells. No difference in cell response and morphology between fibroblast and osteoblasts was recorded for all groove sizes apart from the surface of a 20 μm groove width. A strong connection was noted between the size of the cells and the underlying features since fibroblasts could not recognise the grooves of 20 μm width, while the osteoblasts cells responded very well and grew according to the pattern generated. It was concluded that the cell width is a critical parameter for determining the cell reaction with grooved structures because the width of the fibroblast cell (20 μm) is smaller than that of the osteoblast (26 μm), while the cell's length is larger for fibroblast (124 μm) than that of the osteoblast (111 μm). Therefore, wide grooves cause less cell orientation since the narrow fibroblast did not show a significant response to the 20 μm groove width (Fadeeva et al., 2010). It should be noted here that the fibroblast cells take a rounded shape in a hierarchical structure with very limited growth over the complex hierarchical structure. This is attributed, as previously discussed, to the high roughness values and to the inflexibility of the cell cytoskeleton which prevents the cell from altering their shape and settling between the micro-features. However, osteoblast cells proliferated very well, and its growth was promoted due to its high flexibility and ability to adaptation (Fadeeva et al., 2010).

Liang et al. (2013) studied the generation of different micro/nanostructures using Coliseum/Phosphorus deposition on titanium surfaces. Titanium substrates were immersed

in 2 mg/ml nano-hydroxyapatite suspension with a 2.5 mm solution/air interface. Then, the titanium in the solution was patterned using fs laser producing two surfaces of microgrooves covered by a secondary structural layer of nano peaks and valleys at two different laser fluences. While at low fluences, 10 μm periodic with 1.5 μm depths were formed, 40-50 μm structures with a 4 μm depth were fabricated at high fluences. It was observed that there was no difference in the MC3T3-E1 cells' attachment and proliferation between non-textured and textured Ti at low fluences (low periodicity), while 50% of the seeded cells were attached to the high-periodicity structures indicating a 60% improvement over the polished Ti samples. The results concluded that surfaces with high periodicity exhibited better osseointegration, including active attachment, proliferation and division, with the bone tissues compared to low-periodicity samples and polished Ti. This conclusion was further supported by the high value of the binding force obtained during the test of tibia tissue with bone trabecula formation without fibroblast tissue (Liang et al., 2013).

#### *3.1.3. Effect of laser scanning direction*

LIPSS effect on the human Mesenchymal Stem (hMSCs) cell behaviour was recently investigated. Two types of LIPSS were induced on stainless steel samples in a two-strips pattern using fs laser. One of the directions was parallel to the scanning direction while the second was engraved at 40 deg to the strip direction. It was noted that the cell preferentially adhered to the LIPSS grooves parallel to the strips and high values were recorded for the cell density. The cells preferentially migrated to the fabricated nanopatterns while avoiding the polished or as-received areas since the larger contact area was provided by the former (Martínez-Calderon et al., 2016).

## *3.2. Effect of Surface Chemistry*

Regarding the effect of wettability, Ranella et al. (2010) found that the hydrophobicity of the spiky surfaces was increased with enlarging the spikes' dimensions (width and height). This resulted from the poor cell attachment to the modified surfaces. For comparison, hydrophilic spikes were produced by thickening the oxide layer during the samples' heating in a furnace at 1000$^{o}$C for 30 minutes in ambient atmosphere. However, to generate superhydrophobic spikes, surfaces were coated with saline after the laser treatment.

With increasing the hydrophobicity of the surface, cell's attachment, cultivation and spread were inhibited. According to the surface condition, the cell responded and formed what is known as a polygonal spread (extensive cell-surface interaction) on the hydrophilic surfaces and formed a rounded shape with clustering (weak cell-surface interaction) on the hydrophobic surfaces (Ranella et al., 2010).

Furthermore, Polydimethylsiloxane was linearly textured using fs laser and the effect of textured surfaces were studied on protein adhesion and cell growth. It was found that the cells adhered on the non-textured areas located between the nano-features. The study suggested that the nanostructures actively facilitated the cell adhesion in certain areas over others. This allows the production of complex scaffolds with customised cell adhesion in certain parts (Alshehri et al., 2016).

In addition, it was noted that the replication of spike structure in Si and silicone elastomer switched the surface condition from hydrophilic (spikes on Si) to hydrophobic (silicon elastomer spikes). This replication helped to reduce the fibroblast's proliferation compared to Neuroblastoma's growth, which was promoted on silicone elastomer (Fadeeva et al., 2009b). Koufaki et al. (2011) studied the effect of wettability of replicated spike structure of three types of polymers on cell adhesion. It was concluded that the fibroblast avoids the hydrophobic areas whereas PC12 cells seem to adhere well with high density compared to the non-textured areas (Koufaki et al., 2011). Fadeeva et al. (2010) found that the contact angle of a laser textured Ti with a groove structure increased when it was measured in the direction perpendicular to the slot direction and reduced in the direction parallel to groove after laser processing. This led the cells to spread in a direction parallel to the slot. The authors concluded that the production of the hierarchal structure with super-hydrophobic properties reduced the fibroblast adhesion, while the osteoblast adhesion was enhanced compared to the non-textured Ti surface (Fadeeva et al., 2010).

## 4. Surface Characteristics and Bacteria Aggregation and Adhesion

Statistics showed that 1.5% of the introduced implants in the human body is associated with implant-associated infections (IAI). Although this percentage seems low, such infections can lead to severely detrimental complications to the patients, physicians and the health care system. Different types of bacteria can exist in this kind of infection including Staphylococcus

aureus, a biofilm-forming coagulase-negative Staphylococcus epidermidis (S. epidermidis), or gram-negative species (Zaatreh et al., 2017).

Over the last decades, various materials have been used in manufacturing the implant, bio-chips and devices. Heavy metals such as copper, zinc, silver, arsenic have antibacterial properties and can be used in different applications except for human contact since they are toxic (Medlin, 1997). Therefore, in this review, we will only discuss metals and non-metals that are biocompatible with the human body and do not rise any toxicity issues.

It should be noted here that one deals, in disinfection tests, with three types of bacteria present at the same time: sensitive, moderate and resistant. Sensitive bacteria can easily be killed after a short period of exposure to the antibacterial material, while resistant bacteria can stand toxic environments for longer periods of time. Some antibacterial biomaterials can be bacteriostatic for short periods and bactericidal for long periods. Therefore, care should be taken while carrying out such tests when the antibacterial effect is being evaluated (Price, 2006). Table 2 gives the medical applications for different biomaterials with cell anti-adhesive/antibacterial characteristics.

Surface chemistry and topography have a significant effect on the antibacterial activity of biofilms or antibacterial surfaces. The key for the antibacterial activity of a surface textile is how the bacteria cells attach to and aggregate on the surface. One should note that “adhesion” term describes the attachment of a cell to a substrate while “cohesion” is called after the cell-to-cell attachment or aggregation (Garrett et al., 2008).

A variety of techniques has been used to modify and develop the surface topography and chemistry of biomaterials to change the bacterial response and promoting desirable cell phenotypes. The understanding of the aggregation and the bacterial adhesion mechanisms is governed by several physical, chemical and biological processes.

Fletcher (1980) described the aggregation of bacteria on a biofilm or a textured surface as a process of three stages: firstly, adsorption of bacteria on a collector surface; secondly, the attachment at the interface between bacteria and the collector surface which form a polymer bridges between the bacteria and surface; and Finally, the bacteria growth and division on the collector’s surface. Characklis and Marshal (1990) proposed an expanded process consisting of eight steps: the formation of an initial layer, reversible adhesion of bacteria, irreversible adhesion of bacteria, and the ultimate detachment of cells from a mature biofilm as a preparation for subsequent colonisation (Garrett et al., 2008).

**Table 2.** Various materials with cell antiadhesive/antibacterial characteristics and their applications

| Material | Behaviour (bactericidal/anti fouling) | Application(s) | References |
|---|---|---|---|
| Poly (Ethylene Glycol) /Carboxyl-Containing Ethylene | Anti-fouling | Implants Surface Engineering, Anti-Adhesive Properties, Heparin Surface Treatment, Medical Instrument Protection and Disinfection | (Ackart et al., 1975, Desai et al., 1992, Bridgett et al., 1992, Arciola et al., 1993, Kohnen and Jansen, 1995, Koufaki et al., 2011) |
| Titanium | | Orthopedic and Dental Applications | (Duarte et al., 2009, Cunha et al., 2016) |
| Antibiotics | Bactericidal | Surface Modification of Polymers | (Kohnen and Jansen, 1995) |
| Quaternary Ammonium Compounds | | Nonwoven Polypropylene Fibre Extrusion and Cosmetics | (Nohr and Gavin Macdonald, 1994, Buffet-Bataillon et al., 2012, Timofeeva and Kleshcheva, 2011) |
| Silver | | Medical, Biosensors, Paints, Tissues Dressings and Cosmetics | (Kumar and Münstedt, 2005, Ahamed et al., 2010) |
| Iodine | | Medical and Antibiotics | (Kristinsson et al., 1991) |
| Nylon Film | | Food-Packaging | (Shearer et al., 2000) |

Keselowsky et al. (2003) showed that adhesion of the human cells and bacteria follow similar trends. Positively charged surfaces have greater adhesion than hydrophobic surfaces with existed blood pertains, while hydrophilic surfaces have the strongest cellular adhesion in comparison with the charged or hydrophobic surfaces.

In related research, the conformation and accessibility of ligands were found to considerably affect the irreversible adhesion of microorganism to materials with modulated surface chemistry, and the biofilm will be produced as a result (MacKintosh et al., 2006). The adhesion and growth of bacteria in biofilms are affected by the nanostructured surface. So far, there is no quantitative understanding of the influence of nanoscale surface morphology on prokaryotic cell attachment. Singh et al. (2011) confirmed that the increase of the surface

roughness is proportional to the increase in surface voids aspect ratio and volume which improves bacteria adsorption. When the surface roughness increases up to 20 nm, bacterial adhesion and the biofilm formation are improved. On the other hand, the continuous increase in the roughness leads to considerably weak bacterial adhesion and prevents biofilm formation.

## *4.1. Bacterial Adhesion Mechanism*

The bacteria depends during the adhesion process on the physiochemical reactions that occur between the bacteria's outer surface and the implant or the biochip (Oliveira et al., 2003). There is two different types of bacterial adhesion: reversible and irreversible adhesion.

In reversible adhesion, the bacterial cell is driven towards the substrate's surface either by physical forces that originate from the environment or by the appendages, such as flagella, fimbriae and pili, which pushes the bacteria to overcome the repulsive forces (Garrett et al., 2008). There are many forces bacteria have to resist to achieve its attachment: van der Waals, steric and electrostatic (double layer) interaction forces (Rutter and Vincent, 1980). It should be noted that most bacteria maintain a negative charge on their outer surface which includes an acidic hydrophilic polymer. This may explain the preferable bacteria adhesion to some of the positively charged hydrophobic surfaces (Harden and Harris, 1953). However, once the first layer of the bacteria is established, the surface charge becomes negative, and the following bacteria find it a challenge, in this case, to adhere to the negatively charged layer. This may explain the reduction in the bacterial growth rate on some bio-surfaces in comparison with the initial adhesion phase (Fletcher, 1977). Regardless the type of opposing forces, bacteria will be able to adhere to the surface if they can overcome the hydro- and electro-static forces imposed by the bio-surfaces.

In irreversible adhesion, bacteria overcome the repulsive forces and continue its way until it contacts the bio-surface and becomes immobilised. Once the contact is established, a chemical reaction, such as oxidation or hydration, takes place and the bacteria is attached to the substrate and colonisation begins (Kumar and Anand, 1998, Garrett et al., 2008).

It is important to note that there are many factors contribute to the adhesion process including hydrophobicity/hydrophilicity, surface roughness, the chemical composition of the substratum, electric charge and the surrounding environment (Sousa et al., 2009). Table 3

summarises the different materials, bacteria type and the surface condition and charge investigated so far.

**Table 3.** Effect of surface condition (wettability) and the electric charge on bacterial attachment to various materials

| Material | Bacteria | Surface condition | Surface Electric Charge | Attachment | Ref. |
|---|---|---|---|---|---|
| Chitosan | S. Aureus | Hydrophilic | N/A | High | (Estevam-Alves et al., 2016) |
| Platinum | *Pseudomonas* Sp. | | Positive | Medium | (Fletcher and Loeb, 1979) |
| Germanium | | | Neutral | | |
| Glass | | | Negative | Low | |
| Mica | | | | | |
| Nylon | | | | | |
| Epoxy | | | | | |
| PTFE Teflon | | Hydrophobic | N/A | High | |
| Polyethylene (PE) (Sterilin) | | | | | |
| Polystyrene (PS) | | | | | |
| Poly(ethylene terephthalate) (PET) | | | Neutral | | |
| Silicone Rubber | *Candida, Pseudomonas* Streptococci and Staphylococci | | N/A | | (Sousa et al., 2009, Boswald et al., 1995, Gristina et al., 1988) |
| Acrylic | S. Epidermidis | | | Low | |
| Expanded Polytetrafluorethylene (ePTFE) | | | | High | (Oliveira et al., 2001) |
| Silicone | | | | Medium | |
| Polyethylene (PE) | | | | | |
| Cellulose Diacetate (CDA) | | | | Low | |

| Material | Bacteria | Surface condition | Surface Electric Charge | Attachment | Ref. |
|---|---|---|---|---|---|
| Polystyrene (PS) | Spherical Cocci, Rod-Shaped Bacteria and Stalked Rods | $\gamma_{critical}$= 33 dyn/cm | N/A | High | (Dexter et al., 1975) |
| Cooper | Different Species | $\gamma_{critical}$= 45 dyn/cm | | | |
| Nickel | Rod-shaped Bacteria | | | | |

Some investigations (Busscher and Weerkamp, 1987, Van Oss and Giese, 1995, Wiencek and Fletcher, 1997) stated that hydrophobicity may be the dominant factor in affecting the bacterial adhesion compared to the surface roughness or the chemical composition. It is thought that the interaction between bacteria, whom outer surface has hydrophobic characteristics, and the hydrophobic substrates are the more potent in the long term. This can be related to the hydrophobic interaction that happens when the water or the liquid is expelled from the contact area between the attracting surfaces. This force becomes less effective when one of the surfaces is hydrophilic and the liquids are attracted to the interface (Oliveira et al., 2001).

As known, hydrophobicity can be fully controlled by texturing the surfaces and changing their roughness and topographical characters. Creating superficial features also promotes irreversible adhesion by allowing the bacteria to insert their cells in the holes, grooves and other protrusions fabricated on the surface. This immobilises the bacteria and offers an excellent environment for proliferation given that the size of the bacteria is smaller than the introduced features (Garrett et al., 2008). For instance, it was shown that the nanoscale morphological features have a considerable effect on biofilm formation and bacterial adhesion on the surface of nanostructured titanium oxide (Singh et al., 2011).

To conclude, bacteria can irreversibly adhere to the surfaces once they are textured to achieve the optimum wettability and topography. The following section, therefore, will discuss in some detail the effect of the surface condition on the bacterial adhesion.

## *4.2. Effect of Surface Wettability*

Most bacteria types have hydrophobic properties assigned to their protective wall and adhere more preferably at high rates to silicone than to acrylic; this is due to its higher roughness and

greater degree of hydrophobicity (Sousa et al., 2009). However, an opposite findings were presented by Ranella et al. (2010) who concluded that the low roughness values stimulate the bacterial adhesion regardless the chemistry and wettability of the surface. Schumacher et al. (2007) introduced a relationship between the characters of the fabricated topographical features and the bacterial cell adhesion using the so-called Engineered Roughness Index (ERI). This dimensionless number was found to be inversely proportional to the number of cells which adhered to the considered surface. The higher ERI values for a given surface, the lower the number of cells attached to that surface. Therefore, ERI number rises to a significant value for Sharklets patterned surfaces in comparison to the polished ones, indicating that the cell attachment density on the former is significantly less than other surfaces (Graham and Cady, 2014). Compared to randomly texturised surfaces, uniformly engineered surface with specific dimensions and shapes showed a higher degree of controllable inhibition over initial cell adhesion (Graham and Cady, 2014). However, a contrary finding was concluded by Duarte et al. (2009) who compared the efficiency of textured and as-received titanium surfaces on S. Sanguinis adhesion reduction. The rough surfaces, which have $R_a$ values around 700 nm, were found more prone to bacterial adhesion than smooth polished surfaces with $R_a$ values less than 200 nm. Moreover, a rough surface treated with a metal curet and an air-powder abrasive jet, which is a rough surface, was found to be less susceptible to bacterial adhesion in comparison with Er:YAG laser treated surfaces, which are smoother than the mechanically abraded surfaces (Duarte et al., 2009).

So far, it can be seen that the relationship between surface hydrophobicity and roughness and bacterial adhesion is still under argument by researchers who are investigating different materials with surface conditions and different bacteria types. Therefore, further understanding is needed to clearly identify the bacterial behaviour to contrast surface conditions. In the following subsection, laser surface texturing technique for controlling bacterial behaviour is highlighted.

## *4.3. Effect of Laser Texturing on Bacterial Behaviour*

Various methods have been used to modify the surface chemistry and topography to control and modulate DCs, adverse immune bacteria's interaction with biomaterials and the macrophage phenotype towards anti-inflammatory phenotypes (Rostam et al., 2015).

However, laser ablation is receiving an increasing attention due to being a dry and contactless technique and an environment-friendly method compared to the chemical etching or multilevel surface modification which embraces many chemical reactions with the substrate. Femtosecond laser surface texturing was reported in the field of nano/micro- texturing of biomaterials in many studies including (Nayak et al., 2008, Ionin et al., 2013), which concentrated on low fluence values less than 2.5 J/cm$^2$. Ionin et al. (2013) imprinted hydroxylapatite nano/micro-powder onto the prepared titanium surfaces to produce nano/micro-scale structures using fs laser pulses of fluences 0.5-2 J/cm$^2$. The titanium wetting characteristics were also tuned towards hydrophobicity or hydrophilicity depending on the laser processing parameters especially the scanning speed and the pulse fluence. Despite the success in producing biocompatible micro/nanostructures, the research focused on the hydrophobicity without providing any critical evaluation of the bacterial behaviour and its interaction with the modified surfaces. This issue was also mentioned in (Nayak et al., 2008) which discussed the formation of sharp and quasi-regular pillars on titanium substrates using fluences in the range of 1.5-2.5 J/cm$^2$. Fadeeva et al. (2011) clearly addressed this issue while introducing the lotus leaf-like microstructures on titanium and studied the influence of the superhydrophobic properties on the S. aureus and P. aeruginosa proliferation. P. aeruginosa cells were significantly prevented from fouling to the surface, while S. aureus found a safe environment for adhesion and colonisation in the hydrophobic features. It is pertinent to mention here that Fadeeva et al. (2011) used a very high fluence value of 100 J/cm$^2$ with 50 fs pulses (at 800 nm wavelength) for engraving the titanium substrate. It was indicated that such laser fluence and other parameters are necessary for producing uniform hierarchical structures without a clear explanation of the mechanism behind the formation of such structure.

In later research, a much longer infrared laser pulses of 500 fs were utilised by Cunha et al. (2016) to control the adhesion and formation of S. aureus biofilm on Ti. The surface roughness role in controlling the bacterial adhesion was emphasised by showing that S. aureus adhered preferably to the low-roughness surfaces with $R_a$= 1-4 nm, which was also proved by other investigations (Wua et al., 2011, Ivanova et al., 2010). It can be concluded that the bacteria tend to settle down in concave regions because that provide a larger contact area in comparison with nano-pillars, cones and other microstructures which have a characteristic length smaller than the bacteria size. The little space between the nano/micro-pillars or –

grooves not only prevents the bacteria from penetrating and colonisation but also may kill the bacteria either by penetrating the bacterial cell and damaging the internal components, such as cytoplasm, RNA and DNA, or by tearing the protective cellular wall and damaging the cytoplasm (Cunha et al., 2016, Song et al., 2015). Figure 1 shows an illustration of the potential damage some micro/nanostructures may cause to the bacterial cells.

Perni and Prokopovich (2013) investigated the use of a microsecond laser pulses to ablate medical-graded silicon wafers and to produce conic shape features on the master slide's surface. An Nd:YAG (1 μm wavelength) with 10 kHz pulse repetition rate was used to ablade the material from a 1 mm-thick substrate and to produce 20 μm to 40 μm features with 4, 8 and 13 μm spacing distance between them. The master silicon wafers were subsequently covered with MED-4850 polymer to produce silicone sheets of 1 mm thickness. The resulting silicone will then have conic protrusions on the surface which was later tested against Escherichia Coli or Staphylococcus Epidermidis Bacteria for 5 hours. The results showed that the bacteria aggregated at the circumference of the cones' base and the valleys between the cones rather than the top of the cones. Moreover, less bacterial density was observed using the 20 μm to 40 μm diameter features in comparison with the 25 μm to 35 μm sized features. This selective aggregation can be related to the bacteria size, which is smaller than the cone dimensions, and to the fact that protrusions and areas with low liquid velocity provide a shelter for the bacterial cell to hide and grow, especially in high-velocity fluid flows (Perni and Prokopovich, 2013).

Furthermore, the surface layer of polyethylene terephthalate (PET) was modified using KrF (248 nm wavelength) Excimer laser which generated 0.58 J/cm$^2$ (Gillett et al., 2016). Compared to the research mentioned above, smaller features of 15-20 μm were produced, in a blind-hole form, to test the E. Coli colonisation in comparison with the untreated samples. The authors concluded that the laser engineered surfaces did not hinder the bacterial growth, but in contrary promoted the bacteria's aggregation and proliferation at the edges of the micro features. It should be noted, from the figures provided in this investigation, that the fabricated features are not only much larger that the bacteria size but are also separated by a large areas between each other. Although the surfaces in this research experienced an increase in hydrophobicity, this did not inhibit the cells' attachment, as reported in other investigations (Yan et al., 2011), due to the large smooth areas in which bacteria found a healthy environment for colonisation.

In addition to the YAG and ultrashort pulsed lasers, $CO_2$ laser with 10 µm wavelength was utilised to machine PDMS substrates to investigate E. Coli behaviour in both LB-agar solid and liquid states (Chebolu et al., 2013). The test was conducted using two values for the feature's width: 60 µm and 90 µm. The colonies density was found to be significantly less in the textured surfaces than that of the smooth surface samples (control), although the dimensions of the features produced with the $CO_2$ laser are considered much larger than those fabricated with fs and ps lasers. The main advantage of using $CO_2$ laser and of creating large-sized patterns is promoting the manufacturability and fabricating economic structures on polymers for medical purposes.

In conclusion, various types of lasers of different wavelengths and processing parameters were successfully utilised in the biomaterials texturing for micro- and nanofeatures production. Although the bacteria's proliferation, fouling and colonisation were hindered/eliminated from the laser textured surfaces, the bacterial behaviour control is still a new area to investigate and a better understanding of the bacteria-biomaterial interaction mechanism is needed in order to effectively improve the bio-surfaces in implants and medical devices.

## 5. Conclusions

The biological interaction between the human and bacterial cells and the biomaterial surfaces have been studied for many years. Due to the increased need for biocompatible materials at fast rate and low cost, investigations focused on effective and environment-friendly methods, such as lasers, for producing such surfaces. This chapter reviewed the effect of laser-induced surface structures on the bacterial and cellular behaviour.

It can be concluded that the human cells response differently to the surface structure. Using lasers, it is possible to generate surface topographies of different geometries and sizes which are vital to reduce fibroblast growth and to increase the friendly human cells growth. Lasers were successfully able to produce surface features larger/smaller than the fibroblast size, various features shape, size and distribution and surfaces of high/low roughness, hence, variant wettability. For human cells, it can be concluded that the increase in surface hydrophobicity significantly reduce undesirable cell adhesion.

For bacterial cells, lasers also produced conical and blind-hole features on the biomaterials surfaces to control the surface hydrophobicity and the interaction with the bacteria. The laser-produced spikey features prevented the bacteria aggregation by either killing them or hindering their fouling by reducing the surface-bacteria contact area. Although antibacterial and antifouling behaviours were achieved using various features, the investigations did not explain the mechanism behind such behaviour. Moreover, contrary observations were presented regarding the effect of the surface topographical and chemical properties on the bacterial response.

It can be concluded that the use of laser surface modification as a method for controlling cellular and bacterial behaviour is still under development, and a deep understanding of the phenomena involved is very needed.

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