

THE INFLUENCE OF LIPSS SPATIAL ALIGNMENT AND PERIODICITY ON OSTEOBLASTIC DIFFERENTIATION

X. SEDAO^{1,2*}, M. MAALOUF³, A. A. KHALIL¹, Y. D. MAIO², S. PAPA³, E. DALIX³,
S. PEYROCHE³, A. GUIGNANDON³ AND V. DUMAS⁴

¹ Hubert-Curien Laboratory, University of Lyon, Jean-Monnet University, UMR 5516 CNRS, F-42000 Saint-Etienne, France

² GIE Manutech-USD, 20 rue Benoît Lauras, F-42000 Saint-Etienne, France

³ SAINBIOSE Laboratory INSERM U1509, University of Lyon, Jean Monnet University, F-42270 Saint Priest en Jarez, France

⁴ University of Lyon, Ecole Centrale de Lyon, Ecole Nationale d'Ingénieurs de Saint Etienne, Laboratory of Tribology and Systems Dynamics, UMR 5513 CNRS, F-42100 Saint-Etienne, France

* xxx.sedao@univ-st-etienne.fr

ABSTRACT

Ultrashort pulse lasers have significant advantages over conventional continuous wave and long pulse lasers for texturing of metallic surfaces, especially for nanoscale surface structures patterning. Furthermore, ultrafast laser beam polarization allows precise control of the spatial alignment of the nanotextures imprinted on titanium-based implants surfaces. In this article, we report the biological effect of beam polarization on human mesenchymal stem cells differentiation. We created, on polished Titanium-6Aluminum-4Vanadium (Ti-6Al-4V) plates, Laser Induced Periodic Surface Structure (LIPSS) using linear or azimuthal polarization of Infra-Red beams to generate linear or radial LIPSS, respectively. The main difference between the two surfaces was the anisotropy of the Linear LIPSS and the isotropy of the Radial LIPSS. Seven days post-seeding, cells on the radial LIPSS surface showed the highest extracellular fibronectin production. At 14 days, qRT-PCR showed on the same surface an increase in osteogenesis-related genes such as alkaline phosphatase and osterix. At 21 days, mineralization clusters indicative of final osteoinduction were more abundant on the radial LIPSS. Taken together, we identify that creating more isotropic surfaces than linear enhances cell differentiation resulting in an improved osseointegration. Thus, fine tuning of ultrashort pulse lasers may be a promising new route for functionalization of medical implants.

1. INTRODUCTION

Titanium and related alloys, such as titanium-6aluminum-4vanadium (Ti-6Al-4V), have been used as the main biomaterial for dental and orthopaedic implant devices, which can be attributed to their resistance to corrosion, mechanical strength and high biocompatibility with host tissues. However, patients still face the risk of implant failure because of insufficient bone integration due to fibrous tissue production and/or the occurrence of infection. It remains a challenge to improve osseointegration with good mineralization at the bone-implant interface.

The femtosecond laser (FSL) offers the possibility to texture titanium at nanometric scales with controlled nanopatterns. Particularly, periodic nanostructures such as laser-induced periodic surface structures (LIPSS) are formed during a complex interplay between incoming laser light and surface waves. The periodicity of the LIPSS is closely linked to laser wavelength, and the direction of the LIPSS is determined by laser polarization. As a consequence, LIPSS with desired periodicity and linear or radial organization can be produced by applying FSL light with a well-defined laser wavelength and polarization. In the process of developing next-generation implants, it is of great importance to evaluate biological responses triggered by FSL-induced nanopatterns, which are dependent on surface parameters, including surface isotropy modification.

In this in vitro study, we assessed several stages of the osseointegration process. By providing aligned or disordered textures to the human Mesenchymal Stem Cells (hMSCs), we hypothesized that cells

differentially organize their focal adhesions and, more importantly, their fibrillar adhesion (increased cell contractility), thus accelerating their differentiation toward osteoblasts. Our results clearly indicate that isotropic texturing improved the induction of osteogenesis on titanium surfaces at all investigated time points.

2. EXPERIMENT DETAILS

Titanium samples were textured using FSLs from Amplitude Systems and galvo scanners from Scanlab within the GIE Manutech-USD platform (Saint-Etienne, France). The following laser sources were used: a Tangor HP and a Tangerine FSL (both from Amplitude Laser Groupe, Pessac, France), operating at 1030 nm central wavelength and a pulse duration of around 400 fs. Both lasers exhibited linear polarization states. All samples were placed on XYZ translation stages from Aerotech in order to find the best focusing plane. Scanlab GmbH's intelliSCAN 14 scanners were finally associated with f-theta lenses. Linear LIPSS (IR and GR, in some cases) and IR radial LIPSS were generated. In the later case an s-wave plate was implemented in the beam path of the laser to create a donut-shaped beam that converted the entering linear polarization into an azimuthal polarization.

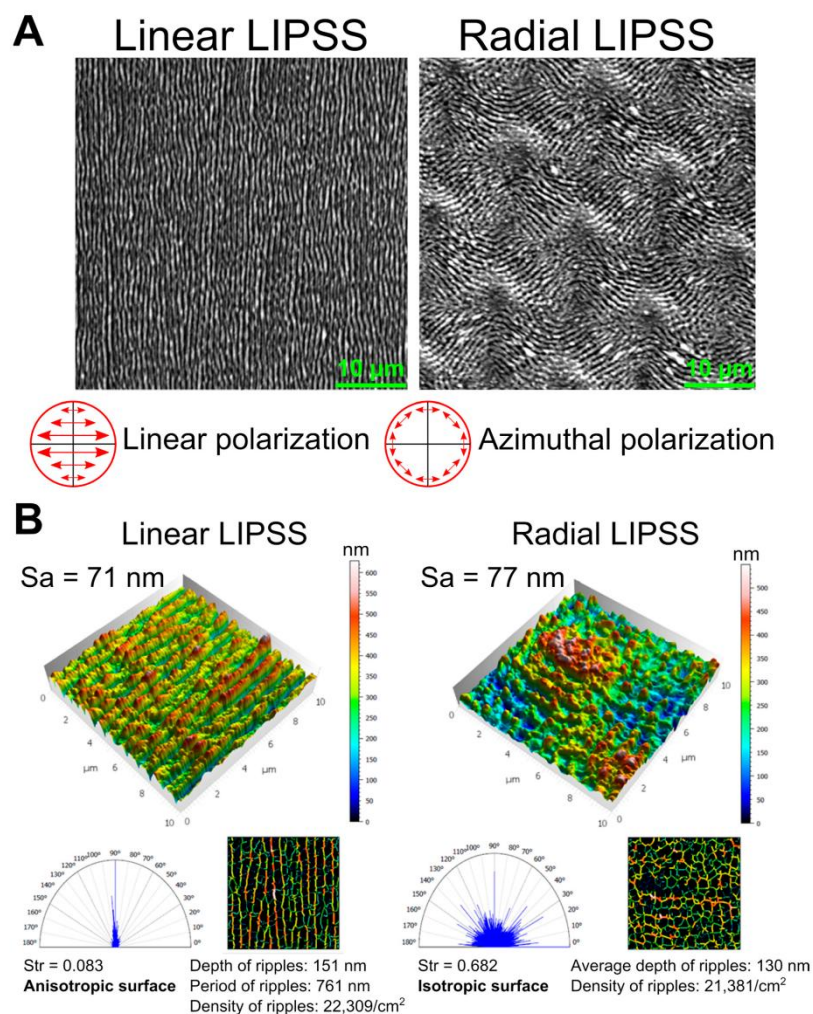


Fig 1. (A) SEM images of the two surface textures with linear or radial LIPSS (magnification 3000×). (B) 3D image reconstruction of the nanometer-scale surface texture with linear or radial LIPSS (data from atomic force microscopy images 10 $\mu\text{m} \times 10 \mu\text{m}$) and surface analysis by Mountain Map® software.

3. RESULTS

The topography of titanium is one of the key features for the acceleration of osteogenic cell differentiation on medical implant devices. Stem cells interact with underlying surface patterns, which

lead to modulating the cell's fate. In this study, we focus our work on two different nanostructures obtained by FSL texturing. The nanoroughness of the two textures was similar, but one texture was anisotropic (linear LIPSS), whereas the other could be considered as isotropic (radial LIPSS). We demonstrate that isotropic texturing improves osteoblastic differentiation compared to anisotropic and polished surfaces. This was conducted by a cross-time investigation and by various operational techniques. Most dominant evidence is that at 21 days' post seeding, mineralization was assessed by calcein blue labelling. Image analysis revealed that hMSCs on the radial LIPSS surface had increased mineralization activity compared to the other two surfaces (Figure 2). This was highlighted by a larger mineralized area (+99% vs. polished, $p = 0.016$; +132% vs. linear LIPSS, $p = 0.002$) and a higher number of mineralized spots (+83% vs. polished, $p = 0.046$; +128% vs. linear LIPSS, $p = 0.006$) on the radial LIPSS surface. The average size of the mineralization spots was not different between the three surfaces.

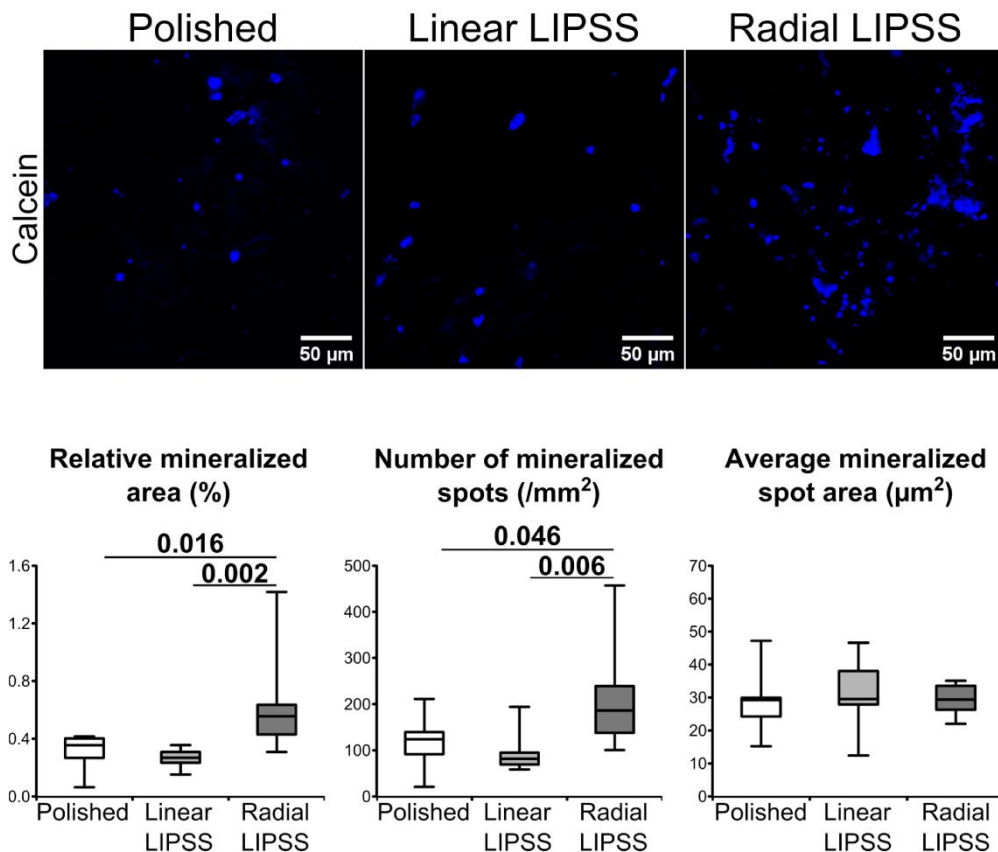


Fig. 2 Mineralization clusters labeled by calcein blue (blue) on the polished linear LIPSS and radial LIPSS surfaces at 21 d post seeding. On the bottom of the image are the results of the different quantifications made for the three surfaces.

This study provides insights into how small changes in laser polarization can modulate hMSC fate choice and extracellular matrix deposition. Nanoscale radial LIPSS generated on titanium alloys by FSLs with azimuthal polarizations exhibited an isotropic distribution that seemed to promote osteoblastic differentiation. Cell contractility and density, fibronectin production, gene overexpression and improved mineralization helped to ensure the consistency of cell evolution analysis over a time period of several weeks. Such dynamic hMSC sensitivity appears very important during implant osteogenesis, as these cells are primarily recruited to the implantation site. As a perspective, texturing titanium surfaces with such small features opens the possibility to couple-enhanced osseointegration with potential antibacterial properties, which are known to be more sensitive at the nanoscale than the microscale.

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