Project LaserImplant

D3.2 Images of osteoblasts on screws of dental implants

Reporting period	from	**01.01.2021**	to	**31.12.2021**
Report completed and released		**30.09.2021** **Martina Muck, Johannes Heitz**		

1. Goals

The deliverable **D3.2** provides a collection of SEM images of osteoblasts on screws of dental implants published on the **LaserImplant** web-site (www.laserimplant.eu).

2. Detailed Description

Introduction

JKU performs osteoblast cell experiments as follows:

Bone-forming cells (osteoblasts) of the commercially available human cell line SAOS-2 (provider DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) are used. The cells are cultivated in an established cell culture medium in an incubator with a water vapor saturated atmosphere with 5% CO_2 content at 37°C and are divided at a ratio of 1:10 once a week. The experiments are performed with typical culture times of about 3 weeks. After disinfection with ethanol, a set of samples is placed in a Petri dish with cells and culture medium, thus the samples are completely covered with liquid. After the chosen cultivation time in the incubator, the cells at the samples are fixated and dehydrated. In detail, the cells are initially fixed overnight with 6% glutardialdehyde (GA; Merck, Darmstadt, Germany) in phosphate-buffered saline (PBS) and subsequently dehydrated with the help of ascending ethanol series (30%, 40%, 50%, 50%. 80%, 90%, 96%, 3 x 100%) for 30 min each. The samples are repeatedly transferred 3 times into hexamethyl-disilazane (HMDS; Merck). After the overnight evaporation of HDMS, the samples are sputter-coated with gold and the cell density is evaluated by means of scanning electron microscope (SEM).

JKU

For the images in this deliverable **D3.2**, **JKU** uses a Yb-based femtosecond laser set-up (Spirit 1040-16 HE, Spectra Physics, wavelength λ = 1040 nm, pulse duration τ = 350 fs) to produce a laser beam that is guided through a system of five mirrors and focused by a lens (100 mm focus length) onto the sample stage, which can be moved linearly in two dimensions. The diameter of the focused laser beam is $2w_0$ = 75 µm ± 5 µm. To be able to produce conical structures with superimposed nanoripples (LIPSS), a parameter set (peak fluence F_0, scanning velocity v_{scan} and line distance ΔS) is determined beforehand based on parameters such as laser type (wavelength and pulse duration), focused beam diameter $2w_0$ and the desired laser frequency *f*.

For cylindrical Ti-based samples, a continuous rotation during laser structuring was chosen, which was investigated only on titanium cylinders of 8 mm diameter until now, but can be adapted to other cylindrical samples such as bone screws and dental implants. A schematic of the spiral approach for area structuring on cylindrical sample is shown in Fig. 3. While rotating, the sample is moved along the *y*-axis with a velocity v_{scan} that leads to the desired line distance ΔS. Pulse repetition rate *f* and *y*-axis velocity can be chosen according to the desired rotation speed.

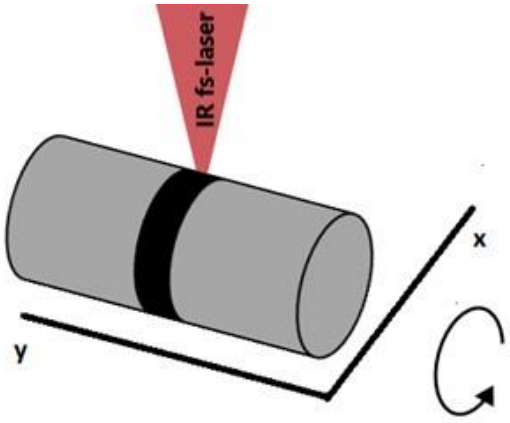

Fig. 1: Schematics of continuous laser processing of cylindrical samples. The laser is focused onto the highest point of the cylindrical sample. For continuous laser processing, the sample is irradiated while being rotated and moved along the *y*-axis, leading to a spiral around the cylinder. Figure adapted from [1].

After testing, a rotation velocity v_{rot} = 2500 ms/round trip was chosen. To achieve microcones with superimposed nanoripples a repetition rate f = 33 kHz was determined. To obtain the desired line separation of ΔS = 20 µm, a scanning velocity v_{scan} = 8 µm/s has to be used. Figure 2 shows an unprocessed dental screw (left image, in casing) and a laser-treated dental screw with 3 rings in black (right image). The shown dental screws were ordered from **DPU**. As a good stability in bone material is required for dental screws, the idea of structuring the surface is to achieve high cell adherence and growth.

Fig. 2: Images of unprocessed dental screw (left image, in casing) and laser-treated dental screw in 3 rings in black (right image).

Figure 3 shows SEM images of the laser-treated titanium surface of the dental screw. It should be mentioned that the laser-treated surface was already pre-processed by sandblasting, which leads to fine bumps at the surface. These bumps can be seen in Fig. 4 in the bottom-left image. The laser-treated surface only shows very few micro-sized bumps, while nano-sized ripples can be found everywhere. For structuring, a peak fluence F_0 = 0.935 J/cm^2 was chosen. The remaining parameters were chosen according to the structuring test of cylindrical samples.

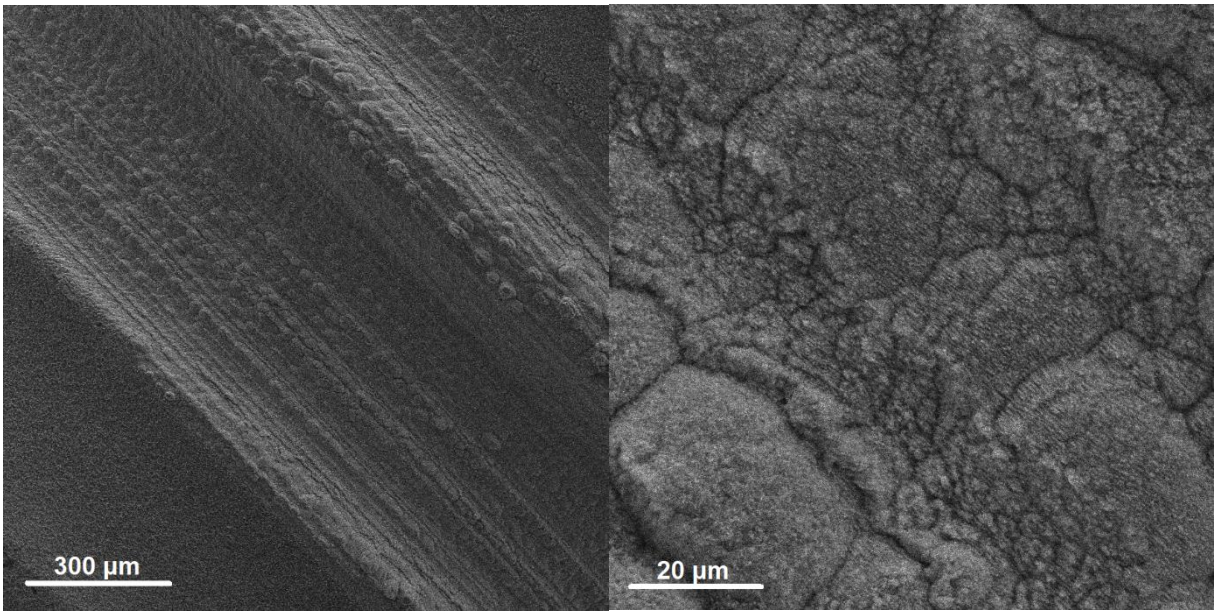

Fig. 3: SEM images of laser-induced ring on sand-blasted titanium dental screw. The left image shows an overview of the laser-induced structures on the screw, where only a few micro-sized bumps can be seen. At a higher magnification (right image) the nano-sized ripples are visible.

As described above, it is intended to achieve high cell adherence and growth on dental screws for good stability in bone material by structuring the surface with laser irradiation. Figure 4 depicts SEM micrographs of the dental screw surface after osteoblast cell growth. In the top row it can be seen that the cells overgrow the whole screw, both unstructured and structured areas. In the bottom-left image a thick layer of cells is shown in the back part of the image (left side), while in front part (right side) the sandblasted surface is visible. In the bottom-right picture a crack in the cell layer is shown, where it is clear, that the osteoblasts grow in dense confluent multilayers, where no structures underneath are visible.

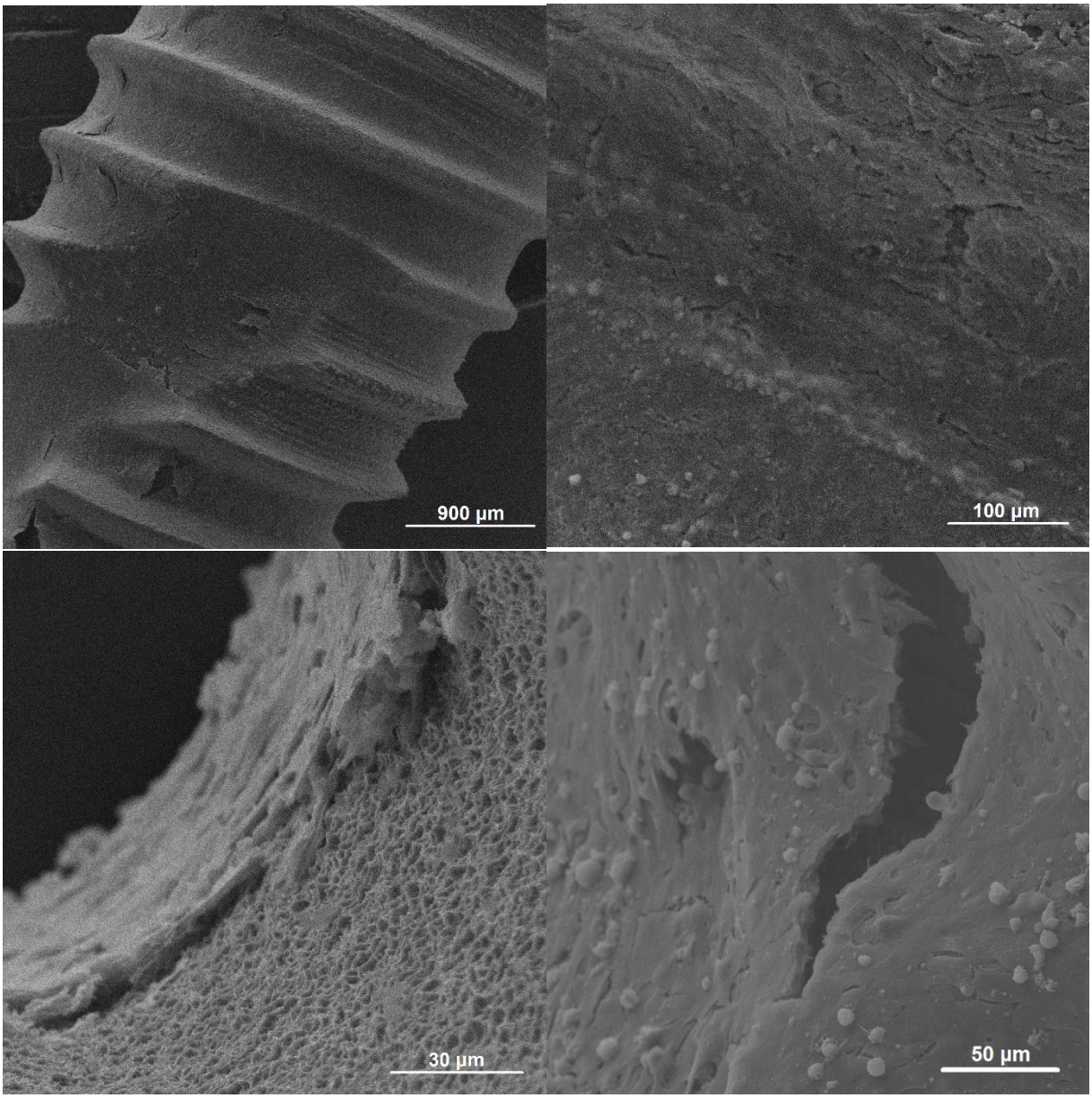

Fig. 4: SEM images of osteoblast cell growth on sand-blasted titanium dental screw. The whole screw surface is covered with dense confluent multilayers of osteoblasts (top-left), where almost no laser-induced structures are visible underneath the cells (top-right). The sand-blasted surface can be seen at one point (bottom-right), where it is also apparent that the cells grow in thick layers (bottom-left and bottom-right).

References

[1] M. Muck, B. Wolfsjäger, K. Seibert, C. Maier, S. Ali Lone, A.W. Hassel, W. Baumgartner, J. Heitz: *„Femtosecond Laser-Processing of Pre-Anodized Ti-Based Bone Implants for Cell-Repellent Functionalization“*, Nanomaterials **11** (2021), 1342. https://doi.org/10.3390/nano11051342

3. Evaluation of Goals and Resulting Actions

The deliverable **D3.2 Images of osteoblasts on screws of dental implants** was finalized in time by m9. A link to this report will be implemented into the Dissemination the **LaserImplant** web-site (www.laserimplant.eu).

This report is part of the project **LaserImplant** that has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 951730.