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Antibacterial surfaces produced by high-average power USP laser

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Abstract

Laser-textured antibacterial surfaces were produced on 316L stainless steel and its polypropylene replica. Antibacterial properties of steel samples were investigated by ISO-standard based tests: best results were observed with 1µm-size surface features, showing reductions of 99.8% for *E.Coli* and 84.7% for *S.Aureus*. Textured polypropylene inserts were produced by injection molding from the textured steel samples and contaminated by standard bacteria live/dead kit. A visible improvement in biofilm attachment was reached on the textured inserts with respect to non-textured reference inserts.

Keywords: antibacterial surfaces; biofilm adhesion; USP laser texturing; surface functionalisation

1. Introduction

Nowadays, the possibility to control specific surface properties by USP laser texturing is well known and new applications of this technique are coming to light to fully exploit its potential. Moreover, the availability of high average-power USP laser sources and high-speed scanning systems, as well as the development of multi-beam processing approaches, is attracting the attention of several industrial fields thanks to the possibility to reach very high-throughput processes. USP laser texturing is a one-step technique which allows tailoring the surface properties of several different types of materials (metals, semiconductors, dielectrics) by generating different types of nano- and micro-structures on the material surface. The size and the

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geometry of these surface structures, which finally determine the surface property, are mainly defined by the total energy, or dose, deposited on the surface during the laser texturing process. The dose is a parameter which itself depends on several process parameters, as for instance the laser fluence and the laser repetition rate. Discrimination of the role of each parameter in the generation of the surface structures is critical from a point of view of process industrialization as the feasibility of upscaling for high throughput is defined by the choice of process parameters.

The main goal of this study is the generation of antibacterial surfaces on steel and its polypropylene replicas to be used in home-appliance and food-production machinery by high-throughput laser texturing process. Initially, a comprehensive investigation of all process parameters was carried on flat mirror-polished 316L stainless steel (SS) samples to define the individual role of surface morphology, wettability and chemical composition on the resulting antibacterial properties. A complete description of this study can be found in Sciancalepore et al., 2018, Gemini et al., 2018 and Gemini et al., 2017 and Lazzini et al., 2017. The best performing surface morphology with respect to antibacterial behavior was selected to be reproduced on mirror-polished demo-size AISI H13 steel molds (hardness: 50+-2 HRC; mirror-polishing quality: Ra=0,07µm, Rz=0,56µm). The polypropylene replicas were later obtain by injection molding. In this work, an overview of the antibacterial properties results obtained on flat mirror-polished 316L SS samples is firstly introduced. Textured surfaces showing the lowest bacterial adhesion on steel, were chosen to be reproduced on demo molds and then transferred on polypropylene inserts by injection molding. Several injection parameters were varied to optimize the transfer of the nano-structures from the steel surface to its polypropylene replica. Live/dead bacteria test were carried on the polypropylene replicas and the biofilm adhesion was evaluated by live/dead microscopy.

2. Laser texturing of 316L SS surfaces and their antibacterial properties

The main results from the initial parametric study carried on flat 316L mirror-polished SS samples highlighted the role of the dose in obtaining different types of surface morphology and different surface wettability. The role of surface chemistry was also investigated as it is known to be essential in driving the surface wettability from an initial hydrophilic state to a superhydrophobic one. Fig. 1 shows a scheme of the laser texturing setup employed for all texturing processes. An Amplitude System SatsumaHP3 laser beam running at 1030nm-central wavelength and 350fs-pulse duration was sent through a telescope to adjust its size before entering a galvanometric scanning system for beam positioning running at speed up to 2m/s. The laser beam was finally focused on the sample surface by a 100mm focal-length lens to obtain a beam diameter of about 20µm in the focal plane.

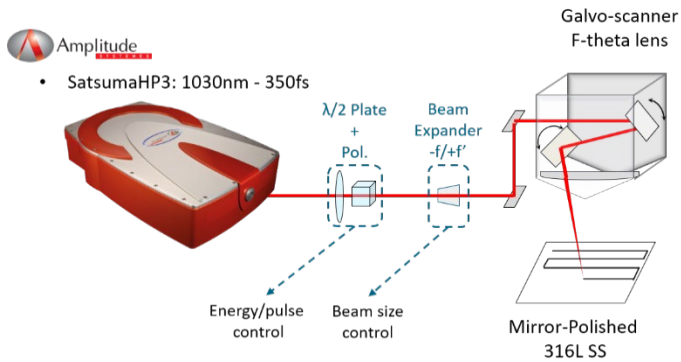

Fig. 1. Scheme of the laser setup employed for USP laser texturing.

Fig. 2 shows representative surface morphologies of the five different textured surfaces selected to carry bacterial adhesion tests:

- Hydrophobic (static contact angle 119±5°) infrared (IR) laser induced periodic surface structure (LIPSS, or ripples) – Fig. 2a
- Aged hydrophobic (static contact angle 119±5°) IR ripples, aged in ambient air for 30 days and contaminated a second time – Fig. 2a
- Hydrophilic IR (static contact angle 26±5°) ripples – Fig. 2a
- IR spikes (static contact angle 160±6°) – Fig. 2b
- Non-textured mirror-polished surface (average areal surface roughness of 30±5nm - static contact angle 98±5°) – Not shown in Fig. 2

Respective results on bacteria adhesion are presented in Fig. 3. Bacterial retention tests were carried according to ISO standard based technique (ISO 22196 and ISO 27447) in terms of viable colony forming units after two hours of immersion in bacterial broth with two different types of bacteria: *Escherichia Coli* and *Staphylococcus Aureus*, in order to account for the specific bacteria geometry and Gram property. All results were normalized with respect to the one obtained on non-textured industrial-standard 316L SS control samples with average roughness of about 0.5µm. More detailed information can be found in Lutey et al., 2018.

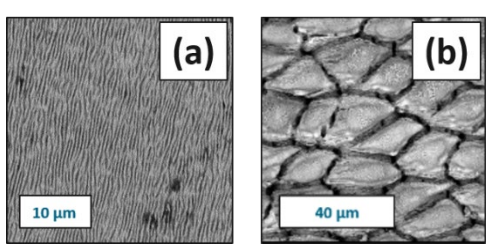

Fig. 2. Representative SEM images of (a) IR ripples and (b) IR spikes on 316L SS surfaces.

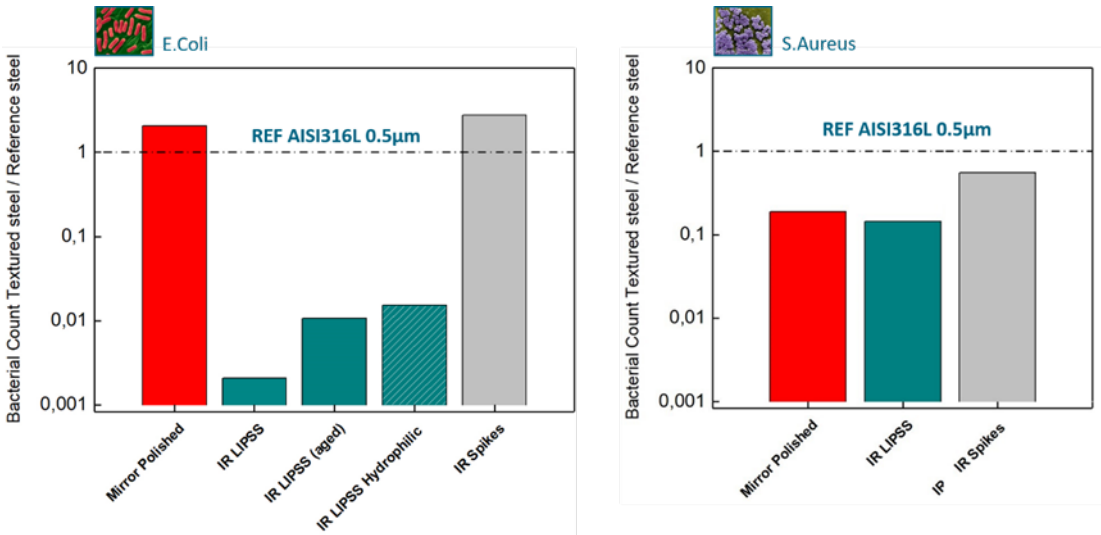

Fig. 3. Bacterial adhesion results on USP laser textured surfaces of 316L SS tested for *E.Coli* retention (left-hand side) and *S.Aureus* (right-hand side).

After contamination, the morphology IR ripple was found to reduce *E.Coli* retention by 99.8% and 99.2%, respectively, and *S.Aureus* retention by 84.7% and 79.9% in terms of viable colony forming units. It was possible to conclude that while for *E.Coli* the bacterial adhesion depends mostly from the relationship between surface morphology and bacteria dimension, for *S.Aureus* it is the surface wettability to drive the bacteria retention. As general rule, low surface roughness together with low surface wettability and small surface-structure size may define a surface state with reduced bacterial adhesion.

3. Injection molding of polypropylene replicas and their antibacterial properties

IR ripple morphology was chosen to be reproduced on demo molds and later on polypropylene replica inserts by injection molding (ALLROUNDER 170 S, clamping force: 150kN). Fig. 4 presents a picture of a representative mirror-polished demo-size AISI H13 steel mold, the laser textured area and the inlet for the polypropylene injection. Several injection parameters were tested to optimize the reproducibility of the IR ripple nanostructures on the polypropylene replica: mold temperature, injection speed and holding pressure were varied in the range 50°C - 85°C, 30cm^2/s - 62cm^2/s, and 550bar - 750bar, respectively. Injection temperature and pression were kept constant at 260°C and 450bar, respectively.

Live/Dead tests were carried on the textured polypropylene replicas and non-texture control polypropylene replica using a Live/Dead Baclight bacterial viability kit from Thermo Fischer. The contaminated replicas were later observed and analyzed by an Olympus Optical microscope Bx53U equipped with a fluorescence module. Fig. 5 presents SEM images of the IR ripple laser-textured demo-mold (left-hand side) and the polypropylene replica (right-hand side) produced at mold temperature of 80°C, injection speed of 62cm^3/s and holding pressure of 550bar. Although some flakes are visible on the polypropylene insert after demolding process, it is possible to observe that the IR ripple nanostructures are reproduced on the polypropylene replica with very high resolution, making the nanostructure transfer process very reliable.

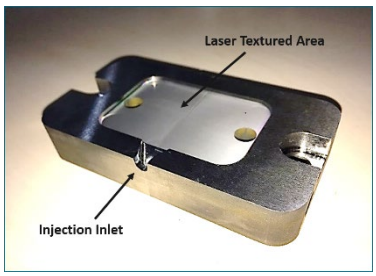

Fig. 4. Picture of a representative demo-size mold. The laser textured area and the injection point are indicated by black arrows.

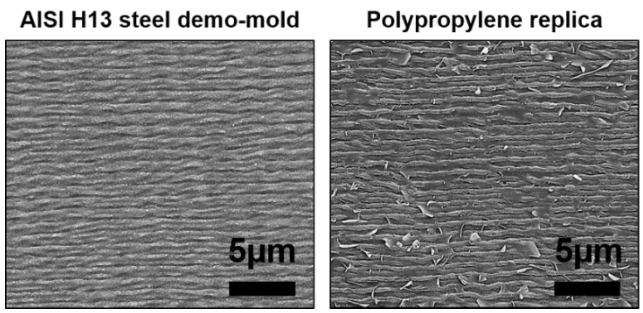

Fig. 5. SEM images of the SS demo-mold (left-hand side) and its polypropylene replica (right-hand side) obtained by injection molding.

Biofilm adhesion was evaluated on several polypropylene inserts obtained with different injection parameters according to ASTM E2871-13 standard guidelines. Fig. 6 presents live/dead analyses of a non-textured control polypropylene replica (left-hand side) and the textured polypropylene replica obtained with the injection parameters previously introduced (Fig. 5 right-hand side). It is possible to observe that the non-textured control insert presents a high density of bacteria clusters spreading homogeneously on the surface and leading to high biofilm formation. The textured replica clearly shows an important improvement with regard the biofilm formation, even if small bacteria clusters are still noticeable.

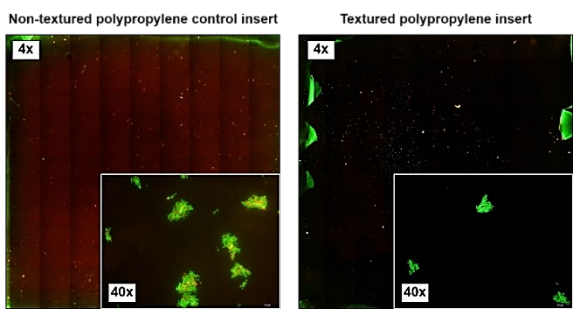

Fig. 6. Live/dead test images for *E.Coli* after 24h of incubation on non-textured polypropylene control insert (left-hand side) and textured polypropylene replica (right-hand side).

4. Conclusions

In conclusion, laser texturing has shown to be a reliable and effective one-step method to produce steel surfaces characterized by highly reduced bacterial retention: low surface roughness together with low surface wettability and small surface-structure size may define a surface state with limited bacterial adhesion. Injection molding proved to be an valid method to transfer laser-generated nanostructures to polypropylene inserts with high resolution and reproducibility. Textured polypropylene inserts also revealed a reduced biofilm formation with respect to the control insert.

Acknowledgements

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