Functionalization of polymeric surfaces for antibacterial purpose

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ABSTRACT

Introduction

This study is a first step in the SAFER project (StAmpaggio di componenti polimerici Funzionalizzato mediante tessitura lasER - Functionalized Moulding of Polymeric Components by Laser Texturing). The goal of SAFER is to develop a process for the production of polymeric parts with a functionalized surface by texturizing injection moulds using laser technology and applying nanostructured coatings.

The primary aim of functionalization is to limit the proliferation of pathogens on surfaces. Secondary benefits to the moulding process are expected, in terms of injection pressure , mould cleanability and anti-stitching effects.

In this first phase, the antibacterial efficacy of hierarchical structures, obtained by laser texturing plastic injection moulds and replicating them on polymeric material samples, was tested.

The literature presents studies on surface functionalization for wettability modification [1] and on how surface structure modification can reduce the adhesion and replication capacity of bacteria [2].

Based on these studies, micro- and nanometric hierarchical structures were defined. The structures were produced on metallic inserts and then replicated using a plastic microinjection press to produce polymeric polypropylene (PP) test disks. Each sample was characterized to verify the accuracy of replication at the micro- and nanoscale. Wettability tests were then carried out to verify that the hydrophobicity

properties obtained by laser texturization were also valid on the polymeric replica. Later, microbiological bacterial growth analyses were performed on each sample to test the effectiveness of the antibacterial action, using three different organisms. The results obtained show an effective reduction in bacterial growth of all microorganisms considered in all treated samples.

Methods

The test disk measures 15 mm in diameter and 2 mm in thickness, with a cavity 0.5 mm deep and 8 mm in diameter on one of the two faces to locate and contain bacterial cultures. The cavity is made in the mould through a special 16MnCr5 steel insert. For the purpose of the study, four inserts were used: one non-textured insert as a reference and three worked with different patterns.

Laser texturing was performed using an EKSPLA Atlantic 50 ultra-short pulse laser. First, micrometric features were created using the second harmonic (Green). Then LIPSS were generated using the first harmonic (IR), capable of providing more regular LIPSS.

In order to obtain the polymeric part with a functionalized surface, three different micrometric patterns were designed, taking into account the size of the bacteria used for microbiological analyses and geometries from the literature.

1. LINEAR: Parallel ridges. Width 30 μm, spacing 20 μm
2. GRID20: Square-based parallelepipeds 20x20 μm, spacing 20 μm
3. GRID60: Square-based parallelepipeds 60x60 μm, spacing 20 μm

Micro-texture was created by overlapping the passes and using the 'dashed line' method: the line is scanned by alternating periods of laser on and periods of laser off, resulting in a regularly dashed line. The final job is shown in Fig. (1). Once the desired feature was obtained, LIPSS were performed, making parallel lines spaced 4 μm to ensure a uniform energy distribution over the surface.

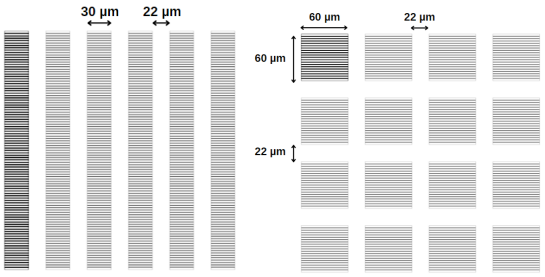

Figure 1. Microtextures. LINEAR (left) and GRID60 (right)

Injection moulding was performed on a *Wittmann Battenfeld Micro Power 15* machine. The entire cavity was obtained on the moving part of the mould, taking into account the need to not damage the replicated geometries during extraction and to maximize replication capacity. The injected volume is approximately 528 mm^3.

The correct replication was verified using a scanning electron microscope (SEM), and the surfaces were observed with a confocal microscope (Sensofar Neox). For

the evaluation of the micrometric structures, the average height H was taken as the distance between the maximum and minimum planes, by averaging over the entire surface. The evaluation of nanometric structures was done considering the average height of the replicated structure Sa, the average height of the ridges on the polymer sample Shh and the average depth of the grooves on the insert Sdd.

Wettability was tested, evaluating the contact angle of the fluid with the surface. The angle assessment was performed by depositing a 15 μL drop of liquid bacterial culture medium on the surface and evaluating the angle formed between the contact plane and the tangent to the surface of the drop at the point of contact.

For microbiological analysis, a viable count was performed using the test disks as seed plates. The pathogens studied were *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.*

Results

The Fig. (2) is a SEM image showing how structures are correctly executed on the inserts and correctly replicated on the polypropylene samples. Table (1) shows the measures from the confocal microscope.

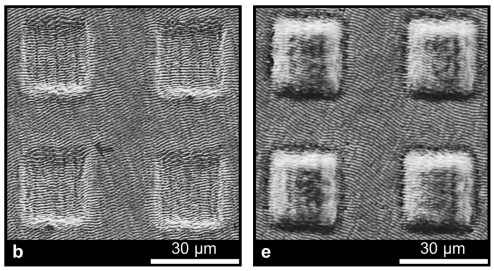

Figure 2. Replication of GRID20. Steel insert (left) and PP replication (right)

Parameters	REFERENCE		LINEAR		GRID20		GRID60	
	Insert	PP	Insert	PP	Insert	PP	Insert	PP
$H\ [\mu m]$	-	-	2.9	2.8	2.4	2.4	3.2	3.5
$Sa[nm]$	426	408	15	12	15	12	15	12
$Shh\ [nm]$	61	59	6	5	5	4	7	5
$Sdd\ [nm]$	63	63	6	4	5	4	7	6

Table 1. Laser parameters

Concerning wettability, the effectiveness of laser processing is evident. The reference sample shows hydrophilic behaviour (contact angle $< 90°$), while all samples with replicated features show hydrophobic behaviour, with a minimum contact angle of $109° \pm 2°$.

Regarding bacterial growth, all textured surfaces led to a reduction in live bacteria compared to the control surface, regardless of the type of bacteria considered. In Fig. (3) the global wettability results and the growth results for *E.Coli* are shown.

Results also show that the correlation between wettability and bacterial growth is present, but not linear. This confirms the observations already made by [3], which

shows that the adhesion of microorganisms depends not only on wettability but also on the topography of the surface. To adhere, the bacterium needs a surface area larger than its size. All organisms tested have a diameter of at least 500 nm. The distance of the replicated LIPSS on the polypropylene disks is approximately 400

$$nm$$

. As expected, the CFUs on the treated surfaces are lower than on the untreated sample.

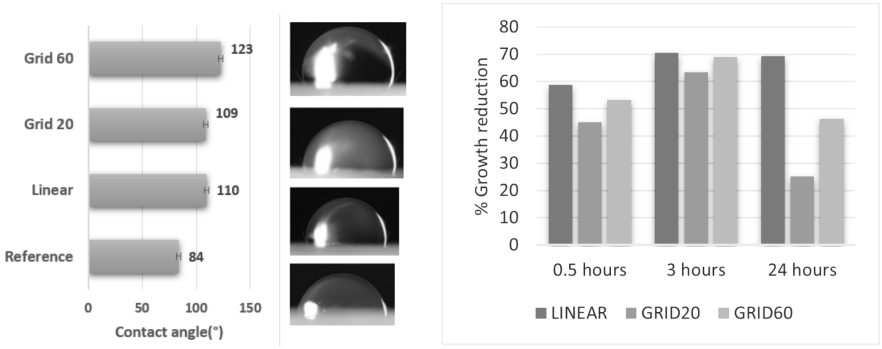

Figure 3. Wettability results (left), growth results for E.coli (right)

Conclusions

Results demonstrate the chance to replicate micro- and nanostructures of different geometries on polymeric samples by injection moulding. This confirms the effectiveness of the combination of laser texturing and plastic injection in the functionalization of surfaces. It also confirms the possibility of realizing functionalized products while minimizing production time and costs for large commercial batches. Among the geometries tested, the parallel ridge geometry (LINEAR) is the most effective in terms of antibacterial action after 24 hour. From the results obtained in [4] using appropriately directed LIPSS, a further improvement in process parameters can be expected with the use of LINEAR-type microtextures. In this first step, nanostructured coatings were not considered. However, the results suggest an higher durability of the coatings, by increasing the surface roughness.

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