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Nondestructive testing in microfabrication using bacteria

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Abstract

Micromanufacturing has increased rapidly at scientific, technological and industrial levels. However, this evolution has not been followed by a parallel development of Non Destructive Testing (NDT) techniques. The available techniques are, generally, unable to detect microdefects. In this work, two types of microgeometries were produced by Micro Powder Injection Molding (μPIM) of stainless steel AISI316L: specimens for tensile tests (simple geometry) and microscrews (complex geometry). During the process optimization, different injection conditions of temperature and pressure were tested, as well as various temperatures for thermal debinding and sintering. Throughout the process, detectable and undetectable defects by NDT techniques were produced, which were used in the assays to assess the role of bacteria in the detection of defects. After adding bacterial suspensions of *Staphylococcus aureus* or *Rhodococcus erythropolis* cells, the microcomponents were subjected to magnetic or electric fields to facilitate mobility of bacteria towards the defects. This new methodology to detect defects produced during microfabrication can be a good solution for inspection of microdefects.

Keywords: Micromanufacturing; non destructive testing (NDT); bacteria.

1. Introduction

Powder technology has opened up new opportunities to produce (micro) components with different types of materials (metallic and ceramic). The Micro-Powder Injection Molding (μPIM), a similar technique to the plastic injection moulding, allows to produce microcomponents with complex geometries from metallic and ceramic powders.

The microcomponents tested in the present study were produced by μPIM in five steps: 1 – characterization of selected raw materials and their interaction (powder and binder); 2 – mixture of the powder and a thermoplastic binder to get a feedstock; 3 – injection moulding into the required shape; 4 – thermal debinding (to remove the binder) and 5 – sintering [1-3]. The optimization of the whole process is an important aspect to minimize the defects introduced in the microcomponents [1]. The selected powders should respect some characteristics in relation to: particle size, particle size distribution, shape and surface (4S's) [4]. The ideal feedstock should be homogeneous and with low viscosity, ensure complete filling, easy demoulding and good shape retention in debinding and sintering steps [3,5,6]. The thermal and rheological properties of the feedstock are also important because their melting and degradation temperatures determine the processing conditions for mixing, injection moulding and debinding [3]. Injection in μPIM requires higher mould temperature, lower injection speed and higher pressure, when compared with PIM [5]. The debinding step should be properly carried out to avoid distortion of the

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microcomponents. After the solid state sintering stage, the components should present homogeneous shrinkage, without visible defects such as incomplete filling, warpage and cracks [2,6].
Micromanufacturing has been developed mainly to decrease the weight of parts/devices, in order to contribute for decreasing the environmental impact. However, they are submitted to the same loads as the macrocomponents used for the same application. The improvement of their performance obliges to a significant decrease of all kinds of defects. The current NDT techniques are not efficient for detecting many microdefects. Thus, the possibility to have new inspection techniques is crucial.
A novel NDT technique was recently proposed where the defects are identified with the aid of bacterial cells [7]. In this study, we assessed the possibility of using this novel technique to locate the defects produced during μPIM.
The main objective of this work was thus to demonstrate the efficacy of bacteria to detect defects, at the micrometre scale, in these microcomponents.

2. Experimental

The proposed feedstock used AISI 316L stainless steel and a commercial binder composed by polyolefin wax. The equipment used for mixing the powders and binder was a Brabender Plastograph mixer, which allowed the monitoring of parameters including: temperature, rotation speed, and torque value. A homogeneous distribution of powders in the binder was sought since it helps to obtain an isotropic shrinkage after sintering. The feedstock was granulated/pelletized to facilitate the feeding of the injector machine. The injection step conditions were optimized until components without injection defects were produced. The injection temperature and pressure were set at 150ºC and 10 MPa, respectively, similar to other research work [8].
During the thermal steps (thermal debinding and sintering) various temperatures were tested, until microcomponents without visible defects were produced. Two types of microgeometries were manufactured: specimen for tensile test (simple geometry) and microscrews (complex geometry – Fig. 1).
Bacterial suspensions of *Rhodococcus erythropolis* or *Staphylococcus aureus* cells were placed on the surface of the samples to be tested, as described previously [7,11]. In summary, the surfaces to be tested were cleaned and the cultures containing bacterial cells stained with fluorescent dyes (viability kit from Molecular Probes, Invitrogen) were poured over the surface. After 4 min, during which the samples could be subjected to electrical or magnetic fields, the bacterial suspension was removed from the surface with precision wipes (Kimtech Science from Kimberly-Clark) and the cells remaining inside the defects were observed by fluorescence microscopy. At the end of the assay, the samples were sterilized by immersion in 70 vol.% ethanol or exposure to UV light for 15 min. The microcomponents with *Rhodococcus erythropolis* were subjected to an electric field with an intensity of about 320 kV/m whilst those with *Staphylococcus aureus* were placed under an alternating magnetic field with a frequency of 1 Hz and a peak intensity of about 40 mT (400 Gauss), to facilitate movement of the bacterial cells towards the defects.
The bacterial cells were stained with a Live/Dead® *Bac*Light™ bacterial viability kit (Molecular Probes, ThermoFisher Scientific) which stains live cells green and dead cells red under fluorescent light [9]. The cells in samples for scanning electron microscopy (SEM- FEI Quanta 400FEG ESEM/EDAX Genesis X4M) observation were fixed and dehydrated by placing the samples in increasing concentrations of ethanol from 10 to 100%.

Fig. 1. Microscrews produced by μPIM.

3. Results and Discussion

To assess the possibility of using bacterial cells to identify defects in microcomponents produced by μPIM, bacterial suspensions of *Rhodococcus erythropolis* or *Staphylococcus aureus* were placed on top of the microcomponents. *Rhodococcus erythropolis* cells can present negative or positive net surface charges, depending on the carbon source used for growth [10] and, thus, the cells respond to electric fields. On the other hand, the cells of *Staphylococcus aureus* used in this study were isolated from mobile phones for their ability to respond to magnetic fields [11]. To improve the entrance of the cells into the defects, the microcomponents were subjected to magnetic or electric fields, depending on the bacteria used in each test. Besides, a maximum deposition time of 4 min was considered to prevent irreversible addition of the cells to the stainless steel.
Fluorescence microscopy allowed the identification of defects not visible by the naked eye since the stained

cells were mainly located inside cracks (Fig. 2). The observation of the cells inside the defects (higher than 600 nm) could be assessed by SEM (Figs. 3 and 4).
The samples that were not used for SEM, were sterilized by immersion in 70% ethanol for 10 min (data not shown). Further cleaning could be achieved by placing the samples in an ultrasound bath. This indicates that, after the inspection process, the inspected microcomponents may be safely used for their final purpose. Nevertheless, sterilization by e.g. autoclave or UV-light should be considered if the final destination is the application in living organisms (such as microscrews for dental implants).

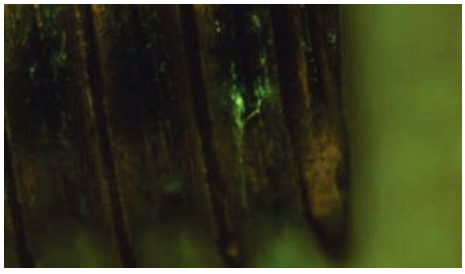

Fig. 2. *Rhodococcus erythropolis* cells inside cracks formed in microscrews produced by μPIM.

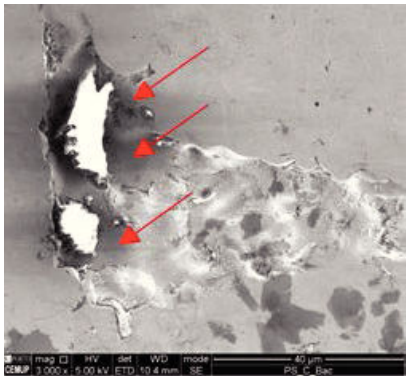

Fig. 3. Example of the bacterial cells in the defects of tensile test specimens.

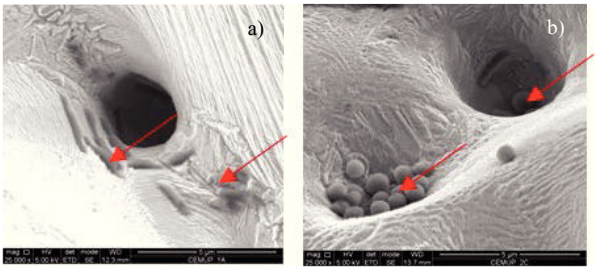

Fig. 4. Example of the *Rhodococcus erythropolis* (a) and *Staphylococcus aureus* (b) cells inside the defects of microscrews for dental implants.

4. Conclusions

This work demonstrated that bacteria could enter the microdefects (≈1 μm) produced. This new methodology, which uses bacterial cells to detect defects produced during microfabrication, should thus be a good solution for inspection and detection of microdefects in microcomponents produced by μPIM.

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