FIB Patterning of Stainless Steel for the Development of Nano-Structured Stent Surfaces for Cardiovascular Applications

M Schmidt¹, F Nazneen², Y Georgiev¹, G Herzog², P Galvin¹, N Petkov,
Electron Microscopy and Analysis Facility (EMAF)¹ and and Life Sciences Interface (LSI)² group, Tyndall National Institute, University College Cork, Cork, Ireland

Corresponding author: michael.schmidt@tyndall.ie; nikolay.petkov@tyndall.ie

Abstract. Coronary artery disease is a major problem worldwide. Stent implantation is a percutaneous interventional procedure that mitigates vessel stenosis, providing mechanical support within the artery. However, stenting causes physical damage to the arterial wall. The research presented here develops novel nano-structured features on stent surfaces to promote rapid endothelial cell adhesion to reduce in-stent re-stenosis. Nano-structured features (concaves) ordered in rectangular arrays were patterned on 316L Stainless Steel (SS) surfaces using focused ion beam (FIB) milling after electropolishing using linear sweep voltammetry and chronoamperometry. Various dose test experiments were performed, aiming at an array of 120 nm diameter holes with pitch of 240 nm and depth of 50-100 nm on sample area of 400 µm × 400 µm. Studies on FIB milling rates were carried out to optimise the time and to create a uniform array of holes. Based on the SEM examination of the hole arrays (plane view and cross section) it can be concluded that a low ion beam current created well shaped uniform concave structures (Gaussian shape) with good depth profile, while a high current resulted in an array of holes with sine profile. Further, a higher current created larger diameter holes with less defined depth profile and deviation from Gaussian shape. We demonstrate that the ability to perform nano-structuring with FIB milling is greatly affected by the polycrystalline nature of SS.

1. Introduction
Coronary artery disease causes 7.2 million deaths worldwide annually [1], resulting from vascular occlusion, myocardial infarction and its complications. Stent implantation is a percutaneous interventional procedure that mitigates vessel stenosis, providing mechanical support within the artery. However, stenting causes physical damage to the arterial wall, leading to inflammation, thrombosis (clotting), and neo-intimal hyperplasia (re-stenosis) [2]. Further improvements in next generation stent coating are needed to enhance the attachment and survival of endothelial cells on the stent surface. The research presented here develops novel nano-structured features on 316L Stainless Steel (SS) stent surfaces to promote rapid endothelial cell adhesion to reduce instant re-stenosis. Based on a literature survey, promising cell responses to nano-structured surfaces were identified including nanopit features [3].

Conventional techniques for topographical surface modification or surface patterning at the nanoscale are lithography based techniques. In technologies typically derived from microelectronics, such as in X-ray lithography or UV lithography, patterns are formed by using a mask [4]. Such lithography processes are time-consuming with many steps, and inherently inappropriate for prototype
designs and processes. Electron beam lithography, and lithography based on scanning tunnelling microscopy or atomic force microscopy are high-resolution mask less procedures, but with a very low throughput and unsuitable for wide surface nano-patterning [5]. Imprint lithography is a high resolution technique for nano-patterning of large surfaces, but it requires moulds and is restricted to polymeric materials which could then be used as etch masks [6]. Patternning by Focus Ion-Beam (FIB) milling offers several advantages for flexible prototyping (i) practically any substrate material that is able to withstand high vacuum conditions of the microscope chamber can be used, (ii) there is high flexibility in the obtainable shapes and geometries by modulating the ion beam current and the patterning conditions, (iii) reduced complexity of the patterning process e.g. it is a single-step process with a possibility of real-time monitoring of the milling progression. Thus for any particulate type of substrate various depths as well as lateral dimensions including the optimal feature size can be obtained at minimum number of processing steps.

In this report we describe the development and the limitations of a new methodology for surface nano-patterning of 316L (SS) stent surfaces based on the use of F. The optimal FIB patterning conditions for achieving reasonably high throughput (patterned rate of about 0.03 mm$^2$ per hour) and nano-size accuracy in dimensions and shapes of the features, are discussed. Additionally we describe a characterisation protocol for analysis of such structures by combination of SEM, AFM and cross-sectional imaging.

2. Experimental

2.1. Focussed Ion Beam (FIB) patterning

The FIB system used in the current study is the FEI Helios NanoLab 600i, which is a Dual beam FIB for localized milling and deposition, transmitting a 30 keV beam of Ga$^+$ ions combined with a high resolution scanning electron microscope (SEM). The resolution of FIB milling is approximately the same as that of the “spot” size (i.e., the diameter of the beam at the sample surface). In our process, tuning the working current from 0.28 nA up to 0.92 nA, the feature size on the surface of the 316L SS stent surfaces changes from 120 nm up to 180 nm.

Electropolished 316L steel substrates were used for this study. Prior to nano-structuring, the polished specimens were cleaned in acetone, ethanol, and finally in ultrapure water via an ultrasonic treatment for 10 min. Nano-structured features (concaves) ordered in rectangular arrays were patterned on 316L steel surfaces using FEI Helios NanoLab 600i system. This system was used because of high beam quality and stage stability. Concave structures of 120 nm and 180 nm diameters with pitch of 240 nm and 360 nm, and targeted depths of 50 - 100 nm were fabricated on three electropolished steel surfaces on areas of 400 µm × 400 µm. Two types of concave arrays were fabricated at 30 keV at two different beam currents and writing times e.g. (i) 120 nm diameter at 0.28 nA beam current and 12 h patterning time and (ii) 180 nm diameter were at 0.92 nA and 6 h writing time.

2.2. Plan view and cross-sectional SEM imaging.

Scanning electron microscope (SEM) was used to analyze the topography of nano textured surfaces of the polished and patterned stainless steel. The SEM images presented were obtained using the SEM at the FEI Helios NanoLab 600i at an electron beam current of 5 kV and 86 pA beam current. Samples for cross-sectional imaging were developed by a standard preparation protocol after depositing protective carbon and Pt layers. The electron beam induced (EBID) carbon deposition supplied necessary contrast difference between the protective Pt and the SS stent surface, hence enabling accurate determination of the concave’s shape and depth. Final polishing was performed at 30 kV and ion current of 93 pA.

2.3. Atomic Force Microscopy (AFM) imaging.

AFM examinations were performed in ambient air with a commercial microscope (Dimension 3100 controlled by a Nanoscope IIIa controller equipped with a phase imaging extender, Digital Instruments, Santa-Barbara, CA, USA) in tapping mode. The silicon cantilevers (Windsor Scientific Ltd, UK) had a <10 nm radius of curvature and a 40N m$^{-1}$ spring constant (nominal values). Topographic images were recorded at a scan rate of 0.5 Hz and a resonance frequency of 300 kHz.
3. Results and Discussion
Before attempting prototyping on large 400x400µm areas used for the biological tests we have performed optimisation tests on relatively small test patterns, one such area is shown in Figure 1 (a). From the known polycrystalline nature of the SS stent material one can assume that when subjected to ion milling or imaging it will show pronounced channelling contrast. It is well known for Si or Cu that it etches and mills faster in preferred directions [7], [8], [9]. Similarly polycrystalline metals will show milling rates that are varying by the different orientation of grains towards the incoming beam.

Figure 1 illustrates how much this anisotropic milling affects the desired outcome of uniform concaves. Shown in Figure 1 are examples from the pre-tests on 10µm x 10µm areas with 120 nm diameter concaves at 240 nm pitch. The structures that appear with the brightest contrast (Figure 1(a), marked as region 1) showed deeper and sharper edges than the structures that appear darker in contrast. The FIB/SEM cross sections across a 10 µm width as depicted in Figure 1 (b) reveal that the difference in depth varies in the range from 20 - 85nm. This variation of more than 4 times indicates that an exact match of the desired depth of 50 - 100nm can only be approximated. Nevertheless the correlation of FIB/SEM cross-sectional imaging with the top-down appearance of known patterned area (grain) was essential in establishing accurate size and shape distribution of the formed concaves.

![Figure 1. a) SEM image of 10µm x 10µm patterned area with 120nm holes. b) Depth profile across a length of 10µm (stretched in height to pronounce region of interest). Clearly to see the difference in depth for different crystal orientations.](image)

4. Summary
FIB has compelling advantages for flexible prototyping compared to other traditional techniques, however the milling rates and the corresponding shape and size of the formed structures is largely affected by the grain size of the polycrystalline stainless steel. Moreover this method is limited to 120 µm.
nm resolution for the desired concave depth and uniform scan size of 200µm x 200µm. Nevertheless formed structures show large variation of concave depths and shapes and as such might serve as a resourceful platform for screening large variations of cell/pattern SS stent interactions.

Figure 2. a) Triangular area covering more than half of the 400 µm × 400 µm square reproduced b) perfect holes, while c-d) shallower partially stigmated holes were reproduced at the corners.

Acknowledgements
This work was supported through 09/SIRG/11621, NBPI, and INSPIRE initiatives funded by the Irish Government PRTLI 4, NDP 2007-2013.

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