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### **16.2** Surface Treatments to Gain Desired Properties: Coating, Polishing, Patterning

There are various ways to improve the stent design in order to make it more effec-48 tive. One way is coating the surface with organic and inorganic materials. This is not 49 trivial with the complex geometry of the stainless steel mesh. Nevertheless this 50 route is extensively studied given its benefit of incorporating drugs into the polymer-51 based surface coating which can be eluted over time into the surrounding vessel 52 material. However, there are several reasons, including concerns about increased 53 risks of late stent thrombosis when using drug-eluting stents [8, 9], why the devel-54 opment focus is likely to return to bare metal or polymer-free stent technologies [3]. 55

While from the invention of stents it was aimed for a surface as smooth and polished as possible in order to minimise abrasion and inflammation of the body tubes, it becomes ever more apparent that a somewhat roughened/textured surface might be a better fit for the task. 59

### 16.3 Biomaterial–Cell Interaction: Advantages of Rough/ Patterned Surfaces

The influence of textured material surfaces on the behaviour of cells has been stud-62 ied for many years by now [10-16]. On one hand, theoretical studies show that cells 63 prefer to grow on rough surfaces in general as it imitates best naturally occurring 64 surfaces [17]. On the other hand, in order to have a better control over and to mini-65 mise the complexity of the experimental conditions, the natural urge to study regu-66 lar patterns led scientists from rough to micro- to nano-patterned surfaces. Especially 67 in tissue engineering where the tissues involved require certain mechanical and 68 structural properties for proper functioning, the trend from micro- to nano-structured 69 surfaces serving as artificially created support systems has become evident within 70 the last decade [18–24]. Also for drug delivery the control over biointerfacial inter-71 actions is often the key to biomedical applications [14]. 72

In particular endothelial, smooth muscle and fibroblast cells play an important 73 role in the healing process and maintenance of cardiovascular systems and thus are 74 likely to be in contact with biomedical implants such as stents and grafts. During a 75 surgical procedure involving the introduction of a stent, vascular tissues in the arter-76 ies may be damaged. Healing of vascular tissues is promoted by the formation of an 77 endothelial cells lining on the stent substrate [25], while the presence of smooth 78 muscle cells and fibroblasts may cause re-stenosis. Micro- and nano-textures on 79 substrates may provide control of cell functions. Such structures could promote bet-80 ter vascular cell adhesion, decrease the need for systemic administration of drugs 81 and reduce the requirement for secondary surgery after stent implantation. 82

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# 16.4 Patterning: Lithography, Electrodeposition, Dip Pen, Pulsed Laser, FIB

Recent advances in micro- and nanotechnology have allowed the patterning of sur-85 faces with the desired textures for cell scaffolding [26–29]. Nano-texturing involves 86 the creation of patterns or features with nanometre precision. The choice of the 87 texturing method depends largely on the nature of the substrate that needs to be 88 modified and on the dimensions of the features expected. Indeed, photolithography 89 was particularly successful for the patterning of features of microscopic dimensions 90 on elastomers such as polydimethylsiloxane (PDMS) [30-33] and on polymers such 91 as polystyrene (PS) [34–37]. E-beam lithography was used for the patterning of 92 submicron features on silicon substrates [38, 39] and on poly(methyl methacrylate) 93 (PMMA) [40]. Features of 350 nm were patterned on PDMS and PMMA substrates 94 by nanoimprint lithography [41]. Metal substrates such as Ti were also textured 95 using micro-machining for feature dimensions in the microscopic range [10, 42]. 96 Cell adhesion, migration, elongation, proliferation and gene expression on textured 97 substrate can be greatly altered depending on the shape and the dimension of the 98 features [40]. 99

The different techniques are compared in Table 16.1. In indirect photolithogra-100 phy methods, patterns are formed over a large area using a mask [43]. Such lithog-101 raphy processes are time consuming with many steps and inherently inappropriate 102 for prototype designs and processes. Electrodeposition is a simple, fast and cost-103 effective method of reproducing nano-structures on many materials using templates 104 made of polymers and metals. However, this method is applicable only for electri-105 cally conductive substrates. Imprint lithography is a high-resolution direct tech-106 nique for nano-patterning of large surfaces, but it requires moulds and is restricted 107 to polymeric materials [44], but this could then be used as etch masks or filled with 108 metal electrodeposition. E-beam lithography and lithography based on scanning 109 tunnel microscopy (STM), atomic force microscopy (AFM) or dip pen are high-110 resolution mask-less procedures, but with a very low throughput and unsuitable for 111 wide surface nano-patterning [45]. Interference lithography can be utilised to create 112 or transfer array patterns on various metallic and polymeric surfaces, but only pat-113 terned features can be reproduced. Microtexturing of surfaces has also been reported 114 by pulsed laser patterning [46, 47]. The feature sizes are however limited to the 115 micron range. 116

Patterning by FIB milling is direct and offers several advantages for flexible 117 prototyping: (1) practically any substrate material that is able to withstand high 118 vacuum conditions of the microscope chamber can be used, (2) there is high flexi-119 bility in the obtainable shapes and geometries by modulating the ion beam current 120 and the patterning conditions, (3) reduced complexity of the patterning process, e.g. 121 it is a single-step process with a possibility of real-time monitoring of the milling 122 progression. Thus for any particulate type of substrate, various depths as well as 123 lateral dimensions including the optimal feature size can be obtained at minimum 124 number of processing steps. 125

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t1.2	Mask/			
t1.3	moulds	Nano-fabrication	Advantages	Drawbacks
11.4	lequileu	techniques	Advantages	Diawbacks
t1.5 t1.6	Yes	Photolithography	Well-controlled features	Requires photoresist, spin coaters and organic solvents Low aspect ratio
t1.7			High throughput	Limited to set of materials
t1.8 t1.9		Electrodeposition	Precise geometries and patterns	Require templates for creating of nano-structures
t1.10 t1.11			Large surface area	Limited to electrically conducting substrates
t1.12		Imprint	High resolution	Requires moulds
t1.13 t1 14		lithography	High aspect ratio	Applied to polymers only
t1.15	No	E-beam	High resolution	No direct writing on substrate
t1 16	110	L'ocum	Precise geometry	Multisten process
t1.17			and patterns	Low throughput
t1.18				Requires vacuum
t1.19				Time consuming
t1.20				Small surface coverage
t1.21				Expensive
t1.22		Interference	No complex optical systems	Limited to patterned array features only
t1.23		lithography		Multistep process
t1.24		STM/AFM/	Very high resolution	Low aspect ratio
t1.25		dip pen		Very low throughput
t1.26				Very small surface area
t1.27		Nanoindentation	High aspect ratio	Wide and shallow features
t1.28			Control over features depth	Slow process
t1.29			Less expensive than FIB	
t1.30			or e-beam writer	
t1.31		Laser patterning	Any material	Wide and shallow features
t1.32 t1.33			High throughput with high power laser	Micron resolution
t1.34		FIB milling	High resolution	Time consuming
t1.35			High aspect ratio	Process requires vacuum
t1.36			High etch rate	Very expensive
t1.37			Any material	Low throughput

t1.1 Table 16.1 Advantages and disadvantages of indirect and direct nano-structuring techniques

### 16.5 FIB Advantages: Fast for Prototyping

Except FIB, none of the texturing techniques mentioned above were suitable to 127 achieve features in the nanoscopic range on a hard substrate as 316L stainless steel. 128 Nanoimprint lithography (NIL) and e-beam lithography (EBL) are able to pattern 129 submicron features, which with NIL were achieved on soft substrates such as polymers and elastomers only and with EBL is a very time-consuming and expensive 131 multistep process. In this research work, FIB milling was used to create 132

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nano-structures onto stainless steel because it is a direct writing process with simple 133 steps, high resolution and aspect ratios. Nano-structured features such as pits were 134 created on 316L steel surfaces. The optimal FIB patterning conditions for achieving 135 reasonably high throughput (patterned rate of about 0.03 mm<sup>2</sup>/h) and nano-size 136 accuracy in dimensions and shapes of the features are discussed. Additionally, a 137 characterisation protocol for analysis of such structures by combination of electron 138 backscattering diffraction (EBSD), FIB, scanning electron microscopy (SEM), 139 atomic force microscopy (AFM) and serial FIB-SEM sectioning is detailed. 140 Furthermore, this chapter reports the comparison of in vitro EC adhesion and growth 141 on FIB nano-structured, unpolished and electropolished 316L steel surfaces. 142

### 143 **16.6 FIB Overview: Ga<sup>+</sup> Beam, Maskless, Pattern Design**

FIB systems usually employ a finely focused beam of gallium ions (Ga<sup>+</sup>) that can be 144 operated at high beam currents for milling or low beam currents for imaging. It can 145 be utilised to remove material locally in a highly controlled manner to the nanome-146 tre scale. When the high-energy Ga ions impinge the sample, atoms from the sample 147 surface are sputtered. In addition, Ga atoms from the ion beam are also implanted 148 into the top few nanometres of the surface, and the surface is made amorphous. As 149 can be seen in Fig. 16.2, the Ga<sup>+</sup> primary ion beam hits the sample surface and sput-150 ters a small amount of material. The primary beam also generates secondary elec-151 trons. When the primary beam strikes the sample surface, the signal from secondary 152 electrons or sputtered ions is collected to form an image of the surface [48]. 153



**Fig. 16.2** (a) Dual-beam FIB schematics. (b) Beam sample interactions: A—incident Ga<sup>+</sup> ions, B—sputtered substrate atoms, C—scattered Ga<sup>+</sup> ions, D—re-deposited Ga<sup>+</sup> ions and substrate atoms, E—in substrate trapped Ga<sup>+</sup> ions and F—secondary electrons

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At a low primary ion beam current, a minuscule amount of material is sputtered, 154 and with existing FIB methods, 5 nm resolution can be attained using Ga ions. 155 However, at higher primary ion beam current, a considerable quantity of material 156 can be taken out, which allows sub-micrometre- to nanometre-scale precision milling of sample surface. 158

FIB originated in the semiconductor industry and has become an important tool for 159 a wide array of applications, ranging from circuit editing, reverse engineering, sample 160 preparation for transmission electron microscopy (TEM), microstructural analysis 161 and prototype nanomachining to name just a few [49]. Many modern FIB instruments 162 supplement the FIB column with an additional SEM column so that it becomes a 163 versatile dual-beam platform as depicted in Fig. 16.2. In nano-patterning, FIB has 164 been used to create nano-structures on Si [50], silicon nitride [48, 51] and glass sub-165 strates [52] and to fabricate platinum nano-structures on peptide-based soft surfaces 166 [53]. Only one study reported the protein adsorption on FIB patterned glass surfaces 167 [52]. To date, no cellular studies have been reported on FIB-structured surfaces. 168 Moreover, this and other aforementioned techniques have not been employed for pat-169 terning the key vascular stent material 316L stainless steel for vascular cell functions. 170 Studies do not exist that determine the EC response on 316L steel with nano-pit fea-171 tures. Endothelial cell studies on unpatterned 316L stainless steel substrates have 172 shown that the grain size and grain boundaries have an impact on their adhesion and 173 morphology [54]. Chemically etched substrates with 16 µm grain size etched have 174 demonstrated cell densities significantly higher than with grain sizes of 31, 47 and 175 66 µm. The authors attribute this increased cell density to greater boundary area and 176 associated higher surface free energy [54]. Cell proliferation was also subject to 177 another study discussing different materials. There the grain sizes varied from 320 nm 178 to 22 µm. Again cell proliferation was reciprocal dependent on the grain size [55]. 179

### 16.7 Crystal Structure of Stainless Steel 316L

Austenitic type 316L stainless steel is commonly used for manufacturing medical 181 implants [56] and was hence selected as the substrate of choice for this study. 182 Austenitic stainless steels have face-centred cubic (fcc) crystal structure, in which 183 the unit cell is a cube with atoms located at the corners and middle of each side 184 (Fig. 16.3a). The presence of higher concentration of Ni in austenitic stainless steels 185 stabilises the fcc crystal structure, because Ni is a fcc crystal itself. This enhances 186 the ductility, i.e. it can sustain large plastic deformation without fracture compared 187 to other stainless steels (maternistic and ferritic phases). 188

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[AU2]

Most metallic materials are composed of many small single-crystalline planes 189 called grains. These materials are referred to as polycrystalline materials (e.g. steel), 190 in which individual grains have identical arrangement of atoms but the orientation 191 of the atom arrangement or crystal structure is different from each adjoining grain 192 (see Fig. 16.3b for visualisation). The interfaces between these grains are grain 193 boundaries, the surface that separates the individual grains [57]. 189



**Fig. 16.3** (a) Schematic face-centred cubic crystal structure and (b) FIB image of polycrystalline 316L used in this study

The austenitic stainless steel function can be affected by two microstructure features: grain (or crystal) size and shape. The general grain size suggested for 316L is 100  $\mu$ m or less [56]. This is because smaller grains have more grain boundaries, which provide resistance to plastic deformation as they are responsible for slip deformation by dislocations.

Depending on the process conditions such as annealing and cold-working, the 200 shape of austenitic stainless steel grains varies. Annealing is a heat treatment process 201 where a material is modified, resulting in changes in its properties, for example, 202 strength and hardness. It is a method that generates conditions via heating to above 203 the recrystallisation temperature, maintaining an appropriate temperature, and subse-204 quently cooling. This method is applied to reduce internal tensions, soften material, 205 enhance ductility, improve the structure by creating it uniform and enrich cold-work-206 ing properties. Austenite grains of the stainless steels under an annealed condition 207 exhibit an equiaxial granular shape (i.e. the grains having axes of equal length). 208

Cold-working produces plastic deformation in the steels and generates a strain 209 hardening effect, which improves both yield strength and tensile strength of steel 210 considerably. However, in cold-worked steel, depending on the amount of cold work, 211 the grains are elongated (i.e. longer in the rolling direction). During large plastic 212 deformation, textured grain structures are produced and preferentially align the grains 213 in specific crystallographic orientations. Hence, cold-worked steel with textured 214 structures demonstrates anisotropic mechanical properties. When employing a cold-215 worked steel for implant fabrication, microstructure analysis is suggested as implants 216 can be better prepared if the loading direction is concurrent to the high strength direc-217 tion in the steel [56]. Hence, it is clear that the microscopic and crystalline structure 218 can play a strong role on the nano-structuring of the stainless steel surface. 219

[AU3]

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#### **16.8** Substrate Preparation: Electropolishing, Cleaning

Electropolished 316L steel substrates were used for this study. Figure 16.4 illus-221 trates the steps involved in the whole sample process flow. Electropolishing of steel 222 was performed with a view to the preparation of these surfaces for nano-texturing. 223 For such applications, a smooth surface is crucial. The composition of this electro-224 lyte solution was 11 M H<sub>3</sub>PO<sub>4</sub>+4.5 M H<sub>2</sub>SO<sub>4</sub> in water. The electropolishing proce-225 dure was conducted in two steps at 80 °C and 5 mV s<sup>-1</sup> [58]. The first step involved 226 scanning of the potential from the open-circuit potential up to the point where the 227 diffusion-limited current region was reached. The linear sweep voltammetry was 228 then stopped, and the selected potential was maintained 10 min using chronoam-229 perometry. This resulted in a smooth and relatively defect-free surface. XPS analy-230 sis of the electropolished surface has shown that the stainless steel was enriched 231 with Cr, P, S, O, Mo and Ni elements. Prior to nano-structuring, the polished speci-232 mens were cleaned in acetone, in ethanol and finally in ultrapure water via an ultra-233 sonic treatment for 10 min. 234

### 16.9 FIB Tests: Challenges with Anisotropic Milling

The FIB system used in the current study is the FEI Helios NanoLab 600i, which is 236 a dual-beam FIB for localised milling and deposition, transmitting a 30 keV beam 237 of Ga<sup>+</sup> ions combined with a high-resolution SEM. In this work, the working current 238 was tuned between 0.28 nA (for 120 nm pits) and 0.92 nA (180 nm) depending on 239 size of the nano-texture. The available pit sizes and the large range of obtainable 240 working currents could make the FIB technique an ideal device for nanomachining 241 in the range from 10 nm to a few micrometres. Nano-structured features (pits/holes) 242 ordered in rectangular arrays were patterned on 316L steel surfaces using FEI 243 Helios NanoLab 600i FIB system. This system was used because of high beam 244 quality and stage stability. 245



**Fig. 16.4** Sample process flow in total. (**a**) Samples as received with rough surface. (**b**) Electropolishing to obtain a smooth surface suitable for nano-structuring. (**c**) Nano-texturing of 316L stainless steel via a focussed ion beam (FIB) milling. (**d**) In vitro endothelial cell adhesion studies

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**Fig. 16.5** SEM images of preliminary FIB tests to determine the feasibility of the prototyping approach. (a)  $10 \times 10 \,\mu$ m area pattern by FIB in preliminary tests with nominal 120 nm wide pits at 240 nm pitch. (b) Detail of another area exhibiting the same pattern. Clearly visible are the differences in appearance of the patterned surface depending on the polycrystallinity of stainless steel

From the literature survey, promising cell responses to nano-structured features were identified including nano-pit features [40, 59]. However, to date no EC studies have been reported on nano-pit structures. Based on this, two pit designs were patterned on three electropolished 316L stainless steel samples on areas of  $400 \ \mu m \times 400 \ \mu m$  using FIB: design A, pits of 120 nm diameter with a pitch of 240 nm and intended depth of 50–100 nm, and design B, pits of 180 nm diameters with pitch of 360 nm and intended depths of 50–100 nm [60, 61].

Before attempting prototyping on large  $400 \times 400 \,\mu\text{m}$  areas used for the biologi-253 cal tests, we have performed optimisation tests on relatively small test patterns; one 254 such area is shown in Fig. 16.5a. From the known polycrystalline nature of the 316L 255 stent material, one can assume that when subjected to ion milling or imaging, it will 256 show pronounced channelling contrast. It is well known for about a century for W, 257 Ag or Cu which are all fcc metals that they etch and sputter faster in preferred direc-258 tions [62-65], as well as Si [66, 67]. Similarly polycrystalline fcc austenitic stain-259 less steel will show milling rates that are varying by the different orientation of 260 grains towards the incoming beam. 261

Figure 16.5 illustrates how much this anisotropic milling affects the desired out-262 come of uniform concaves. Shown in Fig. 16.5 are examples from the pretests on 263  $10 \ \mu m \times 10 \ \mu m$  areas with 120 nm diameter holes at 240 nm pitch. The structures 264 that appear with the brightest contrast showed deeper and sharper edges than the 265 structures that appear darker in contrast. This will be discussed in more detail in the 266 following section describing the correlative microscopy approach. A later section 267 will focus on the study on the patterned substrates used for actual cell adhesion tests 268 and the preliminary FIB tests. 269

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# 16.10Correlative Microscopy: EBSD, FIB, SEM, AFM,<br/>and Serial Sectioning FIB–SEM Towards Better270Understanding of Beam–Substrate Interaction271

In order to gain a better understanding of the beam–substrate interaction during patterning, a correlative microscopy approach was used to illuminate the patterning process from many possible angles [68, 69]. This was also done on a batch of samples that could not be used for the cell adhesion studies basically because of the destructive nature of the last step, the serial FIB–SEM sectioning. 277

The different techniques used for the characterisation of nano-textured, unpolished and electropolished stainless steel surfaces were electron backscattering diffraction (EBSD), FIB, SEM, atomic force microscopy (AFM) and serial FIB–SEM sectioning. Figure 16.6 shows the detailed process flow of the correlative approach. 281

EBSD technique was used to analyse the crystallographic structured surfaces of<br/>the polished stainless steel. EBSD imaging was performed in a Hitachi SEM SU-70<br/>equipped with an Oxford Instruments EBSD attachment AztecHKL at 10 kV under<br/>284<br/>70° tilt angle and step size 2 μm.282<br/>283<br/>284

FIB technique was also used to visualise the crystallographic structured surfaces 286 of the polished stainless steel. FIB imaging was performed as a part of monitoring 287 of the milling at 30 kV accelerating voltage. 288

SEM was used to analyse the topography of nano-textured surfaces of the polished 289 stainless steel. The SEM images presented were obtained using a SEM at the FEI 290 Helios NanoLab 600i at an electron beam current of 5 kV and 86 pA beam current. 291

A commercial atomic force microscope (MFP 3DTM, Asylum Research) in AC 292 mode was used for topography mapping of the films. Olympus AC160TS silicon 293 cantilevers (Al reflex coated, ~300 kHz resonant frequency) were used for imaging. 294 Optimal results were achieved with a medium scan rate of 1 Hz and 256 × 256 pixels 295 image resolution. 296

Samples for serial sectioning were prepared using protective carbon and Pt layers [70]. The electron beam-induced (EBID) carbon deposition supplied necessary contrast difference between the protective Pt and the stainless steel surface, hence enabling accurate determination of the concave's shape and depth. 300



**Fig. 16.6** Process flow in the correlative microscopy approach. (a) The crystallographic grain orientations are measured by EBSD before patterning. (b) Surface patterning using FIB, also gaining FIB SE images as part of the monitoring process. (c) Extensive analysis of the textured steel surface with SEM, AFM and finally destructive serial FIB–SEM sectioning



**Fig. 16.7** Correlative microscopy on the exact same sample location. (a) Inverse pole figure (IPF) as measured by EBSD before surface texturing with (110) and (321) crystal orientation labelled and legend underneath. (b) FIB SE image obtained during patterning as part of monitoring. (c) SE image taken after the patterning—the *red circle* indicates the region used for AFM and serial sectioning (see Figs. 16.8, 16.9 and 16.10 for details). (d) Graphical visualisation of (110) orientation. (e) Graphical visualisation of (321) orientation

EBSD is an SEM-based microstructural-crystallographic technique to measure 301 individual grain orientations, local texture, point-to-point orientation correlations 302 and phase identification and distributions on the surfaces of bulk polycrystals. It is 303 also known as backscatter Kikuchi diffraction (BKD), and both acronyms are being 304 used interchangeably in the literature. EBSD patterns are generated on a phosphor 305 screen by backscatter diffraction of a stationary beam of high-energy electrons from 306 the typically 70° tilted sample surface. Because of the high tilt angle—or the shallow 307 20° angle of the incident beam towards the surface—this technique is very surface 308 sensitive and gives information of the top 20 nm down into the substrate [71]. 309

EBSD mapping was accomplished before the area was patterned in order to 310 determine a correlation between crystal grain orientation on one hand and shape, 311 size and depth of the FIB-milled concaves on the other hand. Figure 16.7 shows the 312 random size and orientation of the crystal grains and illustrates the correlative 313 microscopy approach of three techniques combined in the exact same sample loca-314 315 tion, EBSD, FIB and SEM. In general the intensity of the emitted SE depends on the different inclination of the sample surface towards the incoming beam and crystal 316 orientation [72]. Thus, the grey levels in the SEM image are directly linked to the 317



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surface topography, e.g. the shape of the pits and sidewall profile and the crystal 318 orientation of the surface material. In this way we can correlate the EBSD data to 319 the grey levels in the SEM images. The FIB reveals not surprisingly the same con-320 trast in the SE image as the one taken afterwards in the SEM. The SE yield is inde-321 pendent of the type of the beam; hence the same contrast is achieved. The additional 322 information of the SEM SE image lies in the much higher resolution. The FIB 323 which was run as a monitoring tool only during the patterning process produced one 324 pixel every 360 nm in X and Y direction. The SEM on the other hand was used after-325 wards as an analysis tool with an image resolution of  $4,096 \times 3,775$  pixels which 326 calculates at roughly 3 nm image resolution. Using a low current of 86 pA ensured 327 that the real resolution is not far from this theoretical limit. 328

Two different grains were chosen for additional correlation with AFM and the 329 serial FIB-SEM sectioning based on the crystallographic orientation, a low index 330 grain with (110) orientation and a high index grain with (321) orientation. From as 331 early as the 1920s, it is known that the sputter yield is dependent on the crystal ori-332 entation [62]. It is also known that the SE yield is dependent on the crystal orienta-333 tion [72]. Based on this fact the chosen grains should display a very different 334 behaviour when exposed to the ion beam during sputtering and also to the electron 335 beam in the SEM study. 336

Studying the marked region from Fig. 16.7 across the grain boundary using the 337 AFM (see Fig. 16.8), it appears that there is a difference in hole depth and diameter 338 depending on crystallographic orientation of the patterned surface. Even the shape 339 of the rim is evidently not circular but rather rhombohedral. Because of the high 340 aspect ratio of the pits, the tip could not reach down and probe the full depth of the 341 pits; therefore, the pit depth must be confirmed by the serial sectioning. 342

The first that comes to mind when seeing the rhombohedral shape of the pits is 343 the directional dependence in which atoms are ejected when sputtering at threshold 344 energy [63]. In our study however the energy used to create the patterned surfaces 345 in the FIB was way beyond the threshold energy, which for Cr, Ni and Fe as main 346 elements in 316L lie in the range of 60–90 eV. The directional dependence decreases 347 with higher sputter energies and has no influence on the direction of the sputtered 348 atoms at the 30 keV used here. It is also obvious when looking in detail at the (111) 349 oriented grain at the bottom of the AFM overview scan that shows the same 350 rhombohedral-shaped pits as the whole area around this region instead of the 351 expected triangular shape. As can be seen below in the detailed AFM and SEM 352 studies on the 400  $\mu$ m × 400  $\mu$ m patterns used for the cell adhesion studies, the shape 353 of the holes is solely determined by the ion beam quality (focus, stigmation) at the 354 place of impact on the sample surface. 355

In the SEM surface study as depicted in Fig. 16.9a, it appears as if the holes in both grains seem equal in diameter with only the higher SE yield obvious for the higher index grain. In order to clarify this impression, the region was imaged again after depositing a carbon layer as shown in Fig. 16.9b. Because secondary electrons are emitted from an area very close to the surface of the sample, this amorphous carbon layer masks the crystal orientation of the sample surface, and the image is 361

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**Fig. 16.8** Representative AFM scans of the marked region from Fig. 16.7. (a) Overview scan over the whole  $12 \,\mu\text{m} \times 12 \,\mu\text{m}$  region. (b) Detailed  $2 \,\mu\text{m} \times 2 \,\mu\text{m}$  scan of the area around the crystal grain boundary. (c) Line profile along one row of five holes determining depth and diameter of the holes in the (110) oriented grain. (d) Same line profile determining the depth and diameter of the holes in the (321) oriented grain



**Fig. 16.9** Detailed SEM images of patterned area marked by the *red circle* in previous picture. (a) Freshly patterned surface displays high SE yield for the high index grain and low yield for the low index grain, though the holes seem equal in diameter. (b) Detail of (a) after carbon deposition, the "true" diameter shines through as crystal orientations are hidden behind the amorphous carbon layer

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**Fig. 16.10** Serial sectioning as final destructive step of the correlative microscopy. (a) 3D reconstruction of all the 250 slices. (b–l) Image series illustrating one row of six holes taken every 36 nm (every third image). The *darker left* region is the low index grain (110) oriented, the *brighter right hand side* is the higher index (321) oriented grain. Obvious is the difference in depth by more than a factor of 3 between the two patterned grains

more related to topographical features. Indeed, the difference in diameter of the holes becomes pronounced, and it is visible that the higher index grain has apparently much larger holes than the lower index grain. 363

Serial sectioning of again the same region was performed composing 250 images 365 of 8 rows by 5 holes, with one image taken every 12 nm. This detailed analysis veri-366 fies the difference not only in the diameter but even more pronounced in the depth. 367 Though Fig. 16.10a presents a 3D reconstruction of all the 250 slices, more details 368 can be observed when looking at the individual slices of Fig. 16.10b–1. The diam-369 eter of the pits in the low index (110) oriented grain are  $150 \pm 10$  nm, while the 370 diameter in the (321) oriented grains is only slightly bigger with  $170 \pm 10$  nm. The 371 depth however is much more influenced by the differences of sputter yield depend-372 ing on the crystal orientation, and hence the (110) grain shows only  $55 \pm 5$  nm depth 373 in contrast to the  $200\pm 20$  nm depth of the (321) grain, nearly a factor of 4 374 between them. 375

# 16.11 Prototyping: Samples Prepared for Cell Adhesion 377 Tests and Statistical Pattern Analysis

After the initial tests for the feasibility of the patterning was finished successfully on the batch of samples with small-scale pattern, the "real" prototyping on the  $400 \times 400 \mu m$  patterned areas had been started. As consensus between statistical needs for ideally a high number of samples on one hand and the slow process of patterning with the FIB on the other hand, five samples with each one area of 120 nm pits/240 nm pitch and one area of 180 nm pits/360 nm pitch were manufactured.

The two pit designs, A and B, were created on electropolished stainless steel samples by FIB and are presented in Fig. 16.11. Three things can be observed. First, the square areas that have been milled by the FIB are very different from the electropolished areas. Second, the triangular areas in the centre are much better defined than the areas at the edges. Finally, within and outside these triangular areas, the different colour tones observed are due to the polycrystallinity of the stainless steel as described in detail in the previous section.

Alternatively, AFM was used to evaluate statistically in more depth the information on topography, pit feature dimensions and as a comparison of the results obtained via serial FIB–SEM sectioning. AFM examinations for this analysis were performed in tapping mode using a Dimension 3100 with a Nanoscope IIIa controller equipped with a phase imaging extender (Digital Instruments, Santa-Barbara, CA, USA). The silicon cantilevers (purchased from Windsor Scientific, UK) have a tip radius of less than 10 nm and a 40 N m<sup>-1</sup> spring constant. AFM images were



**Fig. 16.11** Overview of SEM images of (**a**) design A and (**b**) design B showing (1) differences in milled (*inside* the *square* area) and electropolished surfaces (*outside* the *square* area), (2) *triangular* areas covering more than half of the 400  $\mu$ m × 400  $\mu$ m square are much better defined than the areas at the corners in the *squares* and (3) different colour tones illustrate the polycrystallinity of the stainless steel

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**Fig. 16.12** AFM top view and cross-sectional images of pits (**a**, **c**) design A and (**b**, **d**) design B. Variation in depth dimensions from AFM profiles in region I and II is clearly noticed

recorded at a scan rate of 0.5 Hz and a resonance frequency of 300 kHz. Each 399 sample was evaluated over scan fields of  $5 \times 5 \ \mu\text{m}^2$ ,  $1 \times 1 \ \mu\text{m}^2$  and  $500 \times 500 \ \text{nm}^2$ . 400 The pit dimensions of the resulting images were evaluated using scanning probe 401 imaging processor software (version 5.1.5). 402

Four nano-structured samples were used for the measurements. Four topographic 403 measurements were performed in different random fields per substrate. Figure 16.12 404 shows the 2D AFM images and profiles of designs A and B on  $5 \times 5 \ \mu m^2$  areas. 405 A similar trend was noticed with AFM data. Region I showed shallower structures 406 than region II, Fig. 16.12a, b. From the AFM profiles across a 5 µm width as depicted 407 in Fig. 16.12c, d, the average depth recorded in region I for design A and design B 408 was  $24 \pm 18$  nm and  $26 \pm 20$  nm (N=115), whereas in region II the average depth 409 was  $68 \pm 48$  nm and  $64 \pm 8.5$  nm (N=115). This variation of more than four times 410 indicates that an exact match of the desired depth of 50-100 nm can only be 411 approximated. 412

The images presented in Fig. 16.13a, b are of a closer SEM inspection of 413 designs A and B on triangular areas of  $5 \times 5 \ \mu m^2$ . The pit structures within the 414 triangular zones are circular as wanted for pit designs A and B (Fig. 16.13a, b). 415



**Fig. 16.13** SEM images of circular pits of (**a** and **b**) design A and B reproduced within the triangular areas



**Fig. 16.14** AFM topographies of uniformly fabricated structures of  $(\mathbf{a}, \mathbf{c}, \mathbf{e})$  pits design A and  $(\mathbf{b}, \mathbf{d}, \mathbf{f})$  design B pits reproduced within the triangular areas (scale:  $5 \times 5 \ \mu\text{m}$ ,  $1 \times 1 \ \mu\text{m}$  and  $500 \times 500 \ \text{nm}$ ). Panels (**g**) and (**h**) represent the AFM profiles of (**e**) and (**f**) across the length of 500 nm

Smaller areas of about  $150 \times 150 \,\mu\text{m}^2$  showed circular shapes when imaged in topdown direction in the whole patterned area in the preliminary tests.

However, from the AFM images the shapes of the pits in design A in triangular
areas are more elliptical (Fig. 16.14a, c, e), whereas those of design B are closer to
perfect holes (Fig. 16.14b, d, f). The pit structures for designs A and B outside the
triangular areas exhibited deformed circular shapes as demonstrated in Fig. 16.15a, b.
From the stigmated nature one can conclude that there are problems with the focusing and astigmatism caused by the FIB optics. This is not surprising as the 200 times
magnification is close to the lower limit of the FIB Helios column.

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**Fig. 16.15** AFM pictures of unevenly patterned pit structures of (**a** and **b**) designs A and design B reproduced outside the triangular areas (scale:  $500 \times 500$  nm) and (**c** and **d**) profiles of (**a**) and (**b**) across the length of 500 nm

From the AFM profiles, the uniformity and distribution of the pits A and B 425 parameters (or dimensions) such as diameter, depth and pitch produced by FIB technique were evaluated. The A and B pit dimensions were measured using a Nanoscope 427 imaging probe software as described above. 428

The distribution curves of A and B pit dimensions were plotted and presented in 429 Fig. 16.16. Figure 16.16a shows that when seeking to produce design A diameter 430 pits (N=162), the result was far from uniform. Although the highest number of pits 431 did fall within the 116–120 nm range, the graph shows there were considerable 432 variations in resulting pit sizes. 433

In seeking to produce design B diameter pits (N=90), Fig. 16.16b shows a similar pattern to the result shown in the previous graph. The highest number of pits was between 181 and 185 nm range with the lowest amount of pits registering in the ranges above 180 nm. 437



**Fig. 16.16** Distribution curves of pit diameter of (a) and (b) 120 and 180 nm with a pitch of (c) and (d) 240 and 360 nm and depth of (e) and (f) 50-100 nm

The required A pitch range here (Fig. 16.16c) was 240 nm (N=128). Most pits registered between 251–255 and 241–245 nm then at 246–250 nm range. However, only four pits were reported at 240 nm.

In looking for a design B pitch level of 360 nm (N=150 nm) presented in Fig. 16.16d, only 13 pits fell within this range. The majority of pits registered at pitches higher than the required level of 360 nm, falling between 371 and 380 nm, and one pit range as high as 411–420 nm.

In searching for a design A depth of 50 or 100 nm (N=95), Fig. 16.16e shows that one pit reported at 46–50 nm range and four pits reported at 96–105 nm range. However, considerable variation is still noted in the dimensions of all pits.

448 Again looking for a design B depth of 50 or 100 nm (N=110), nine pits were 449 recorded within the 46–55 nm range (Fig. 16.16f). Nine were found to have a depth

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t2.2 t2.3	Dimensions	Sites number	Mean±SD (nm)	Lowest dimension value (nm)	Highest dimension value (nm)
t2.4	Diameter	N=162	$97 \pm 8.5$	46	138
t2.5	Pitch	N=128	$250 \pm 11$	213	290
t2.6	Depth	N=95	$65 \pm 24$	19	116

t2.1 Table 16.2 Dimensions of 120 nm pits obtained with FIB patterning

t3.1 Table 16.3 Dimensions of 180 nm pits obtained with FIB patterning

Dimensions	Sites number	Mean±SD (nm)	Lowest dimension value (nm)	Highest dimension value (nm)
Diameter	N=90	155±11	10	221
Pitch	N=150	$375 \pm 14$	323	411
Depth	N=110	$84 \pm 36$	15	165

in the 96–105 nm range. However, some pits were also recorded above the desired 450 100 nm range which should have minor influence as long as they are deep enough 451 to influence the cell adhesion. 452

The variations in pit dimensions demonstrated that FIB milling rate is greatly 453 influenced by the polycrystalline structure of stainless steel and beam quality. 454

Tables 16.2 and 16.3 summarise the obtained pit dimensions of design A and B diameter (N=162 and 90; scan area,  $1 \times 1 \mu m^2$ ) and pitch and depth (N=128 and 150 diameter (N=162 and 90; scan area,  $5 \times 5 \mu m^2$ ) created by FIB milling. For the required 120 nm diameter of 97±8.5 nm (N=162), pitch of 250±11 nm diameter of 250±11 nm (N=128) and depth of  $65\pm24$  nm (N=95), whereas for the 180 nm pit, the average pit had a diameter of  $375\pm14$  nm (N=150) and depth d

Nevertheless, correlation of FIB–SEM and AFM 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 pit structures. 464

All these variations in pit dimensions reported in stainless steel samples can be due to the atomic arrangement or random orientation of crystallographic structures and nonuniformity in grain size, though the shape of the incident beam at the point of impact on the sample surface will have the greatest effect of all the factors, followed by the crystallographic orientation of the grain at the surface. 469

### **16.12** In Vitro Cell Studies: Endothelial Cell Adhesion Tests 470

Finally, in vitro human EC culture and EC adhesion and densities studies were performed on unpolished, electropolished and nano-textured stainless steel surfaces. 472

Figure 16.17 shows the fluorescence images of the EC adhesion and densities 473 after 1–5 days on unpolished, electropolished and design A and B surfaces. Very 474 little significant difference in EC adhesion between these surfaces is revealed. 475



**Fig. 16.17** Fluorescent images of EC cultured on (**a**) unpolished (**b**) electropolished, (**c**) design A pits and (**d**) design B pits on stainless steel surfaces after 1, 3 and 5 days

However, the morphology of ECs appears to be greatly defined on pits design A and
B relative to electropolished and unpolished samples. After 1 day, EC densities
were significantly lower on nano-structured 316L steel substrates when compared
to unpolished and electropolished control samples. EC adhesion density was significantly greater after 5 days compared to 1 day for all substrates tested.

To confirm the significant difference in EC adhesion and densities after 1–5 days involving these surfaces as demonstrated by fluorescence data, EC counts were performed on each three substrate of interest (N=12).

### 484 **16.13 Conclusion**

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 are largely affected by the grain size of the polycrystalline

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316L stainless steel and stability of the ion beam quality over large areas. Moreover 488 this method is practically limited to 120 nm resolution for the desired pit depth and 489 uniform scan size of 200  $\mu$ m × 200  $\mu$ m. Nevertheless formed structures show large 490 variation of pit depths and shapes and as such surfaces might serve as a resourceful 491 platform for screening large variations of cell/pattern stainless steel interactions. 492 However, the FIB nano-pits design A and B created on polycrystalline stainless 493 steel surfaces demonstrated low EC adhesion and proliferation relative to unpol-494 ished and electropolished specimens. There was no significant difference in EC 495 adhesion and proliferation between unpolished-electropolished samples and design 496 A and B pits. Further morphological examination of EC response on nano-structured 497 steel surfaces would verify the mechanism for low EC adhesion and proliferation on 498 these surfaces. Nano-patterning the stainless steel surfaces by FIB is time consum-499 ing and expensive, especially when patterning large areas. The precision and repro-500 ducibility of this technique is greatly affected by the polycrystallinity of stainless 501 steel and a stable beam quality over large sample areas. 502

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### **References**

1.	Dugdale, D.C.: Stent, in Medicine Plus, 2012. http://www.nlm.nih.gov/medlineplus/ency/article/002303.htm	513 514
2.	Balamurugan, A., Rajeswari, S., Balossier, G., Rebelo, A.H.S., Ferreira, J.M.F.: Corrosion	515
	aspects of metallic implants—an overview. Mater. Corrosion 59(11), 855–869 (2008)	516
3.	O'Brien, B., Carroll, W.: The evolution of cardiovascular stent materials and surfaces in	517
	response to clinical drivers: a review. Acta Biomater. 5(4), 945–958 (2009)	518
4.	Clerc, C.O., Jedwab, M.R., Mayer, D.W., Thompson, P.J., Stinson, J.S.: Assessment of	519
	wrought ASTM F1058 cobalt alloy properties for permanent surgical implants. J. Biomed.	520
	Mater. Res. <b>38</b> (3), 229–234 (1997)	521
5.	Craig, C., Friend, C., Edwards, M., Cornish, L., Gokcen, N.: Mechanical properties and micro-	522
	structure of platinum enhanced radiopaque stainless steel (PERSS) alloys. J. Alloys Compd.	523
	<b>361</b> (1), 187–199 (2003)	524
6.	Craig, C., Friend, C., Edwards, M., Gokcen, N.: Tailoring radiopacity of austenitic stainless	525
	steel for coronary stents. In: Medical Device Materials: Proceedings from the Materials &	526
	Processes for Medical Devices Conference 2003, 8–10 September 2003, Anaheim, California.	527
	2004. American Society for Metals	528
7.	Heublein, B., Rohde, R., Kaese, V., Niemeyer, M., Hartung, W., Haverich, A.: Biocorrosion of	529
	magnesium alloys: a new principle in cardiovascular implant technology? Heart 89(6), 651-	530
	656 (2003)	531

512

- 532 8. Lüscher, T.F., Steffel, J., Eberli, F.R., Joner, M., Nakazawa, G., Tanner, F.C., Virmani, R.:
  533 Drug-eluting stent and coronary thrombosis biological mechanisms and clinical implications.
  534 Circulation 115(8), 1051–1058 (2007)
- 9. Daemen, J., Wenaweser, P., Tsuchida, K., Abrecht, L., Vaina, S., Morger, C., Kukreja, N., Jüni,
  P., Sianos, G., Hellige, G.: Early and late coronary stent thrombosis of sirolimus-eluting and
  paclitaxel-eluting stents in routine clinical practice: data from a large two-institutional cohort
  study. Lancet 369(9562), 667–678 (2007)
- 10. Chou, L., Firth, J.D., Uitto, V.-J., Brunette, D.M.: Substratum surface topography alters cell
  shape and regulates fibronectin mRNA level, mRNA stability, secretion and assembly in
  human fibroblasts. J. Cell Sci. 108(4), 1563–1573 (1995)
- 542 11. Boyan, B.D., Hummert, T.W., Dean, D.D., Schwartz, Z.: Role of material surfaces in regulat543 ing bone and cartilage cell response. Biomaterials 17(2), 137–146 (1996)
- 12. Craighead, H.G., James, C.D., Turner, A.M.P.: Chemical and topographical patterning for
   directed cell attachment. Curr. Opin. Solid State Mater. Sci. 5(2–3), 177–184 (2001)
- 546 13. Curtis, A., Wilkinson, C.: Nantotechniques and approaches in biotechnology. Trends
  547 Biotechnol. 19(3), 97–101 (2001)
- 548 14. Koegler, P., Clayton, A., Thissen, H., Santos, G.N.C., Kingshott, P.: The influence of nanostructured materials on biointerfacial interactions. Adv. Drug Deliv. Rev. 64(15), 1820–1839 (2012)
- 15. Nikkhah, M., Edalat, F., Manoucheri, S., Khademhosseini, A.: Engineering microscale topog raphies to control the cell–substrate interface. Biomaterials 33(21), 5230–5246 (2012)
- 16. Chen, L., Han, D., Jiang, L.: On improving blood compatibility: from bioinspired to synthetic
  design and fabrication of biointerfacial topography at micro/nano scales. Colloids Surf. B
  Biointerfaces 85(1), 2–7 (2011)
- 17. Gentile, F., Tirinato, L., Battista, E., Causa, F., Liberale, C., di Fabrizio, E.M., Decuzzi, P.:
  Cells preferentially grow on rough substrates. Biomaterials 31(28), 7205–7212 (2010)
- 558 18. Kasemo, B.: Biological surface science. Surf. Sci. **500**(1–3), 656–677 (2002)
- 19. Duncan, A.C., Weisbuch, F., Rouais, F., Lazare, S., Baquey, C.: Laser microfabricated model
   surfaces for controlled cell growth. Biosens. Bioelectron. 17(5), 413–426 (2002)
- 20. Berry, C.C., Campbell, G., Spadiccino, A., Robertson, M., Curtis, A.S.G.: The influence of
  microscale topography on fibroblast attachment and motility. Biomaterials 25(26), 5781–5788
  (2004)
- 564 21. Yim, E.K.F., Leong, K.W.: Significance of synthetic nanostructures in dictating cellular
   565 response. Nanomedicine 1(1), 10–21 (2005)
- Falconnet, D., Csucs, G., Grandin, H.M., Textor, T.: Surface engineering approaches to
   micropattern surfaces for cell-based assays. Biomaterials 27(16), 3044–3063 (2006)
- 568 23. Yu, L.M.Y., Leipzig, N.D., Shoichet, M.S.: Promoting neuron adhesion and growth. Mater.
   569 Today 11(5), 36–43 (2008)
- 24. Roach, P., Parker, T., Gadegaard, N., Alexander, M.R.: Surface strategies for control of neuronal cell adhesion: a review. Surf. Sci. Rep. 65(6), 145–173 (2010)
- 572 25. Nazneen, F., Herzog, G., Arrigan, D.W., Caplice, N., Benvenuto, P., Galvin, P., Thompson, M.:
  573 Surface chemical and physical modification in stent technology for the treatment of coronary
  574 artery disease. J. Biomed. Mater. Res. B Appl. Biomater. 100, 1989–2014 (2012)
- 26. Norman, J.J., Desai, T.A.: Methods for fabrication of nanoscale topography for tissue engineering scaffolds. Ann. Biomed. Eng. 34(1), 89–101 (2006)
- 577 27. Buzea, C., Beydaghyan, G., Elliott, C., Robbie, K.: Control of power law scaling in the growth
  578 of silicon nanocolumn pseudo-regular arrays deposited by glancing angle deposition.
  579 Nanotechnology 16(10), 1986 (2005)
- 28. Dolatshahi-Pirouz, A., Hovgaard, M.B., Rechendorff, K., Chevallier, J., Foss, M., Besenbacher,
  F.: Scaling behavior of the surface roughness of platinum films grown by oblique angle deposition. Phys. Rev. B 77(11), 115427 (2008)
- 583 29. Tseng, A.A., Notargiacomo, A.: Nanoscale fabrication by nonconventional approaches.
  584 J. Nanosci. Nanotechnol. 5(5), 683–702 (2005)

- 16 FIB Patterning of Stainless Steel for the Development of Nano-structured Stent...
- 30. Sarkar, S., Dadhania, M., Rourke, P., Desai, T.A., Wong, J.Y.: Vascular tissue engineering: 585 microtextured scaffold templates to control organization of vascular smooth muscle cells and extracellular matrix. Acta Biomater. 1(1), 93–100 (2005) 587
- 31. Houtchens, G.R., Foster, M.D., Desai, T.A., Morgan, E.F., Wong, J.Y.: Combined effects of microtopography and cyclic strain on vascular smooth muscle cell orientation. J. Biomech. 589 41(4), 762–769 (2008) 590
- 32. Biela, S.A., Su, Y., Spatz, J.P., Kemkemer, R.: Different sensitivity of human endothelial cells, smooth muscle cells and fibroblasts to topography in the nano-micro range. Acta Biomater. 592 5(7), 2460–2466 (2009) 593
- 33. Green, A.M., Jansen, J.A., Van der Waerden, J.P.C.M., Von Recum, A.F.: Fibroblast response 594 to microtextured silicone surfaces: texture orientation into or out of the surface. J. Biomed. 595 Mater. Res. 28(5), 647–653 (1994) 596
- 34. Walboomers, X., Croes, H., Ginsel, L., Jansen, J.: Contact guidance of rat fibroblasts on various implant materials. J. Biomed. Mater. Res. 47(2), 204–212 (1999)
   598
- 35. Walboomers, X., Croes, H., Ginsel, L., Jansen, J.: Growth behavior of fibroblasts on microgrooved polystyrene. Biomaterials 19(20), 1861–1868 (1998)
   600
- 36. Walboomers, X., Ginsel, L., Jansen, J.: Early spreading events of fibroblasts on microgrooved 601 substrates. J. Biomed. Mater. Res. 51(3), 529–534 (2000) 602
- 37. Walboomers, X., Monaghan, W., Curtis, A., Jansen, J.: Attachment of fibroblasts on smooth and microgrooved polystyrene. J. Biomed. Mater. Res. 46(2), 212–220 (1999)
  604
- Loesberg, W.A., te Riet, J., van Delft, F.C., Schön, P., Figdor, C.G., Speller, S., van Loon, J.J.,
   Walboomers, X.F., Jansen, J.A.: The threshold at which substrate nanogroove dimensions may
   influence fibroblast alignment and adhesion. Biomaterials 28(27), 3944–3951 (2007)
- 39. Dalby, M.J., Gadegaard, N., Riehle, M.O., Wilkinson, C.D.W., Curtis, A.S.G.: Investigating filopodia sensing using arrays of defined nano-pits down to 35 nm diameter in size. Int. J. Biochem. Cell Biol. 36(10), 2005–2015 (2004)
- 40. Dalby, M.J., Gadegaard, N., Wilkinson, C.D.: The response of fibroblasts to hexagonal nano-topography fabricated by electron beam lithography. J. Biomed. Mater. Res. A 84(4), 973–979 (2008)
  612 (2008)
- 41. Yim, E.K.F., Reano, R.M., Pang, S.W., Yee, A.F., Chen, C.S., Leong, K.W.: Nanopatterninduced changes in morphology and motility of smooth muscle cells. Biomaterials 26(26), 615 5405–5413 (2005)
- 42. Lee, S.W., Kim, S.Y., Rhyu, I.C., Chung, W.Y., Leesungbok, R., Lee, K.W.: Influence of microgroove dimension on cell behavior of human gingival fibroblasts cultured on titanium substrata. Clin. Oral Implants Res. 20(1), 56–66 (2009) 619
- 43. Ito, T., Okazaki, S.: Pushing the limits of lithography. Nature **406**(6799), 1027–1031 (2000) 620
- 44. Chou, S.Y., Krauss, P.R., Renstrom, P.J.: Imprint of sub-25 nm vias and trenches in polymers. 621 Appl. Phys. Lett. 67(21), 3114–3116 (1995)
   622
- 45. Raffa, V., Castrataro, P., Menciassi, A., Dario, P.: Focused Ion Beam as a Scanning Probe:
  Methods and Applications. Applied Scanning Probe Methods II, pp. 361–412. Springer, New York, NY (2006)
- 46. Iordanova, I., Antonov, V., Gurkovsky, S.: Changes of microstructure and mechanical properties of cold-rolled low carbon steel due to its surface treatment by Nd:glass pulsed laser. Surf. Coat. Technol. 153(2–3), 267–275 (2002)
- 47. Duncan, A.C., Rouais, F., Lazare, S., Bordenave, L., Baquey, C.: Effect of laser modified surface microtopochemistry on endothelial cell growth. Colloids Surf. B Biointerfaces 54(2), 630 150–159 (2007)
- 48. Kalantar-Zadeh, K., Fry, B.: Nanotechnology Enabled Sensors. Springer, New York, NY (2007) 632
- 49. Volkert, C., Minor, A.: Focused ion beam microscopy and micromachining. MRS Bull. 32(5), 633 389–395 (2007)
- 50. Lanyon, Y.H., De Marzi, G., Watson, Y.E., Quinn, A.J., Gleeson, J.P., Redmond, G., Arrigan,
  635
  D.W.: Fabrication of nanopore array electrodes by focused ion beam milling. Anal. Chem.
  636
  79(8), 3048–3055 (2007)
  637

- 638 51. Choi, C.H., Heydarkhan-Hagvall, S., Wu, B.M., Dunn, J.C., Beygui, R.E., Kim, C.J.: Cell growth as a sheet on three-dimensional sharp-tip nanostructures. J. Biomed. Mater. Res. 639 640 A 89(3), 804-817 (2009)
- 52. Raffa, V., Vittorio, O., Pensabene, V., Menciassi, A., Dario, P.: FIB-nanostructured surfaces 641 and investigation of bio/nonbio interactions at the nanoscale. IEEE Trans. NanoBiosci. 7(1), 642 643 1-10 (2008)
- 53. Joshi, K., Singh, P., Verma, S.: Fabrication of platinum nanopillars on peptide-based soft struc-644 tures using a focused ion beam. Biofabrication 1(2), 025002 (2009) 645
- 54. Choubey, A., Marton, D., Sprague, E.A.: Human aortic endothelial cell response to 316L stain-646 less steel material microstructure. J. Mater. Sci. Mater. Med. 20(10), 2105–2116 (2009) 647
- 55. Misra, R.D., Nune, C., Pesacreta, T.C., Somani, M.C., Karjalainen, L.P.: Understanding the 648 impact of grain structure in austenitic stainless steel from a nanograined regime to a coarse-649 650 grained regime on osteoblast functions using a novel metal deformation-annealing sequence. Acta Biomater. 9, 6245–6258 (2013) 651
- 56. Russell, A., Lee, K.L.: Structure-Property Relations in Nonferrous Metals. Wiley-Interscience, 652 New York, NY (2005) 653
- 57. Shi, D.: Introduction to Biomaterials. World Scientific, London (2006) 654
- 58. Nazneen, F., Galvin, P., Arrigan, D.W., Thompson, M., Benvenuto, P., Herzog, G.: 655 Electropolishing of medical-grade stainless steel in preparation for surface nano-texturing. 656 657 J. Solid State Electrochem. 16(4), 1389–1397 (2012)
- 59. Dalby, M.J., Berry, C.C., Riehle, M.O., Sutherland, D.S., Agheli, H., Curtis, A.S.G.: Attempted 658 endocytosis of nano-environment produced by colloidal lithography by human fibroblasts. 659 Exp. Cell Res. 295(2), 387-394 (2004) 660
- 60. Schmidt, M., Nazneen, F., Georgiev, Y., Herzog, G., Galvin, P., Petkov, N.: FIB patterning of 661 stainless steel for the development of nano-structured stent surfaces for cardiovascular applica-662 663 tions. J. Phys. Conf. **371**(1), 012065 (2012)
- 61. Nazneen, F., Schmidt, M., McLoughlin, E., Petkov, N., Herzog, G., Arrigan, D., Galvin, P.: [AU4] 664 665 Nano-texturing of medical-grade 316L stainless steel by focused ion beam for endothelial cell studies. J. Nanosci. Nanotechnol., 2013. 666
- 62. Feitknecht, W.: Über den angriff von krystallen durch kanalstrahlen. Helv. Chim. Acta 7(1), 667 825-842 (1924) 668
- 63. Wehner, G.K.: Controlled sputtering of metals by low-energy Hg ions. Phys. Rev. 102(3), 669 690-704 (1956) 670
- 64. Wehner, G.K., Rosenberg, D.: Angular distribution of sputtered material. J. Appl. Phys. 31(1), 671 177-179 (1960) 672
- 673 65. Sigmund, P.: Theory of sputtering. I. Sputtering yield of amorphous and polycrystalline targets. Phys. Rev. 184(2), 383-416 (1969) 674
- 66. Bean, K.E.: Anisotropic etching of silicon. IEEE Trans. Electron Dev. 25(10), 1185-1193 675 676 (1978)
- 67. Seidel, H., Csepregi, L., Heuberger, A., Baumgärtel, H.: Anisotropic etching of crystalline sili-677 con in alkaline solutions I. Orientation dependence and behavior of passivation layers. 678 J. Electrochem. Soc. 137(11), 3612-3626 (1990) 679
- 68. Schmidt, M., Nazneen, F., Herzog, G., Arrigan, D., Galvin, P., Keeney, L., Petkov, N., Holmes, 680 [AU5] 681 J.D.: to be submitted.
- 69. Schmidt, M., Nazneen, F., Herzog, G., Arrigan, D., Galvin, P., Dickinson, C., de Silva, J.P., [AU6] 682 Scanlan, D., O'Hara, N., Cross, G.L.W.: Correlative microscopy study of FIB patterned stain-683 684 less steel surfaces as novel nano-structured stents for cardiovascular applications. MRS Proc 2012. 1466(1). 685
- 70. Giannuzzi, L., Stevie, F.: A review of focused ion beam milling techniques for TEM specimen 686 preparation. Micron **30**(3), 197–204 (1999) 687
- 71. Schwartz, A.J., Kumar, M., Adams, B.L., Field, D.P.: Electron Backscatter Diffraction in 688 Materials Science. Springer, New York, NY (2009) 689
- 72. Stark, Y., Fromter, R., Stickler, D., Oepen, H.P.: Sputter yields of single-and polycrystalline metals 690 for application in focused ion beam technology. J. Appl. Phys. 105(1), 013542-1-013542-5 (2009) 691