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# Roughness Statistical Influence on Cell Adhesion Using Profilometry and Multiscale Analysis

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**Summary:** In this study, two series of 11 samples of TiAl6V4 titanium alloy and 316L stainless steel have been polished in an isotropic manner at different levels in order to quantify the influence of biomaterial roughness on cell behavior. Topography was measured by a tactile profilometer and a multiscale analysis has been carried out. Human osteoblasts have been cultured on those samples. It appears that roughness has no reproducible effect on the cell behavior except an influence on cell orientation on the wider grooves. As a conclusion, biomaterial surface damage, in the roughness range between  $R_a = 0.01$  and  $0.1 \mu\text{m}$ , has no influence on cell-adhesion mechanisms when roughness is isotropic and groove width is inferior to a critical value. SCANNING 00: 1–9, 2012. © 2012 Wiley Periodicals, Inc.

**Key words:** surface analysis, metrology, life sciences, polishing technique, computer simulation

## Introduction

Metallic materials like stainless steel 316L, titanium, titanium alloys, and chromium/cobalt alloys are frequently used in the biomedical field for the manufacture of prostheses (Geetha *et al.*, 2009).

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The influence of metallic surface topography on cell behavior has been widely studied (Martin *et al.*, '95; Lincks *et al.*, '98; Soboyejo *et al.*, 2002; Kim *et al.*, 2005; Chen *et al.*, 2007; Le Guehennec *et al.*, 2007; Giljean *et al.*, 2010; Bigerelle *et al.*, 2011a) using various processes such as plasma spraying, sandblasting, acid etching, anodization, etc. To obtain isotropic topographies over a wide range of dimensions from sub-micron to several microns, researchers generally compare surfaces obtained by various processes (Martin *et al.*, '95; Lincks *et al.*, '98; Soboyejo *et al.*, 2002; Kim *et al.*, 2005; Chen *et al.*, 2007; Le Guehennec *et al.*, 2007; Giljean *et al.*, 2010). However, various processes lead to different topographies in term of amplitude and, above all, in term of morphology, rendering the results noncomparable over the range of roughness.

In addition to good biocompatibility and adequate mechanical properties, a biomaterial must have good resistance to corrosion and wear. Failure to comply with this condition can cause a release of metal ions in the body, especially in the blood, and can cause allergic or toxic reaction (Hallab *et al.*, 2005). Despite of those problems of wear and corrosion resistance, machining (Sinnott-Jones *et al.*, 2005) and polishing (Zhang *et al.*, 2009) are currently used as manufacturing process of implants. These processes generate different surface topographies that directly affect the cellular response and therefore the lifetime of an implant.

The purpose of this study is to develop biocompatible metal surfaces obtained by polishing with different grades of polishing paper, in order to correlate, using a statistical analysis, the influence of a complete range of roughnesses measured by tactile profilometry on cell adhesion. Compared to other studies (Martin *et al.*, '95; Lincks *et al.*, '98; Soboyejo *et al.*, 2002; Kim *et al.*, 2005; Chen *et al.*, 2007; Le Guehennec *et al.*, 2007; Giljean *et al.*, 2010), only one manufacture process is used here to obtain a range of roughnesses while avoiding any chemical artifact.

## Materials and Method

### Sample Preparation

Two rods of 30 mm in diameter, of 316L stainless steel and TiAl6V4 titanium alloy, both respecting requirements of biomedical field, were used in this study. In each rod, 22 samples were cut and polished with 11 different silicon carbide paper grades. The 11 grades were 80, 120, 180, 220, 320, 500, 800, 1,000, 1,200, 2,400, and 4,000. Finally, two series of 11 samples denoted, respectively, “series 1” and “series 2” were obtained for each material with 11 different roughnesses. Polishing was achieved on a Pedemax 2 automatic polishing machine (Struers SAS, Chagny sur Marne, France) with two off-centered rotating movements at a speed of 300 rotations per minute to obtain isotropic roughness. For each sample, a new silicon carbide paper was used with water lubrication under 150-N load during 3 min.

### Roughness Measurements

To quantify the roughness, a tactile profilometer Tencor P10 (KLA-Tencor, Milpitas, CA, USA) was used. The vertical sensitivity of the device is about 10 and 50 nm in the plane. Measurements were achieved with a stylus having a radius of 2  $\mu\text{m}$ . First, 3D measurements were realized in order to check for the isotropy of the roughness. As the roughness of the different samples was demonstrated to be isotropic (data not shown), 30 2D profiles were randomly recorded on each sample at a speed of 200  $\mu\text{m/s}$  with a sampling frequency of 1,000 Hz in order to obtain 25,000 points on 5-mm length (0.2  $\mu\text{m}$  between each recorded point) under 5-mg load.

### Multiscale Roughness Analysis

One of the major problem concerning roughness measurement analyses is the determination of the evaluation length from which roughness parameters are computed (Bigerelle and Iost, 2001). The methodology was fully described elsewhere (Giljean *et al.*, 2007). In a few words, roughness profiles were cut in equal length windows whose length corresponds to the evaluation length  $\varepsilon$ . On each window, the profile was redressed by a first-order polynomial using a least-square linear regression. Then a  $C^0$  continuity was imposed between each window and finally a B-spline was obtained to correct the profile in order to suppress form and wave geometrical defects. Example of correction is given in Figure 1. The original profile and the B-spline obtained for  $\varepsilon = 131 \mu\text{m}$  are plotted on the left and the obtained roughness profile is plotted on the right. On those corrected profiles,

more than 100 roughness parameters were calculated. Amplitude, frequency, hybrid, and fractal parameters were considered and calculated for  $L$  ranging from 0.2 to 5,000  $\mu\text{m}$ . For each samples, 30 profiles were measured and each roughness value corresponds to the mean obtained on the 30 different profiles at a given evaluation length.

### Cell Culture

Human mesenchymal stem cells (hMSCs) were prepared from the bone marrow of healthy patients as previously defined (Anselme *et al.*, 2002). hMSCs were cultured in direct contact with the surfaces in Iscove-modified Dulbecco medium + 10% fetal bovine serum + penicillin-streptomycin. On each sample,  $10^4$  cells per  $\text{cm}^2$  were deposited and cultured in an incubator at 37°C with 5% of  $\text{CO}_2$  during 48 h. Because of the large size of the sample (diameter 30 mm, height 20 mm), sterile pots with appropriate size were used and agar was deposited around the samples up to 1 mm from the surface to limit cell loss at the edges. After culture, samples were rinsed in phosphate-buffered saline (PBS) and fixed at least 30 min in paraformaldehyde 2% in  $\text{NaK}_2\text{P}$  0.2 M buffer. After rinsing with PBS, and permeabilization with Triton 0.2% in PBS, the actin cytoskeleton was labeled with 0.4  $\mu\text{g/ml}$  FITC-phalloidin (Sigma, France) and the nuclei of cells were labeled with 100 ng/ml DAPI (4,6-diamidino-2-phenylindole; Sigma). Then a drop of PBS-glycerol was deposited on the samples before being placed under a microscope glass coverslip in order to avoid surface drying problems. Finally, samples were observed with an epifluorescence microscope Olympus BX51 (Olympus, France). The number of cells was determined by counting the number of nuclei labeled by DAPI on fluorescent pictures using the Image J 1.40 g software and was converted to a number of cells by square millimeter. The area covered by cells, or cell spreading, was measured on pictures with actin labeling using the Image J software. Then the surface covering rate was calculated in percentage of total area.

### Statistical Analysis

First, a classical variance analysis was performed to test if there exists a significant difference in adhesion due to material or the roughness produced by polishing. To test the repeatability, two runs were considered. A three-parameter variance analysis was performed with the three factors: materials, run, and grit paper and their associated interactions.

Second, an original method using two runs of bootstrap was developed to find which of the various roughness parameters is the most relevant to

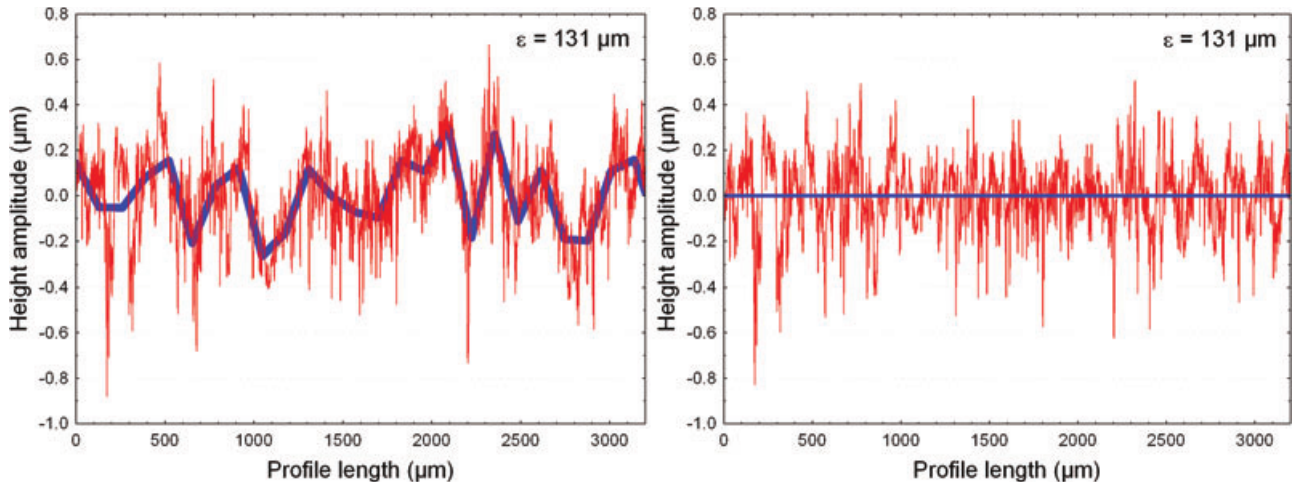


Fig 1. Example of a profile correction to suppress form and wave geometrical defects.

characterize the morphology of a polished surface with regard to cell adhesion. The main purpose of this investigation is to propose a methodology in order to answer this question without any preconception on the possible relevance of any roughness parameter. Thus, only a statistical approach using intensive computer calculations seems appropriate to treat the experimental data. A specific software program was developed and tested to study the relevance of about one hundred roughness surface parameters. An index  $P(q, \varepsilon, M, r)$  was defined to correlate the surface parameters  $q$ , evaluated at the evaluation length  $\varepsilon$  on materials  $M$  and run  $r$  with the cell-adhesion level. The run value is 1 or 2 according to the sample series tested (see “Sample preparation” section). The conventional statistical theory used was the linear correlation analysis. To study the relationships between the surface roughness parameters and the cell-adhesion level, the main problem, which have to be solved, could be summarized as follows: how to correlate a roughness parameter with cell-adhesion level when  $X$  roughness measurements and  $Y$  cell adhesion measurements was separately made on each sample at different points? The most common alternative is to search for a possible correlation between roughness parameters and cell-adhesion level means (number of adhered cells). In this study, the bootstrap theory was used to consider the influence of experimental variations. In this case,  $N$  ( $N > 500$ ) equivalent computational sets of data are generated from the experimental set of data by  $N$  random drawing and replacing. For each newly generated set of data, both the means of the roughness parameter and number of adhered cell can be estimated. The analysis was conducted for every roughness parameter, at every scale, for each material and for the two runs and is illustrated in the “Results” section using an example on the roughness parameter  $R_a$ .

## Results

The results of the multiscale analysis of roughness are plotted in Figure 2 for two roughness parameters arbitrarily chosen: the amplitude roughness parameter  $R_k$ , which represents the intermediate roughness without taking into account highest peaks and lowest valleys frequently used when studying wear phenomena like polishing, and the frequency roughness parameter  $S_m$ , which represents the mean distance between peaks. Figure 2 shows a typical evolution of roughness parameters versus the evaluation length (Bigerelle *et al.*, 2011b). Roughness values increase, as the evaluation length increases, up to a macroscopic threshold beyond which roughness is constant. The threshold value depends on the polishing paper grade: the rougher the samples are, the more the threshold is reached for high values of evaluation length. Figure 2 demonstrates the importance of the choice of the evaluation length in order to characterize the influence of the roughness on a physical, chemical, or biological phenomenon.

After 2 days of culture, cells are labeled with DAPI to highlight the nuclei and with FITC-phalloidin for the cytoskeleton. Then samples are observed with the epifluorescence microscope. Figure 3 shows the results obtained superimposing the image of DAPI, the image of FITC, and the image of the topography for both 316L stainless steel and TiAl6V4 titanium alloy for four different paper grades. The quite white (blue in color reprint) points represent the nuclei of cells while the light gray (green in color reprint) areas represent the cytoskeleton of the cells. The mean cell size ranges between 50 and 200  $\mu\text{m}$ . Visually, no significant difference can be observed between the two materials 316L and TiAl6V4. The material does not appear to have a significant influence in terms of cell number and surface-covering rate. Similarly,



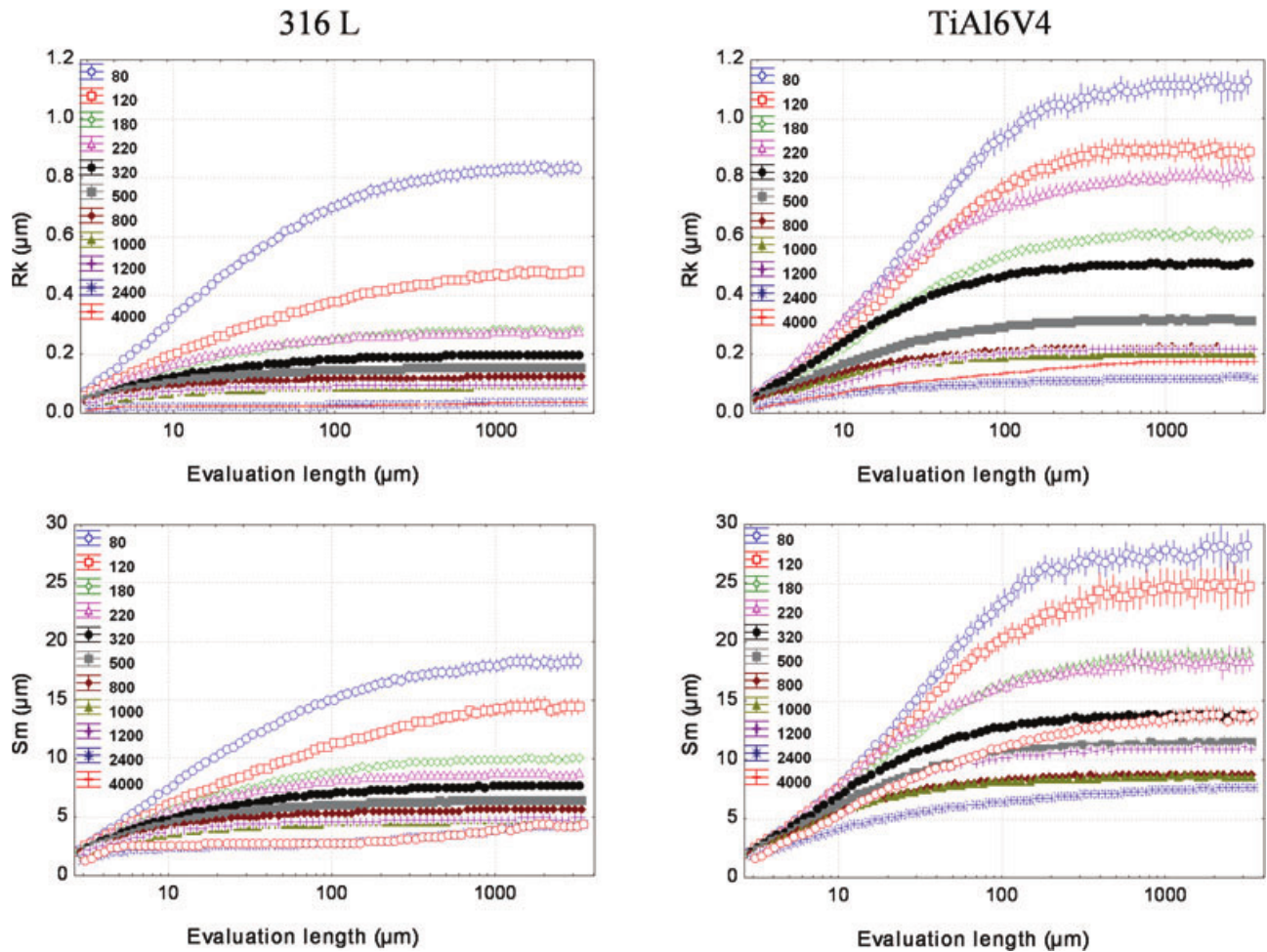


Fig 2. Evolution of the roughness amplitude parameter  $R_k$ , and the roughness frequency parameter  $S_m$  for each paper grade and for both materials versus the evaluation length.

no influence of the paper grade can be noticed except for the paper grade 80 where, on the two materials, cell alignment on the widest grooves (which are also the deepest) can be noted. Measurement of the size of the largest scratches was performed in another study (Giljean *et al.*, 2008). The width of the grooves was measured on the two materials and ranged between 11–13  $\mu\text{m}$  on 316L and 12–13  $\mu\text{m}$  on TiAl6V4. Quantification by image analysis of cell number was performed and converted to a number of cells per square millimeter. The results are presented in Figure 4. The  $x$ -axis scale was, first, arbitrarily chosen and the “infinite  $R_a$ ” denoted  $R_{a\_inf}$  was selected, which represents the macroscopic value of the sample roughness beyond the threshold (Fig. 2). A large variability on both the number of cells, and covering rate of the surface is observed. This variability demonstrates that the influence of the roughness on the behavior of a population of cell is low. The number of cells appears to be invariant regardless of the material and the roughness value and is confirmed hereafter by statistical analysis. Those results show

that the cell spreading (ratio of the surface covered by cells) is constant regardless of the observed sample. To confirm those observations, a variance analysis was conducted to test the influence of the grit paper and the materials on cell behavior. The results of the variance analysis are summarized in Table I. There is no effect on the different materials on cell adhesion ( $F = 1.25$ ,  $p = 0.26$ ) and for the two runs ( $F = 0$ ,  $p = 0.97$ ). The cell adhesion depends on the grit paper ( $F = 14.44$ ,  $p = 0$ ). However, this result depends on the Materials (interaction Materials  $\times$  Grit Paper,  $F = 8.25$ ,  $p = 0$ ) and also of the Run (Run  $\times$  Grit Paper,  $F = 22.26$ ,  $p = 0$ ). These interactions mean that roughness induced by polishing with a given size of grit depends on the material. This result is logical in the point of view of the abrasion process (Bigerelle *et al.*, 2008; Bigerelle *et al.*, 2011b). The Run  $\times$  Grit Paper interaction means that abrasion process is not a repetitive process for a given paper and can be explained by the fact that the grit size effect on abrasion process depends on the wear of the grits of the paper and debris accumulations that are difficult to control

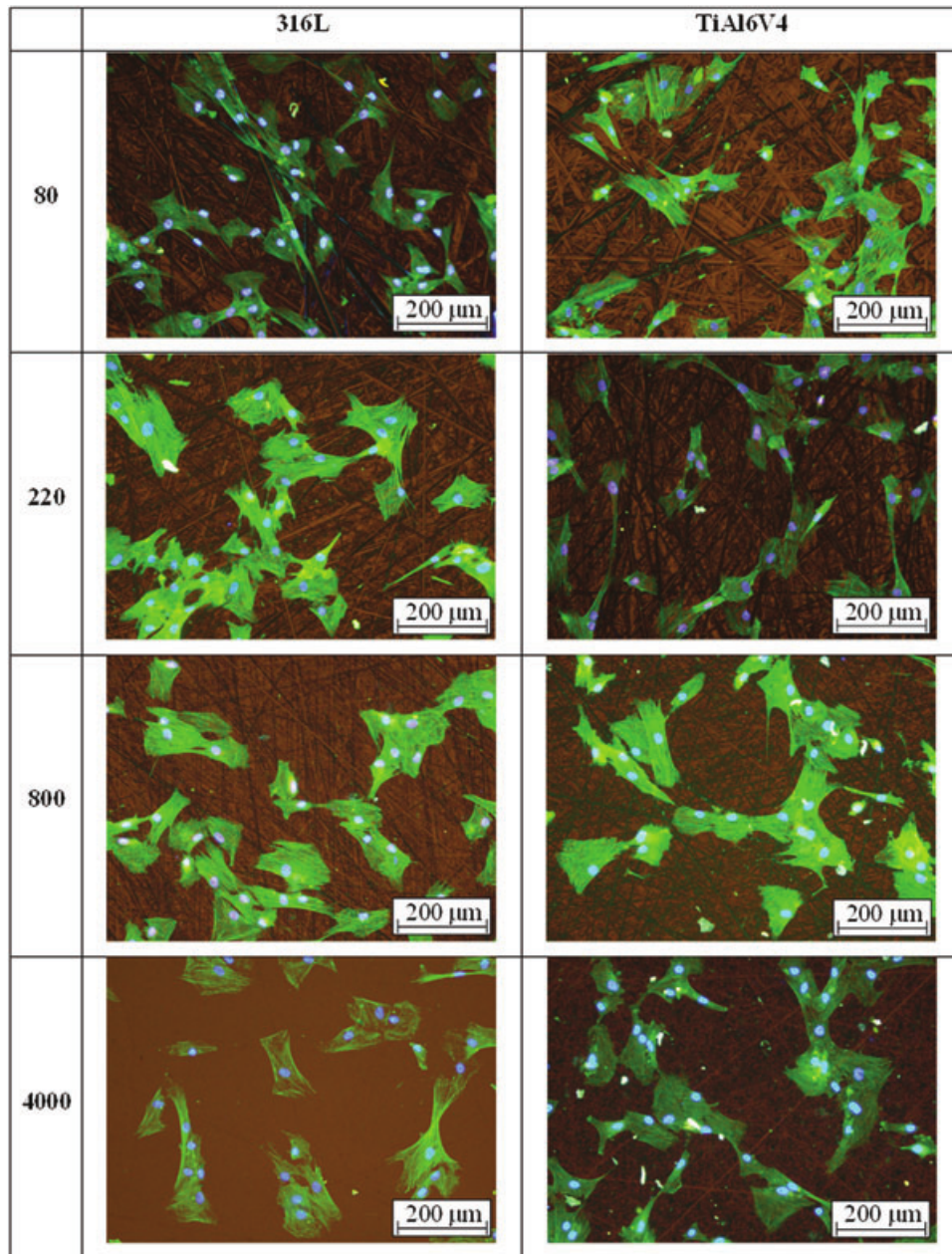


Fig 3. Human bone cells cultured during 2 days on both materials and for four different polishing paper grades.

during experiments (Bigerelle *et al.*, 2012). This fact is also confirmed by the high three levels of Materials  $\times$  Run  $\times$  Grit Paper interactions ( $F = 11.25, p = 0$ ). As a consequence, cell adhesion depends on the grit paper but the relations between grit paper and cell adhesion do not clearly appear. Thus, an original method, using the bootstrap theory was developed to find if one roughness parameter evaluated at a given scale could characterize cell adhesion. A first bootstrap was computed for each  $(q, \varepsilon, M, r)$ . Figure 5 represents an example of the BPDF (bivariate probability density function) of the first bootstrap (100,000 calculations) on the 11 paper grades for  $(R_a, 500, Ti, 1)$ , which represents the roughness parameter  $R_a$

evaluated at  $500 \mu m$  on titanium sample of the series 1. Then the means of the roughness value and the number of cells is obtained for each paper grade thanks to the first bootstrap. From the first bootstrap set (11 sets of bivariate data corresponding to the 11 grit papers in Fig. 5), 11 data are taken randomly with replacement in the BPDF. For example, two data from the paper grade 4,000, zero for the paper grade 2,400, one for the paper grade 1,200, etc. A linear relation was then retained to test the correlation between roughness and cell number. A slope value  $a(q, \varepsilon, M, r)$  is determined by the least-square method. By reproducing a high number of time the first and second bootstrap, it can be then possible to plot the



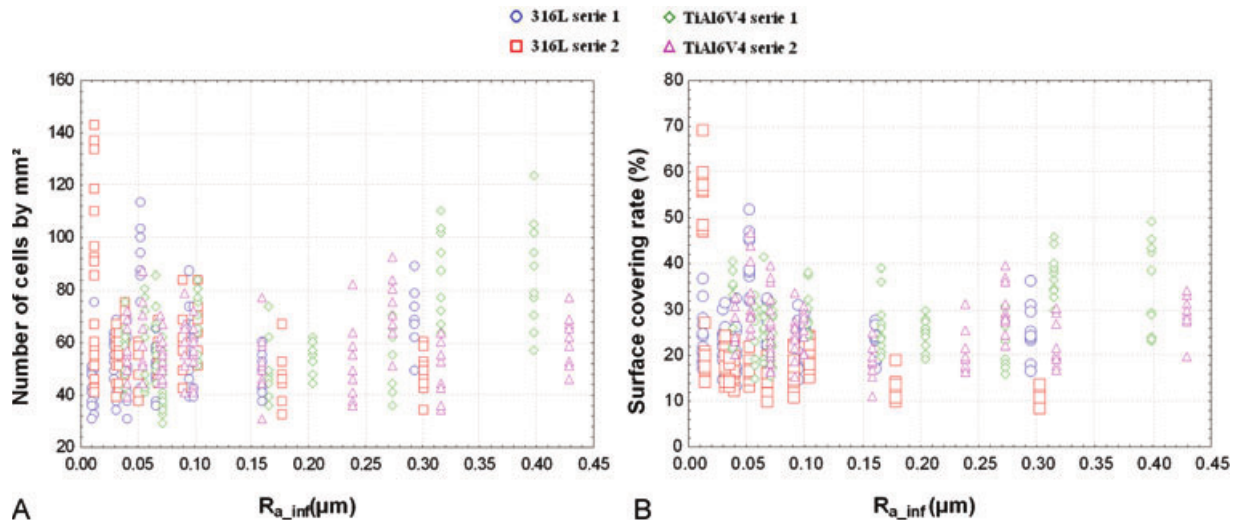


Fig 4. Cell culture results versus the infinite roughness amplitude value  $R_{a\_inf}$ . Panel A shows the number of cells by squared millimeters; Panel B shows the surface-covering rate.

bootstrap probability density function (PDF) of the slope  $a(q, \varepsilon, M, r)$ . Figure 6 corresponds to an example of the histogram (PDF estimation) of the slope  $a(R_a, 500, \text{Ti}, 1)$ . A total of 7% of the correlation between roughness and cell adhesion has negative slope. This means that it is impossible to affirm without an error of 7% that the slope is positive. As the usual critical value of reject used is 5%, the linear relation is rejected meaning that no correlation exists between the  $R_a$  evaluated at the scale of 500  $\mu\text{m}$  and the cell adhesion for titanium sample of the first run. To find if it does not exist a relevant scale with less than 5% of negative slope, the above analysis was reproduced at each scale (5–2,000  $\mu\text{m}$ ). All the PDF were plotted using the Box and Whisker plot (median  $\mu$  and the two quantile 2.5% (sup<sup>97.5%</sup> and inf<sup>2.5%</sup>). Figure 7 represents Box and Whisker for the  $R_a$  parameter for the two runs and both materials. As it can be observed, the zero value is always belonging to the (sup<sup>97.5%</sup>, inf<sup>2.5%</sup>) intervals meaning that the null value of the slope cannot be rejected. One can finally conclude that it does not exist a relation between  $R_a$  and cell adhesion for the two materials and regardless of the scale. This method was applied on the 100 roughness parameters and no relation was found between cell adhesion and roughness (data not shown).

## Discussion

The observation of cell cultures on both materials and roughness levels shows only an orientation of cells along the largest grooves of samples polished with paper grade 80 (Fig. 3). It was reported (Curtis and Wilkinson, '97) that the cell orientation on a topography formed with grooves depends on the

depth (Clark *et al.*, '91; Walboomers *et al.*, '99; Loesberg *et al.*, 2005) and width of the grooves (Clark *et al.*, '92). The preferred orientation of cells on an anisotropic-organized grooved topography was studied many times and is still under debate because the orientation depends on how the topography is measured but also depends on the type and size of cells (Anselme and Bigerelle, 2011). In addition, observations of cell behavior on grooves and the “contact guidance” of cells along the grooves were mainly done on unidirectional grooves whose spacing and width, and even depth, were fully controlled by photolithography. Using that technique, Lenhart *et al.* (2005) showed on primary osteoblasts that, after 2 days of culture on substrates with 500-nm spaced grooves, the cells were statistically more oriented on 150-nm depth grooves compared with 50-nm depth grooves. Dalby *et al.* (2006) observed an alignment of human bone marrow cells on silicon wafers with grooves of 5  $\mu\text{m}$  in width and 500 nm in depth. A less-obvious alignment was observed with grooves of 50  $\mu\text{m}$  in width and 300 nm in depth. The influence of grooved topography on MG63 cell-type was also studied by Ismail *et al.* (2007), which showed a preferential alignment of cells on grooves of 2- and 4- $\mu\text{m}$  widths compared with 8- and 10- $\mu\text{m}$  widths, which less orientated the cells. Unlike all previous works, they therefore showed that increasing the width of the grooves has a negative effect on the ability to orientate the cells. It appears that a grooved topography orientates the cells when the grooves are about the magnitude of nanometer in depth and micrometer in width. Using unidirectional polishing, Deligianni *et al.* (2001) showed that the short-time attachment of human bone marrow cells, after 30 min and 2 h was higher on the roughest polished surfaces ( $R_a$  of 0.3, 0.49, and 0.87  $\mu\text{m}$ ).

TABLE I Variance analysis results for three parameters: materials, run, and grit paper

	SS	DF	MS	F	p
Intercept	1,530,003	1	1,530,003	1,622	0.000000
Material	181	1	181	1,25	0.263437
Run	0	1	0	0,00	0.972614
Grit paper	20,805	10	2,081	14,44	0.000000
Material $\times$ Run	800	1	800	5,55	0.018922
Material $\times$ Grit Paper	11,900	10	1,190	8,26	0.000000
Run $\times$ Grit Paper	32,069	10	3,207	22,26	0.000000
Material $\times$ Run $\times$ Grit Paper	16,216	10	1,622	11,25	0.000000

SS = sum of squares, DF = degrees of freedom, MS = mean square.

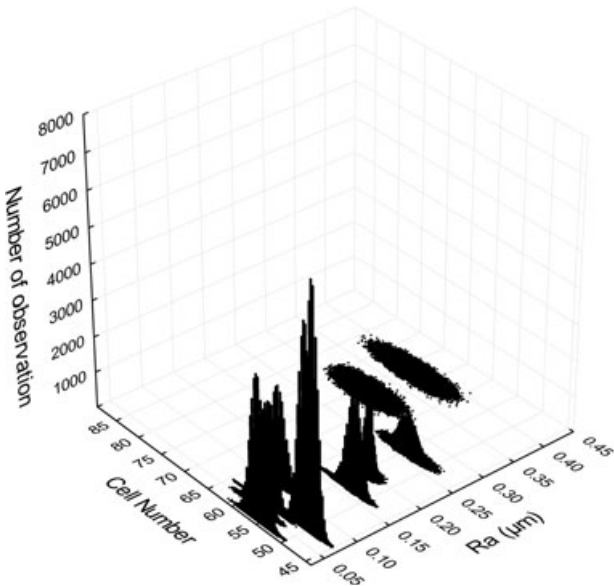


Fig 5. Example of the BPDF (bivariate probability density function) of the first bootstrap (100,000 calculations) on the 11 paper grades for ( $R_a$ , 500, Ti, 1), which represents the roughness parameter  $R_a$  evaluated at 500  $\mu\text{m}$  on titanium sample of the series 1.

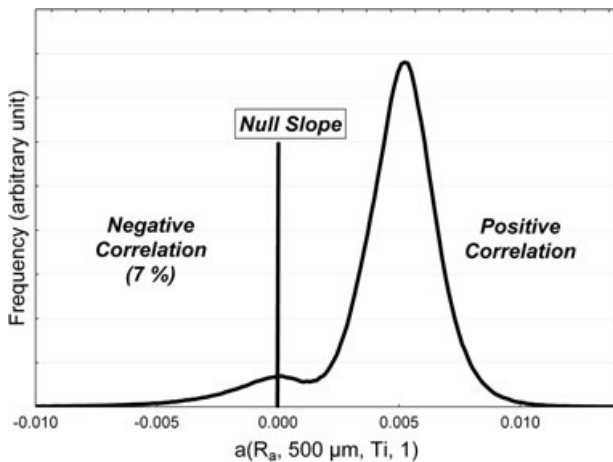


Fig 6. Histogram (PDF estimation) of the slope  $a(R_a, 500, \text{Ti}, 1)$ .

Therefore, the influence of roughness of polished surfaces on cell behavior appears when polishing is unidirectional. Nano- and microroughness clearly influenced cell behavior on anisotropic grooved surfaces. On isotropic surface, only a preferential orientation on some cells on the widest grooves on paper grade 80 was observed (Fig. 3). Multidirectional nature of the topography would cause an isotropic cell spreading except for a critical width of scratches (about 11–13  $\mu\text{m}$ ) beyond which the actin filaments are oriented preferentially in the direction of grooves. However, the results presented in Figure 4 show that cell spreading is constant regardless of the material and roughness. This has been statistically proven and confirmed on all roughness parameters calculated in this study (Figs. 4–7).

To explain this absence of influence of roughness on cell behavior of isotropic-polished surfaces, a much wider roughness range produced by electroerosion ( $R_a = 1.2$  and 21  $\mu\text{m}$ ) was tested (Bigerelle *et al.*, 2011a). It was found that cell behavior, also defined as cell number and surface-covering rate, depends on the roughness. Curves of these parameters versus the roughness present a U-shape with a minimum value for roughness close to the characteristic size of a cell ( $S_m = 110 \mu\text{m}$ ,  $R_a = 4.5 \mu\text{m}$ ). Thus, the roughness range on isotropic-polished surfaces obtained in this study may be probably not large enough or too low to show any influence on cell adhesion.

When looking for cell response to topography, the case of isotropic and anisotropic roughnesses has to be distinguished. Cells respond to nanoroughness on anisotropic grooved topography and cells do not respond to isotropic wear under a critical width of grooves of about 11–13  $\mu\text{m}$ , which can only be obtained using polishing technique with a paper grade of 80 or less.

## Conclusion

A study of the effect of roughness on the response of human osteoblasts was conducted with the help of isotropic polishing technique, tactile profilometry,



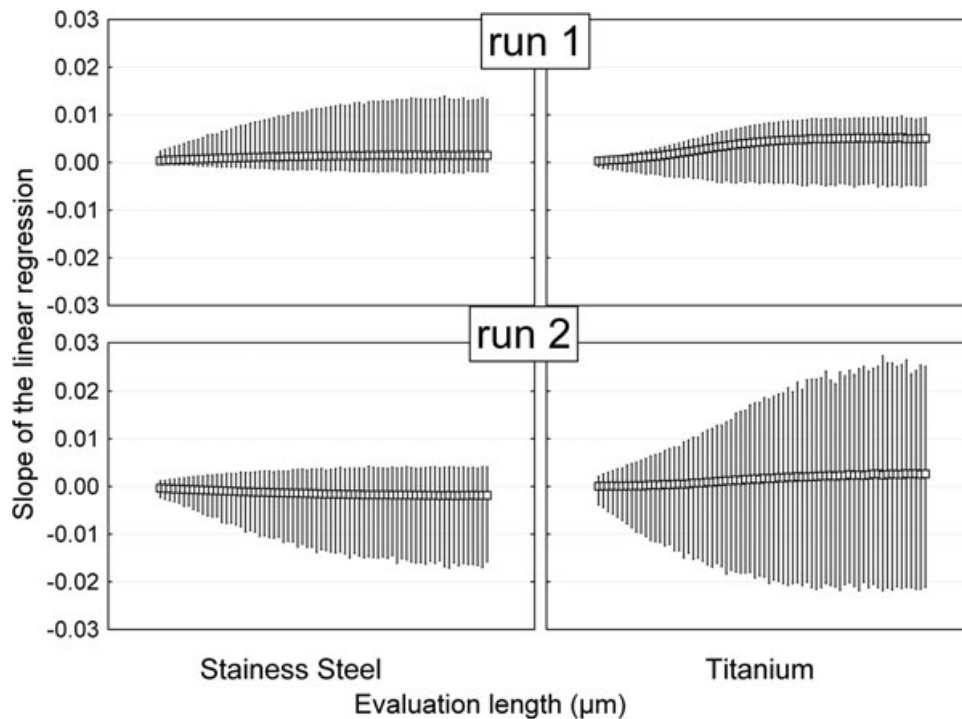


Fig 7. Plot of Box and Whisker of the slope of the linear regression versus the evaluation length for both runs and materials. The slope corresponds to a linear relation of the number of cells versus the roughness parameter  $R_a$ .

multiscale analysis, and statistical calculations. The range of roughnesses was produced by polishing at different paper grades and quantified by a multiscale roughness analysis. Cell response was quantified by image analysis. No influence of the roughness of biomaterials on cell behavior was found both in terms of number of cells or surface-covering rate. The roughness range studied (between  $R_a = 0.01$  and  $0.1 \mu\text{m}$ ) was too limited to observe any effect of roughness on cell behavior. A similar study of the roughness effect on contact angle measurements on the same samples was carried out and also showed an absence of influence of the roughness produced by isotropic polishing. Finally, this study shows that cell-adhesion mechanisms are not influenced by the roughness of biomaterial surface, under a critical value of groove width of about  $11\text{--}13 \mu\text{m}$ , in case of isotropic wear.

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## References

- Anselme K, Bigerelle M. 2011. Role of materials surface topography on mammalian cell response. *Int Mater Rev* 56:243–266.
- Anselme K, Broux O, Noël B, Bouxin B, Bascoulergue G, Dudermeil A-F, Bianchi F, Jeanfils J, Hardouin P. 2002. In vitro control of human bone marrow stromal cells for bone tissue engineering. *Tiss Eng* 8:941–953.
- Bigerelle M, Giljean S, Anselme K. 2011a. Existence of a typical threshold in the response of human mesenchymal stem cells to a peak and valley topography. *Acta Biomater* 7:3302–3311.
- Bigerelle M, Giljean S, Mathia TG. 2011b. Multiscale characteristic lengths of abraded surfaces: three stages of the grit-size effect. *Tribol Int* 44:63–80.
- Bigerelle M, Hagege B, El Mansori M. 2008. Mechanical modelling of micro-scale abrasion in superfinish belt grinding. *Tribology International* 41:992–1001.
- Bigerelle M, Iost A. 2001. A new method to calculate the fractal dimension of surfaces. Application to human cell proliferation. *Comput Math Appl* 42:241–253.
- Bigerelle M, Mathia T, Bouvier S. 2012. The multi-scale roughness analyses and modeling of abrasion with the grit size effect on ground surfaces. *Wear* 286–287:124–135.
- Chen J, Mwenifumbo S, Langhammer C, McGovern JP, Li M, Beye A, Soboyejo WO. 2007. Cell/surface interactions and adhesion on Ti-6Al-4V: effects of surface texture. *J Biomed Mater Res B* 82:360–373.
- Clark P, Connolly P, Curtis AS, Dow JA, Wilkinson CD. 1991. Cell guidance by ultrafine topography in vitro. *J Cell Sci* 99(Pt 1):73–77.
- Clark P, Connolly P, Moores GR. 1992. Cell guidance by micropatterned adhesiveness in vitro. *J Cell Sci* 103:287–292.
- Curtis A, Wilkinson C. 1997. Topographical control of cells. *Biomaterials* 18:1573–1583.

- Dalby MJ, McCloy D, Robertson M, Wilkinson CD, Oreffo RO. 2006. Osteoprogenitor response to defined topographies with nanoscale depths. *Biomaterials* 27:1306–1315.
- Deligianni DD, Katsala N, Ladas S, Sotiropoulou D, Amedee J, Missirlis YF. 2001. Effect of surface roughness of the titanium alloy Ti6Al4V on human bone marrow cell response and on protein adsorption. *Biomaterials* 22:1241–1251.
- Geetha M, Singh AK, Asokamani R, Gogia AK. 2009. Ti based biomaterials, the ultimate choice for orthopaedic implants—a review. *Prog Mater Sci* 54:397–425.
- Giljean S, Bigerelle M, Anselme K. 2007. A multiscale topography analysis of grinded stainless steel and titanium alloys. *Proc IMechE Part B* 221:1407–1420.
- Giljean S, Najjar D, Bigerelle M, Iost A. 2008. Multiscale analysis of abrasion damage on stainless steel. *Surf Eng* 24:8–17.
- Giljean S, Ponche A, Bigerelle M, Anselme K. 2010. Statistical approach of chemistry and topography effect on human osteoblast adhesion. *J Biomed Mater Res A* 94:1111–1123.
- Hallab NJ, Anderson S, Stafford T, Glant T, Jacobs JJ. 2005. Lymphocyte responses in patients with total hip arthroplasty. *J Orthop Res* 23:384–391.
- Ismail FSM, Rohanizadeh R, Atwa S, Mason RS, Ruys AJ, Martin PJ, Bendavid A. 2007. The influence of surface chemistry and topography on the contact guidance of MG63 osteoblast cells. *J Mater Sci Mater Med* 18:705–714.
- Kim HJ, Kim SH, Kim MS, Lee EJ, Oh HG, Oh WM, Park SW, Kim WJ, Lee GJ, Choi NG, Koh JT, Dinh DB, Hardin RR, Johnson K, Sylvia VL, Schmitz JP, Dean DD. 2005. Varying Ti-6Al-4V surface roughness induces different early morphologic and molecular responses in MG63 osteoblast-like cells. *J Biomed Mater Res A* 74:366–373.
- Le Guehennec L, Soueidan A, Layrolle P, Amouriq Y. 2007. Surface treatments of titanium dental implants for rapid osseointegration. *Dent Mater* 23:844–854.
- Lenhert S, Meier MB, Meyer U, Chi L, Wiesmann HP. 2005. Osteoblast alignment, elongation and migration on grooved polystyrene surfaces patterned by Langmuir-Blodgett lithography. *Biomaterials* 26:563–570.
- Lincks J, Boyan BD, Blanchard CR, Lohmann CH, Liu Y, Cochran DL, Dean DD, Schwartz Z. 1998. Response of MG63 osteoblast-like cells to titanium and titanium alloy is dependent on surface roughness and composition. *Biomaterials* 19:2219–2232.
- Loesberg WA, Walboomers XF, van Loon JJ, Jansen JA. 2005. The effect of combined cyclic mechanical stretching and microgrooved surface topography on the behavior of fibroblasts. *J Biomed Mater Res A* 75:723–732.
- Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford J Jr., Dean DD, Cochran DL, Boyan BD. 1995. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63). *J Biomed Mater Res* 29:389–401.
- Sinnett-Jones PE, Wharton JA, Wood RJK. 2005. Micro-abrasion-corrosion of a CoCrMo alloy in simulated artificial hip joint environments. *Wear* 259:898–909.
- Soboyejo WO, Nemetsky B, Allameh S, Marcantonio N, Mercer C, Ricci J. 2002. Interactions between MC3T3-E1 cells and textured Ti6Al4V surfaces. *J Biomed Mater Res* 62:56–72.
- Walboomers XF, Monaghan W, Curtis AS, Jansen JA. 1999. Attachment of fibroblasts on smooth and microgrooved polystyrene. *J Biomed Mater Res* 46:212–220.
- Zhang H, Brown LT, Blunt LA, Jiang X, Barrans SM. 2009. Understanding initiation and propagation of fretting wear on the femoral stem in total hip replacement. *Wear* 266:566–569.