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Human primary osteoblast behaviour on microrough zirconia-toughened alumina and on selectively etched microrough zirconia-toughened alumina

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ABSTRACT

Hip arthroplasty cementless acetabular components require excellent mechanical properties, biocompatibility, low friction and good osseointegration with surrounding bone tissue. Zirconia-toughened alumina (ZTA) fulfils these demands but requires combination with a rough metal shell for adequate osseointegration. Surface modifications of ZTA could allow a metal-free solution, thus preserving the bone stock for an eventual revision surgery. In this study, selective chemical etching proved to be an innovative method for the introduction of nano-features on micro-rough surfaces obtained by injection moulding. Results suggest that micro-roughness, fluorine enrichment and nano-porosity at the surface of ZTA play a synergistic role on human osteoblast (hOb) maturation. Among the tested groups, hydrofluoric acid etched "medium" roughness (Sa = 330 nm) ZTA showed the highest and/or earliest ALP expression at both the protein and gene level, while microroughness alone induced only minor effects on hOb maturation on ZTA.

1. Introduction

Prostheses with various designs are available for the hip, knee and shoulder joints. In the case of total hip replacements, each device is composed of a femoral stem, a femoral head, and an acetabular cup consisting of an insert (made of ceramic or cross-linked polyethylene) and a metal shell. The first ceramic material exploited in hip arthroplasty was the alumina, afterwards, in the nineties, yttria-stabilised zirconia (Y-TZP) was introduced with the intent to the decrease the revisions for fracture of the ceramic component, because of its high fracture toughness [1]. Nonetheless, Y-TZP is sensitive to low temperature degradation. This issue has been addressed by the latest development of bioceramic prosthesis, which is now composed of zirconia-toughened alumina (ZTA), the gold standard ceramic bearing material in hip arthroplasty today. The current ceramic-on-ceramic (CoC) bearings used for cementless hip prostheses need an osseointegrative metal shell on the acetabular side to offer an adequate bone in-growth. In addition, the modularity allows a better positioning of the acetabular cup and a further fixation with the screws; as a downside, the thickness of the acetabular cup cannot be further reduced, otherwise the metal shell would deform, compromising the efficiency of the bearing. As a palliative solution, pre-assembled ceramic cups are offered into the market. However, the thickness of the cup could be further reduced if the ceramic component would be directly implanted without the need of an osseointegrative metal shell. This would allow a metal-free solution with a reduced loss of the bone stock and the possibility to use larger ceramic ball heads, which would decrease the risk of dislocation. At the same time, the low friction arthroplasty guaranteed by CoC with a larger range of motion (ROM) may offer an improvement of the gate, thus enhancing the quality of life of active patients. Furthermore, such solution could be used for a CoC hip resurfacing arthroplasty, which would give the opportunity to have an all-ceramic answer to the hip arthroplasty market. Promising results were obtained on porous ZTA coatings, with a primary stability comparable to metal shells with plasma sprayed titanium coatings [2].

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The concept of "osseointegration" was first introduced by Brånemark in 1952 and was defined as the formation of a direct interface between an implant and bone, without intervening soft tissue. The future generation of ceramic implants targets the need for joint prostheses with increased ROM, which will have a substantial impact on the quality of life, especially for the young and more active patients. If adequate osseointegration could be achieved by solely the bioceramic component, the external metal part is no longer required and the overall thickness of the acetabular part of the prostheses could be reduced, thus making space available for a bigger ball with a larger ROM. Ideally, surface modifications technologies should allow changing the outer surface of the cup in contact with bone (for improved osseointegration) without affecting the inner surface of the cup in contact with the ball (preserving low friction) and without affecting the resistance to fracture.

Surface properties are orchestrating the first events after implantation, such as water adsorption, protein adsorption from blood and interstitial fluid, cell attachment, followed by the long-term integration into the native tissue [3]. Surface roughness affects osteoblast proliferation, differentiation, matrix synthesis and local factor production [4,5]. The majority of currently marketed oral implants are moderately rough, with a arithmetic average (R_a) in the range of 1–1.5 μm [6]. Surface modifications can be obtained with a broad range of techniques, among which blasting, acidic and alkaline treatment, coating and surface functionalization. Both mechanical and chemical surface modifications techniques (such as machining, grinding, polishing, blasting or acidic and alkaline treatment) have been used to improve the integration of alumina and zirconia with bone tissue, especially zirconia implants for the dental field [7,8].

Chemical etching has been used to increase the roughness and the total surface area of an implant and most commonly utilized acid agents are hydrofluoric, nitric and sulfuric acid, or a combination of different acid solutions. The obtained roughness is a function of different parameters, *e.g.* solution concentration, temperature and etching time. *In vivo* studies have shown that osseointegration of acid-etched zirconia is comparable to that of acid-etched titanium [9]. Combinations of sand blasting and chemical etching have been used to produce surfaces with both micro- and nano-scale roughness. In the dentistry field, the majority of commercially available sand-blasted implant surfaces are subsequently acid-etched. *In vivo*, low-pressure injection moulded acid-etched zirconia showed similar osseointegration as sandblasted and acid-etched titanium [9]. Recently, chemical etching has been applied to microrough ZTA to achieve a selective removal of the zirconia phase from the surface, leading to an increase of the nano-roughness and the generation of surface porosity, without affecting the pre-existing micro-topography [10].

One of limitations in the surface modification field is represented by the difficulty in modifying only one surface parameter at a time. Indeed, the majority of the roughness modification methods are influencing surface chemistry as well. Injection-moulding has been recently proposed for the fabrication of micro-rough zirconia [11]. With this technique, a wide range of micro-topographies can be obtained without affecting surface chemistry. Moreover, compared to sandblasting or grinding, this technique does not introduce additional surface defects. When implanted in rabbit tibia, micro-rough injection-moulded implants showed a significantly higher removal torque compared to smooth controls [11]. Another limitation is that most studies report on surface roughness focusing on R_a, which is a two-dimensional profile parameter. Nonetheless, as roughness is a scale and resolution-dependent measurement, R_a is not sufficient to characterize surface roughness. Wennerberg et al. and more recently Deltombe et al. and Flamant et al. have suggested using at least an amplitude, spatial, hybrid and functional parameter to characterize surface roughness of materials [12–14].

The objectives of this study were: (i) to determine the optimal microroughness range of injection moulded ZTA and (ii) to assess whether further selective chemical etching of the microrough surface is beneficial for human primary osteoblast proliferation and maturation. Cell behaviour was investigated on five different micro-rough surfaces without and with chemical etching and surface roughness was assessed using amplitude, spatial, hybrid and functional parameters. Primary human osteoblasts obtained from hip joints undergoing arthroplasty were chosen as a highly clinically-relevant cell source for this study. Cell proliferation, gene expression and alkaline phosphatase activity were followed up to 30 days of culture.

2. Materials and methods

2.1. Microrough and selectively-etched microrough zirconia-toughened alumina (ZTA)

Zirconia-toughened alumina (ZTA discs – 20 mm diameter and 2 mm height) were produced from ceramic slurries by injection moulding as previously described [10]. Samples with same composition but five different grades of surface micro-roughness were obtained (Table 1): mirror-polished after injection ("polished", S_a = 13 nm), no treatment following injection in the unmodified mould ("as sintered", – S_a = 203 nm) and increasing rough surfaces obtained by mould modification. The different rough surfaces, "low", "medium" and "high" (S_a = 176 nm, S_a = 330 nm and S_a = 410 nm) were obtained by sandblasting the mould surface. The topographies of ZTA discs are reported in Fig. 1 and white-light interferometry analyses (Veeco Wyko 9300NT, area of observation: 150 μm × 150 μm obtained by stitching of four images acquired at magnification 50x, resolution: 758 × 758 pixels) are summarized in Table 1 [10]. Samples from all groups were either tested as sintered or following further surface modification by selective chemical etching. In the latter case, samples were immerged in a 40% hydrofluoric acid (HF) (QP Pancreac, Spain) bath at room temperature for 4 days (4 mL of acid per sample). Following etching, samples were cleaned twice with fresh DI water in an ultrasonic bath (ten minutes each time). Half of the samples underwent an additional treatment in 37% hydrochloric acid (HCl) for one hour. Therefore, for each roughness, three types of samples were obtained: microrough, microrough with HF etching, microrough with sequential HF and HCl etching. The HF treatment led to the selective removal of zirconia grains on the sample surface, thus creating a superficial zirconia-depleted nano-porous network. Since the alumina matrix was not affected, the nano-scale roughness introduced during the etching process was superposed on the pre-existing micro-roughness [10]. Following chemical attack, yttrium fluoride (YF_3) crystals were found on "polished" and "medium" surfaces (Fig. 1), while no crystals were observed on "as sintered" and "low" and few on the "high". YF_3 crystal removal could be achieved with HCl (Fig. 1). AFM analyses (Veeco Dimension 3100; area of observation: 50 μm x 50 μm, resolution: 512 × 512 pixels) are summarized in Table 2 [10].

The following parameters were evaluated: Sa (average of height values), Sds (number of summits per unit area), Sdr (percentage of additional surface area contributed by the texture as compared to an ideal plane the size of the measurement region) and Sci (measure of the volume that the surface would support from 5% to 80% of the bearing ratio relative to Sq = standard deviation of height values).

2.2. Cleaning and sterilization ZTA samples

Prior cell culture testing, ZTA discs were cleaned in order to remove organic and inorganic contaminant molecules and ster-

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