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BASIC RESEARCH

In Vitro Investigation of Biofunctional Response of Fibroblasts/Osteoblasts Toward Roughened, Injection-Moulded Zirconia Implant Material

Ahmad Jum'ah, Jordan University of Science & Technology, Jordan

David Wood, University of Leeds, UK

Abstract

Rough implant surface is a pre-requisite for optimum healing and osseointegration. Achieving such surface profile is challenging in zirconia implant materials. This study aimed to compare the surface characteristics of a SLActive-like titanium (Ti) surface and an MDS zirconia surface as well as the biofunctional response of fibroblasts and osteoblasts toward these surfaces. Confocal Laser Scanning Microscopy (CLSM) was used to determine surface roughness parameters. SEM was used to visualise surface topography of both surfaces. Doubling time and cell viability for HGF and G292 cells were determined using flow cytometry.

Ti surface appeared significantly rougher than MDS on SEM images. There was no significant difference in the fold increase of surface area, Havg, Ra and Rq values between MDS and Ti surfaces. The mean doubling time of both cell types seeded on TCP and MDS-Zr was lower than that for cells seeded on Ti. All studied surfaces exhibited high cytocompatibility.

Methods and Materials

CLSM was used to obtain 3D topographic maps and to determine surface roughness parameters for acid-etched, injection-moulded zirconia (MDS) and sandblasted, acid-etched cpTi (Ti) discs (n=10 p/g). Scanning electron microscope (SEM) was used to examine surface topography of representative samples of each group.

Human gingival fibroblasts (HGF) and human osteosarcoma cells from G-292 cell line were seeded on three discs from each group with seeding density equals to 5×10^4 cell/well. Tissue culture plastic was used as a control (TCP). The cells were incubated for 24 hours, 1 week, 2 weeks and 3 weeks periods. Proliferation of both cell types was calculated by means of cell number and doubling time. The percentage of viable, pre-apoptotic, apoptotic and dead cells as a result of necrosis was determined using flow cytometery technique. SPSS statistics, version 19 (IBM, USA) was used to compare findings among the experimental groups. Significance level was set at 0.05

Results

Ti surface appeared significantly rougher than MDS on SEM images (**Figure 1**). There was no significant difference in the fold increase of surface area, Havg, Ra and Rq values between MDSand Ti surfaces (ANOVA: $p>0.05$). Ti surface demonstrated the higher mean maximum peak height (Rp) and maximum valley depth (Rv) (Kruskal-Wallis: $p<0.05$). **Table 1.** The mean doubling time of HGF and G-292 cells seeded on TCP and MDS-Zr was significantly lower than that for cells seeded on Ti (ANOVA: $p<0.05$) (**Figure 2**). Total cell counts varied according incubation period. HGF and G-292 counts were significantly higher in MDS-Zr/TCP compared to Ti at 24 hours and 1 week. After 2 and 3 weeks incubation, difference in total cell counts became statistically insignificant. All studied surfaces exhibited high cytocompatibility after all incubation periods (>87% viability). MDS surface induced the least amount of HGF necrosis. Ti had higher viability percentage compared to MDS-Zr and TCP at 2 weeks ($p<0.05$). **Figure 3**

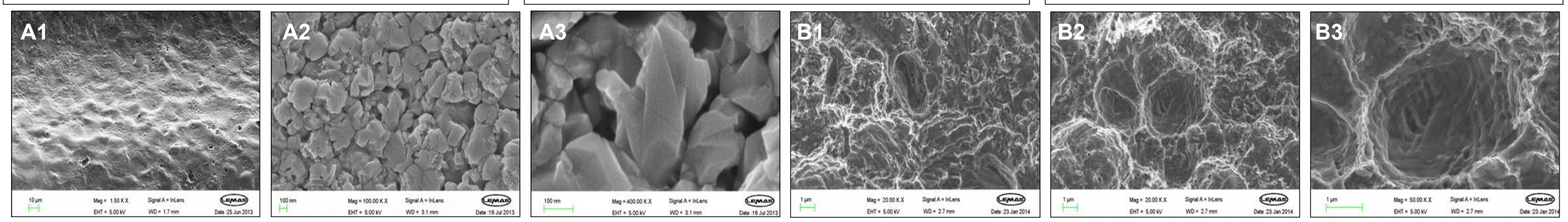

Figure 1. A1-3, SEM images of MDS zirconia implant surface. B1-3, Sandblasted, etched Ti implant surface

Background and Aim

High strength & exceptional biocompatibility qualified zirconia to be one of the leading bioceramics. Zirconia may outperform titanium as an implant material. This can be attributed to optimum aesthetics, antibacterial properties and periointegration capacity[1]. Achieving a rough zirconia surface is major challenge due to insolubility, high surface hardness and Young's modulus. Injection of molten zirconia in a roughened mould may overcome such problem.

The present study aimed to compare the surface characteristics of a SLActive-like titanium surface and an MDS zirconia surface as well as the biofunctional response of fibroblasts and osteoblasts toward these surfaces.

Parameter	Havg	Ra	Rq	Sa/S
MDS	40.3 (6.5)	2.2 (0.2)	2.5 (0.3)	1.6 (0.1)
Ti	37 (5.5)	2.9 (0.3)	3.3(0.4)	1.5 (0.2)

Table 1. Surface roughness parameters

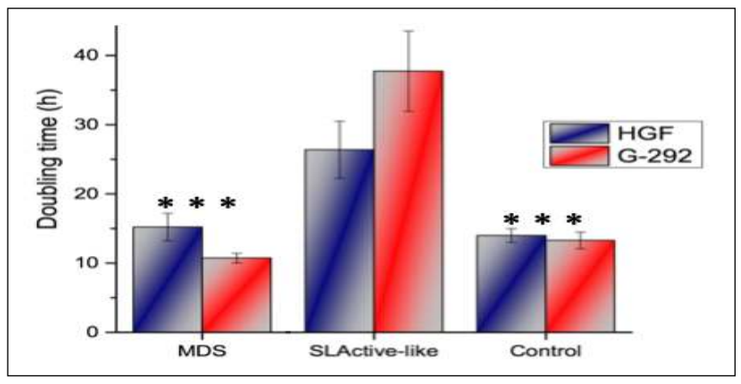

Figure 2. Doubling time

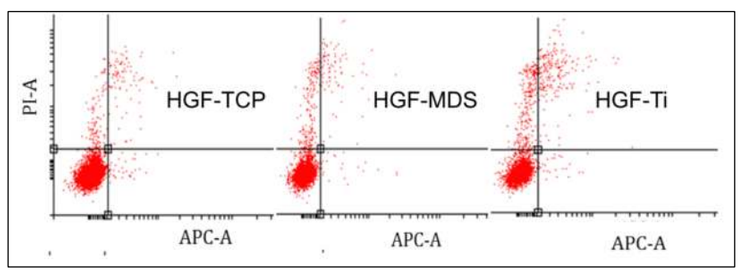

Figure 3. Flow Cytometry output for HGF at 24h

Conclusion

The MDS-Zr surface exhibited moderate roughness and high biocompatibility when tested with HGF and G-292 cells. The findings of this study indicates that a rough zirconia implant surface can be achieved using injection moulding and acid etching. The short doubling time indicates high osteoconductive capacity. Shorter healing times, faster loading and better osseointegration in compromised surgical sites are all theoretical advantages for such surface that should be further explored and verified.

References

1 Rimondini *et al,* Bacterial colonization of zirconia ceramic surfaces: an *in vitro* & *in vivo* study.Int J Oral Maxillofac Implants. 2002 Nov-Dec;17(6):793-8.

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