An *in vitro* investigation of human gingival fibroblast proliferation on dental titanium implants treated with selective laser melting technology

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Implant surface operations are often performed subsequent to the lathing procedure of titanium and titanium alloys. These kind of operations can also be carried out by laser metal sintering [1]. This method is based on high power laser beam conjoining an implant stem designed electronically with the aid of a computer program with the metal dust bed as thin layers. As the last layer agglomerates, the surface becomes rough [2,3]. Thus this method may be considered as a surface covering technique to increase implant-bone contact. Laser metal sintering method can be divided into two sub-groups. Selective laser sintering (SLS) technology partially melts metal particles while selective laser melting (SLM) method melts all of them [4,2]. Therefore, SLM provides less porous structure than SLS [5].

In the present study, proliferation of human gingivial fibroblast (HGF-1) cells on a rough implant surface obtained by covering Grade 5 lathed titanium material surface, titanium-6-aluminium-4-vanadium (Ti-6Al-4V) alloy, by Grade 23 (ELI) titanium alloy using SLM technology was investigated. In order to compare the results, cell adhesion and proliferation on covered surfaces, surfaces treated with sand-blasted, large grid, acid etching (SLA) technique and both SLA and SLM techniques were also evaluated. The main aim was to provide a novel surface procedure providing more biocompatible external structure with a biomechanically intact inner structure and increasing cell proliferation.

48 titanium Grade 5 machined discs with 5 mm of diameter was divided into four groups. Group 1 wasn't subjected to any surface treatment. Group 2 was treated with sand blasting, large grid, acid etching (SLA). Group 3 was treated with selective laser melting technology (SLM). Group 4 was treated with both SLA and SLM. The surface morphology of the samples were evaluated with scanning electron microscopy (SEM) and optic profilometer. For further assays involving cell proliferation and cell labeling on the aforementioned disc samples, human gingival fibroblast (HGF-1) cell line was purchased. HGF-1 proliferation was assessed with MTT assay. These results were also confirmed under fluorescence microscope when cells were stained by fluorescent dye Dil for 48 hours.

When surface roughness of the discs were examined, average Ra values of group 3 and 4 which were treated with SLM process were 2.308 and 3.467, respectively. These values were observed to be higher than the values of control group which was not treated with either SLA or SLM (Ra=0.592) and group 2 which was only handled with SLA procedure (Ra=1.783). The difference of the Ra values of group 2, 3 and 4 compared to control was shown to be statistically significant ($p < 0.05$) (Table 1).

MTT proliferation assay was carried out for each of the pre-determined hour with the aim of examining the impact of the surface morphology of the titanium discs on HGF-1 cell adhesion and proliferation. Based on 48 h MTT results, higher cell proliferation was observed on group 4 and 3 (Fig 3A), respectively while after 72 h of incubation, cell viability was higher on group 2 and group 4 (Fig 3B).

Lastly, HGF-1 cells were incubated with Dil dye in order to interpret cell proliferation qualitatively on the discs. Data showed that after 48 h of incubation, the highest cell density was observed on group 3 while on control group, cell density was the lowest (Fig. 4).

Group	Average	St. Deviation	Min. Value	Max. Value
1	0,592	0,079	0.5	0.7
2	1.783	0,094	1.7	2.0
3	2.308	0,010	2.1	2.5
4	3.467	0,107	3.3	3.7

Table 1. Ra values obtained from three different areas of disc surface for each group ($n=12$).

Fig 1A-1B. MTT proliferation assay performed on 48 (A) and 72 (B) hours. Data obtained after 48 h indicated higher cell viability within each group in reference to control. 72 h results showed high survival rate on both groups 2 and 4. $n=8$, $\pm$ SD, $*p < 0.05$ vs. control, $**p < 0.01$ vs. control and $***p < 0.001$ vs. control.

Sources:

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