Effect of Antimicrobial Photodynamic Therapy (aPDT) on Osteoblast Adherence and Growth in Vitro

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Introduction

Antimicrobial photodynamic therapy (aPDT) involves the use of non-toxic dyes as photosensitizers, which were excited by visible light of the correct wavelength. In the presence of oxygen the excited photosensitizers transfers energy to molecular oxygen producing highly reactive oxygen species such as singlet oxygen. All studies that have examined the killing of antibiotic resistant bacteria by aPDT have found them to be equally as susceptible as the non-resistant strains [1]. The benefit of aPDT with phenothiazine chloride and low intensity laser application on alveolar ostitis, periodontal disease, periimplantitis and infected root canals has been proven recently (fig. 1) [2-4]. In addition to decontamination of the infection side the effects on reattachment properties of osteoblast like cells on titanium implant surfaces have not been elucidated yet. The aim of this study was to evaluate the differences in cell adherence and growth on implant surfaces after aPDT in vitro.

Material and Methods

For simulation of aPDT in periimplantitis large grid blasted and high temperature etched titanium disc specimens with 10 mm diameter (Bredent, Senden, Germany) were used (fig. 2). The specimens were rinsed in PBS (control), treated with a special phenothiazine chloride (HELBO®*Blue Photosensitizer*, HELBO Photodynamic Systems, Wels, Austria) according to manufacturer instructions for 60 sec without laser light exposure (group 1) or treated with phenothiazine chloride and laser light exposure (HELBO®*TheraLite Laser*, 660 nm, 60 mW/cm^2) for 60 sec (group 2) (fig. 3). After rinsing with PBS, 1000 Saos2 cells (fig. 4) were seeded on the pretreated or control specimens. On day 1, 2, 4 and 8 cell viability (WST-1) and alkaline phosphatase levels were analyzed by ELISA. The apoptotic cells on different treated titanium specimen were stained using the TUNEL technique and counted [5]. The number of adherent cells was quantified with PHOTOSHOP analysis after Ethidium-bromid staining according to literature [6]. For morphological analysis of osteoblast like cells the specimens were assessed by SEM. All experiments were carried out in low adherence multiwell dishes and were repeated twice.

Results

Cell viability assay (WST-1) as well as activity of osteoblast derived alkaline phosphatase showed no significant differences between control and treatment groups (fig. 5, 6). The apoptotic index calculated revealed no differences between control and treatment groups either (fig. 7). Quantitative assessment of the growing cells on the different treated surfaces by Photoshop identified almost identical pixel values (fig. 8-11). The morphological analysis of adherent Saos2 cells on the specimens revealed wide spreaded cells with philopodia (fig. 12-14).

Conclusion

In the present study, we have investigated the influence of two different pretreatment regimens at etched and blasted titanium surfaces on the in vitro proliferation, differentiation, morphology and apoptosis of osteoblast-like SaOS-2 cells. In comparison to the untreated control specimens no statistical significant differences were shown at surfaces treated either with HELBO®*Blue Photosensitizer* alone or with HELBO®*Blue Photosensitizer* and laser light exposure from HELBO®*TheraLite Laser*. It can be concluded that by using an appropriate technology aPDT does not impair the adherence, proliferation and differentiation properties of titanium surfaces in vitro.

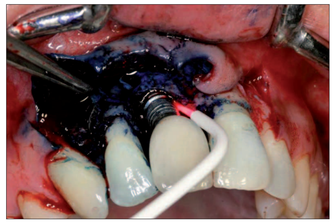

Fig. 1: aPDT (HELBO-therapy) at a maxillary incisor after staining

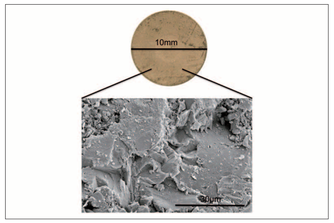

Fig. 2: Grid-blasted and etched titanium specimens with 10 mm diameter (Bredent Medical, Senden, Germany)

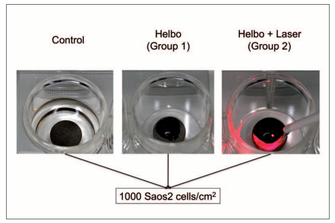

Fig. 3: Experimental set-up: left control group (only PBS cleaning); center HELBO group (HELBO treatment 60 sec); right laser group (HELBO treatment, and laser 60 sec each)

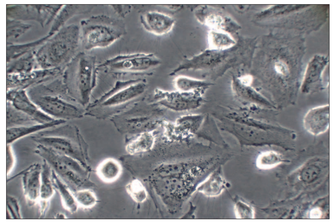

Fig. 4: Saos2 cells in culture (200x magnification)

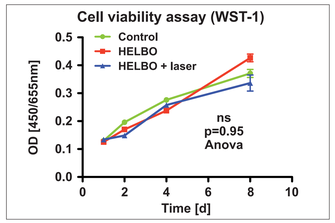

Fig. 5: Cell proliferation assay (WST-1) results (1, 2, 4 and 8 days)

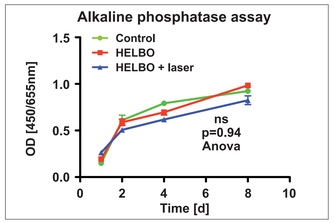

Fig. 6: Osteogenic differentiation assay (AP) results (1, 2, 4 and 8 days)

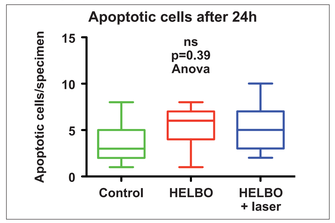

Fig. 7: Apoptotic cells on the specimen after 24h, stained with TUNEL technique

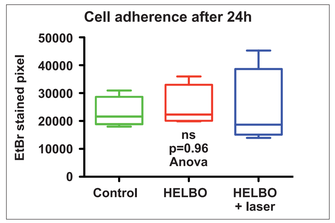

Fig. 8: Cell adherence after 24h, evaluated after staining with Ethidiumbromid

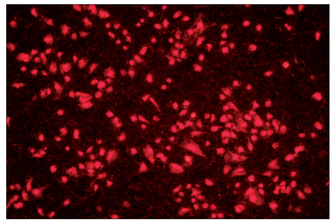

Fig. 9: Ethidiumbromid staining, control-group (100x magnification)

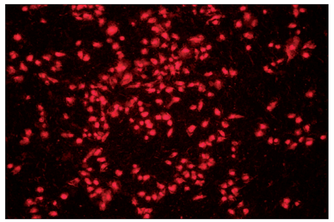

Fig. 10: Ethidiumbromid staining, HELBO-group (100x magnification)

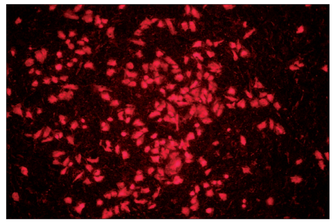

Fig. 11: Ethidiumbromid staining, HELBO and laser-group (100x magnification)

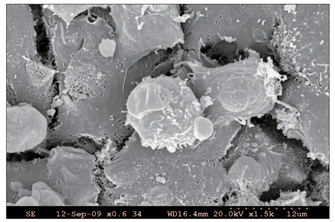

Fig. 12: SEM control-group day 8 after seeding (1500x magnification)

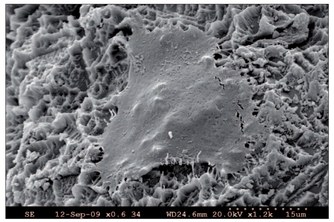

Fig. 13: SEM HELBO treatment day 8 after seeding (1200x magnification)

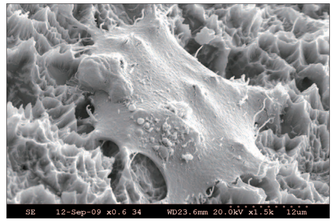

Fig. 14: SEM HELBO and laser treatment day 8 after seeding (1500x magnification)

References

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