VIA MEDICA

SHORT COMMUNICATION

Vol. 65, No. 1, pp. 78–80

ISSN 0015–5659
www.fm.viamedica.pl

The survival and proliferation of fibroblasts on orthodontic miniscrews with different surface treatment: an *in vitro* study

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[Received 21 December 2005; Accepted 8 February 2006]

It is of fundamental importance for prosthodontic and orthodontic applications that there is a short osseointegration time of dental implants without inflammation of the surrounding tissue. In addition to the chemical properties of the implant material, the surface morphology is an equally critical parameter. The objective of this work was to study the effect of two simple surface treatments on the survival and proliferation of fibroblasts.

Three groups of orthodontic miniscrews (Mondeal®) were used. One group was given an airflow (EMS, Schweiz) treatment, the second was sand-blasted in the area of the threading and a third group served as a control. After preparation sterilised screws were cultured in vitro *with fibroblasts (L-929). The metabolic cell activity on the implant surface was determined after 24, 48 and 120 hours using the alamarBlue assay and a count of DAPI labelled fibroblasts was performed with a fluorescence microscope.*

After 24 hours, but not at 48 hours and 120 hours, the metabolic activity of the fibroblasts was slightly decreased for the airflow screw group. Generally, no significant difference was found regarding metabolic activity and proliferation of fibroblasts within the different groups.

Key words: orthodontic miniscrews, surface treatment, cell culture, fibroblasts, surface colonisation

INTRODUCTION

As a result of both its mechanical properties, which provide sufficient stability under mechanical loading, and its excellent biocompatibility, titanium has been the material of choice for medical and dental implants for a considerable time [1].

Successful placement of dental implants depends on short osseointegration times and as little inflammation as possible in the surrounding tissue [1, 2].

Currently two different techniques are applied to improve the osseointegration. One is surface modification with chemical/biochemical means and the other approach uses controlled formation of defined surface structures [2].

To initiate osseointegration the surface of a dental implant needs to be colonised with various interacting cells and molecules [7].

Following implant placement a thin layer of tissue can usually be found between the titanium surface and the alveolar bone (contact osteogenesis) [3, 4].

For orthodontic applications the request to load the implant shortly after placement requires fast

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colonisation of the implant surface and as little inflammation as possible in the surrounding tissue. It is known that the surface structure of the implant is one critical parameter for this process to succeed and a certain surface roughness appears to be of advantage [5, 6]. The objective of this work was to study *in vitro* the effect of two different surface treatments of titanium implants on the colonisation rate with fibroblasts.

MATERIAL AND METHODS

AlamarBlue assay

In the study three groups of orthodontic miniscrews (Mondeal®) were used. One group was given an airflow treatment (EMS, Schweiz) [2], for the second the threads were sand-blasted [3] and a third served as control group [1]. Subsequently all screws (autoclaved) were incubated (37°C, 5% CO_2) with fibroblasts (L-929, 1×10^5 Zellen) in four well culture plates.

The survival rate/metabolic activity of the fibroblasts was measured 24 h, 48 h and 120 h after incubation using the alamarBlue assay. The alamarBlue assay can be used to quantify the proliferation rate of cells and the relative toxicity of a test substance. AlamarBlue is a growth indicator which is soluble in water. The assay is based on the natural metabolic activity of the cells chemically reducing the alamarBlue indicator. Analysis was performed photometrically at two different wavelengths (570 nm and 630 nm).

Fluorescence cell labelling

The surface colonisation was determined by fluorescence cell labelling. After incubation with fibroblasts (L-929) for 4 h, 24 h and 45 h miniscrews were rinsed with phosphate buffer and fixated with methanol. The screws were air-dried and labelled with DAPI, a fluorescent dye which binds to the DNA. In this way all cell nuclei at the implant surface were labelled and the number per unit area counted using a fluorescence microscope.

RESULTS

In all groups the incubation with the miniscrews did not negatively affect the survival of the fibroblasts in comparison with cells cultured without implants. After 24 h the cells on the sand-blasted surface showed a slightly but not significantly higher metabolic activity. Generally, no significant differences were seen between the groups at 24 h, 48 h and 120 hours of culture (Fig. 1, 3).

As seen in Figure 2, the number of cells visualised at the implants increased in time, indicating fibroblast proliferation in all groups. The increase in cell number was significant for all implants after 45 hours of culture. However there was no significant difference in cell number between the three groups, even after 45 hours of culture

CONCLUSION

The survival and metabolic activity of fibroblasts was not reduced in either of the test groups. The steady increase in the amount of cells on implants indicates that the cells (L-929) proliferate well on all the surfaces investigated.

In summary, the simple methods of surface treatment tested did not result in a significantly higher number of fibroblast on the implants. Complementing studies using osteoblasts, which are of greater importance for the osteointegration of implants, should be carried out to further investigate the potential for achieving a better and quicker colonisation of implant surfaces treated with these simple methods.

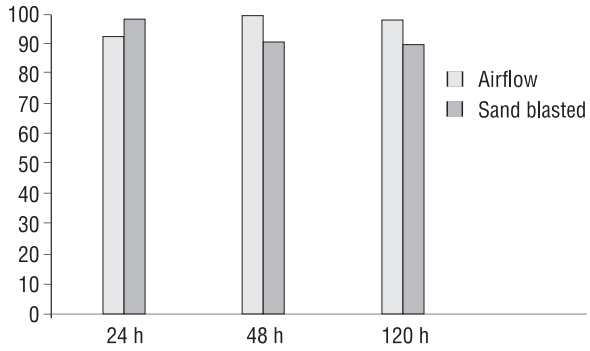

Figure 1. The time course of the metabolic activity of L-929 cells grown on differently treated implants in comparison with cells grown on untreated implants measured using the alamarBlue assay.

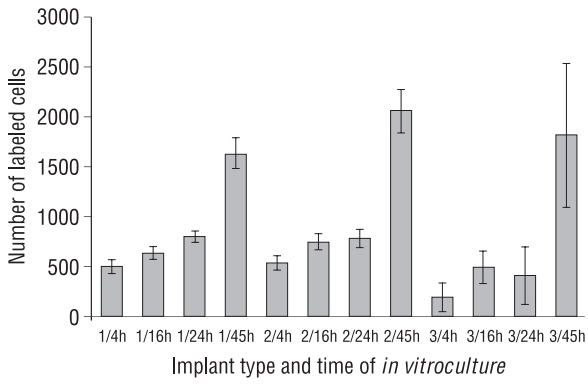

Figure 2. Number of DAPI labelled fibroblasts at differently treated implant surfaces (1 untreated; 2 airflow; 3 sand blasted) at 4 h, 16 h, 24 h and 45 hours of *in vitro* culture.

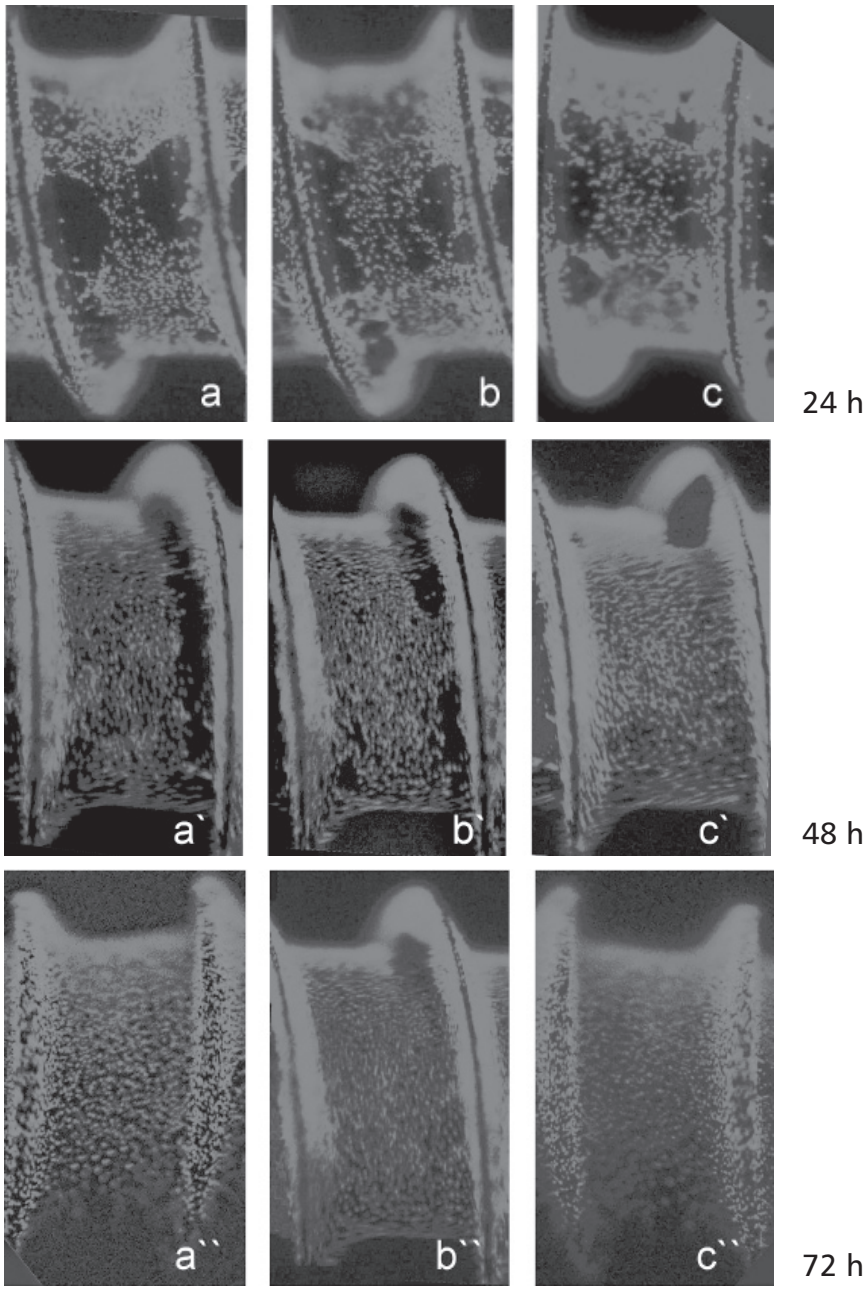

Figure 3. a, a', a'' — untreated miniscrews (controls); b, b', b'' — airflow treated miniscrews; c, c', c'' — sand-blasted miniscrews.

ACKNOWLEDGEMENTS

The authors would like to thank Sabine Hanisch for her technical assistance in carrying out the cell culture experiments.

REFERENCES

1. Albrektsson T (1985) CRC Critical Reviews in Biocompatibility 1: 53.
2. Böing J (2003) Modifizierung von Glas- und Titanoberflächen zur Verbesserung der Biokompatibilität. Dissertation, RWTH Aachen.
3. Brånemark PI, Adell R, Breine U, Hansson BO, Lindstrom J, Ohlsson A (1969) Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plast Reconstr Surg, 3: 81–100.
4. Davies JE (1998) Mechanisms of endosseous integration. Int J Prosthodont, 11: 391–401.
5. Pfeiffer F (2004) Untersuchung des Adhäsionsverhaltens von Gingiva-Fibroblasten auf mikrostrukturierten Titanoberflächen. Dissertation, EKU Tübingen.
6. Thull R (2003) Surface modifications to improve biocompatibility and mechanical properties of orthopaedic implants. Orthopäde, 32: 51–59.
7. Thull R (2002) Physicochemical principles of tissue material interactions. Biomol Eng, 19: 43–50.