Improvement of Epidermal Adhesion by Surface Modification of Craniofacial Abutments

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Craniofacial implants may present peri-implant inflammation because there is no close adhesion of the epithelium to abutments and because of bacteria infiltrating the subcutaneous tissue through the gap. Therefore an attempt was made to improve adhesion of epithelium to abutments. In an in vitro model, adhesion of epithelial cells (HaCat cells) to nonmodified and 3 modified Brånemark System abutment surfaces was quantified. It was found that more cells were adherent in sequence at silicone-coated surfaces, sandblasted surfaces, and collagencoated (Types I and IV) surfaces than on nonmodified abutments. It was concluded that it is possible to improve epidermal adhesion to abutments through modification of abutment surfaces. (INT J ORAL MAXILLOFAC IMPLANTS 2000;15:247–251)

Key words: abutments, adhesion, craniofacial implants, HaCat cells

Implant-anchored facial prostheses fabricated with silicone or polymethyl methacrylate offer coverage of facial defects with secure retention and good esthetics. Abutments are connected to the osseointegrated implants and carry the frameworks in the form of bars or magnets that retain facial prostheses.¹ The abutments penetrate the skin permanently. Around the abutments is a problem zone, where inflammation may occur^{2,3} (Fig 1). The causative factors in this situation are: (1) thickness of peri-implant tissue, (2) mobility of peri-implant skin, (3) nonadhesion of skin to abutments, (4) accumulation of bacteria, and (5) the patient's hygiene.

These factors have been addressed in various ways. The thickness of peri-implant tissue can be

most cases, the implants can be placed in immobile skin areas, and the patient's hygiene can be optimized by giving precise instructions for reducing bacteria and the nutritional supply of the bacteria. This study was performed in an attempt to solve the problem of the nonadhesion between skin and

altered by reducing it surgically to a minimum. In

abutments. Attachment of keratinocytes to the abutment was sought by testing different modified abutment surfaces to prevent bacterial invasion of the peri-implant gap. An in vitro model was developed, to enable quantification of the adhesion of HaCat cells to the modified abutment surfaces.

MATERIALS AND METHODS

Eighty craniofacial abutments from the Brånemark System (Nobel Biocare, Göteborg, Sweden) with a diameter of 4.5 mm and a length of 4.0 mm were used. Abutments with a nonmodified (polished) surface were compared with 3 surface modifications: sandblasted, silicone-coated, and collagen A-coated (containing 95% collagen I and 5% collagen IV). With the exception of the nonmodified abutment surface, the abutment types with surface modifications were manufactured especially for this assay. All 3 surface modifications were first sandblasted (Widder 100, Sapi, Noerdlingen, Germany) with 100- to 200-µm grain.

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Fig 1 Abutment with bar demonstrating peri-implant inflammation.



Fig 2 Abutments in 96-well plate.



Fig 3 Study protocol.

One third of the abutments thus treated remained in this condition. Another third was modified further with a coating of silicone, consisting of a primer (Wacker Priming Coat G790, Ottobrunn/Riemerling, Germany) and an air-drying silicone coating (Rehau 1511/THF 14, Rehau, Germany). The rest of the sandblasted abutments were coated with collagen A (containing 95% collagen I and 5% collagen IV, Biochrom AG, Berlin, Germany). The collagen was applied by submerging the abutments for 1 hour at 37°C in 100 μ L of a solution containing 50 μ g collagen A.^{4,5}

The conditions for cultivating the abutments in 96-well plates dictated halving the abutments. For every surface type, 20 abutment halves were tested. Before each run, they were cleaned mechanically by using a fine brush (Elmex Interdental Brush, Wybert, Loerrach, Germany) and ultrasonic cleaner (Sonorex, Bandelin, Berlin, Germany). They were then sterilized. All abutments underwent "glow discharge" treatment (Ar, atmosphere at 1 bar, Plasma Cleaner Sterilizer PDC-32G, Harrick, Ossining, NY) to increase surface tension.⁶ Twenty abutment halves per surface type were put into 96 micro titer plates (Falcon 3072, Becton, Dickinson and Co, Franklin Lakes, NJ) with the outer surface facing up (Fig 2).

The cell type used in this study was an immortalized (transformed) cell line of human keratinocytes (HaCat cells). This cell line is not tumorigenic. In the first test, every well was filled with 2×10^4 HaCat cells. This corresponded to a cell density of 40% of the well bottom surface with reference to the data given by the producer, indicating the maximal possible number of cells per well bottom surface (Fig 3). A concentration of 2×10^4 HaCat cells per well and 3 days of cultivation were selected to study the behavior of cells with respect to their adhesion and their reproduction on the abutment surface.

After 3 days of incubation at 37° C, 5.0% CO₂ atmosphere, and submersion in 200 T nutrition solution (RPMI 1640, PAA-Laboratories GmbH, Linz, Austria), the abutments were rinsed with a buffer solution (Dulbeccos phosphate buffered saline, Biochrom) to remove the nonadhering or dead cells. The HaCat cells adhering to the abutments were removed with a trypsin solution (0.05% trypsin plus 0.02% EDTA in PBS, Biochrom). From 5 abutments of each surface modification, the cells collected were counted in a Neubauer counting chamber under a microscope (Olympus CK 2,

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Fig 4 Results after 3 days (40% cell density). P = nonmodified; S = sandblasted; S + Si = sandblasted and silicone-coated; S + Col A = sandblasted and collagen A-coated.

Tokyo, Japan). The counting results of 5 abutments had to be taken to reach a higher level of statistical certainty. The means were calculated with all the numbers of 1 surface modification.

To ensure that all adhering cells had been removed and were not still adhering to the inner surface of the abutments, the abutments were stained with trypan-blue. This control step showed that all adhering cells had been detached and no cells remained on the undesired surfaces of the abutments. By visual control, the existence of cells under the abutments could be excluded as well.

This test was repeated with 4×10^4 HaCat cells per well, corresponding to a cell density of 80% of the well bottom surface. In this run the cells were incubated for 1 day. This run was performed to see how well the cells behaved with respect to their adhesion when they were cultivated with double density for only 1 day. With this run it was possible to investigate only the instant adhesion of the applied cells.

The statistical analysis was performed with the Student-Newman-Keuls test with significance level of P < .05.

RESULTS

The run with 2×10^4 cells/well (corresponds to 40% cell density at the beginning of a run) showed the least number of cells adhering to the abutments with the nonmodified surface. There were 1.2 times more adherent cells counted on the surfaces that had been coated with silicone. The abutments that had been sandblasted showed 2.3 times more cells than those with the nonmodified surface. The highest cell count was taken from the surfaces that had



Fig 5 Results after 1 day (80% cell density). P = nonmodified; S = sandblasted; S + Si = sandblasted and silicone-coated; S + Col A = sandblasted and collagen A-coated.

been sandblasted and coated with collagen A. Here, 4 times more cells adhered than on the nonmodified abutments (Fig 4).

The run was repeated with 4×10^4 HaCat cells/well (corresponds to an 80% cell density at the beginning of a run). Analysis of the results showed the same ranking order for the 4 abutment types. Related to the nonmodified surfaces, surfaces sandblasted and coated with silicone showed 1.6 times more adhering cells. Surfaces that were only sandblasted showed 2.1 times more cells than the nonmodified abutments. Surfaces sandblasted and coated with collagen A showed 3.4 times more cells, the highest quantities among the modified surfaces (Fig 5).

Both assays showed that the nonmodified abutment surfaces provided the least possibility for cell adherence. This may be improved by roughening the surface. No essential improvement was obtained by applying a silicone coating to the roughened surface. The greatest adherence of the HaCat cells to the abutments was achieved by sandblasting the abutments and applying a collagen coating.

The statistical analysis of the results in the 40% run showed significant differences between all surfaces except that of the nonmodified to the siliconecoated abutments. The statistical analysis of the results in the 80% run showed significant differences between all surfaces.

DISCUSSION

The nonadherence of peri-implant tissue to abutments is a reason for the formation of a periimplant gap. This gives bacteria the opportunity to invade this space, and peri-implant inflammation around craniofacial implants may occur. Abutment

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surfaces were modified to improve the adherence of keratinocytes to abutments in the hope of obtaining a more stable connection.

Sandblasted abutments were used because studies have demonstrated the enhanced adhesion of cells to roughened surfaces.⁷ The present result, with sandblasted surfaces with twice as many cells adhering, conforms with the earlier observation that physical surface modifications enhance the adherence of cells. The reason for this is that the physical modification of the abutments (sandblasting) leads to a magnification of the surface. This provides a greater possibility for cells to find adherence by forming a cell morphology that adapts to these surface irregularities.

The silicone surface modification was chosen because silicone surfaces had been tested to facilitate keratinocyte adhesion.⁸ Of the different biomaterials examined, silicone rubber showed the most favorable results with respect to the quantitative analysis of the cell-covered substrate surface, as well as cytomorphologic findings.⁸ The reason for the slight improvement of cell adhesion in this study may be that the silicone surface offers only a small increase in opportunities for cells to interdigitate between the micro structures, compared with a pure titanium surface. Furthermore, there was no specific recognizable adherence mechanism.

Collagen A-coated surfaces were used because some investigators had tested these matrix proteins in similar studies before. Donaldson and Mahan reported that epidermal cells showed attachment, spreading, and migration on collagen surfaces.9 Scharfetter-Kochanek et al demonstrated the influence of HaCat cell migration by collagen I.10 Clark et al had the same results.¹¹ The collagen A-coated (95% collagen I plus 5% collagen IV) abutments reached the highest count. This can be attributed to their biologic impact on the cells of the soft tissue and epithelium. These molecules are able to intensify the adhering mechanism of cells, because collagen is the main component of the basal lamina of the epithelium. The cells are able to make a connection between extracellular collagen and membrane proteins, so that an intense adherence results.

Cell adhesion was tested in a cell culture model, and the cell type used in this study was an immortalized (transformed) cell line of human keratinocytes (HaCat cells). This is a nontumorigenic cell line. Nühlen and Grosse-Siestrup declared keratinocytes as the most important cells in the connection between skin and abutment.¹² Use of dermal specimens of human origin is limited by the difficulties in obtaining this tissue type and the technical difficulties involved in its laboratory cultivation. Skin specimens of mammals are easier to obtain but have the same difficult cultivation, since they tend to be colonized by unwanted microorganisms and fungi. For the laboratory work, preference was therefore given to a freely reproducible 1-cell culture of a transformed (tumor) cell line. It must be mentioned that the optimization of cells for laboratory experiments entails more and more differences in cell origin characteristics.^{13,14} An important difference is the reduced or nonexisting contact inhibition by adjacent cells. Final preference was given to the HaCat cells.

Both kinds of keratinocyte adhesion—the adhesion during cell reproduction and the adhesion under "overcrowded" conditions—were supported by the 3 surface modifications. But it was assumed that competition for adherence-stimulating surfaces may occur between desired and undesired cell species. For example, extracellular matrix proteins such as collagen stimulate not only wanted cell species. Holgers et al could prove that fibronectin enhanced the adherence of *Staphylococcus aureus* to the abutment surface.¹⁵ This germ is the most important pathogenic one in peri-implant inflammation.¹⁶

It can be deduced from the in vitro model that clear improvement of the adhesion of HaCat cells to abutments is possible by modifying the surface physically or biochemically. In the authors' estimation, the chemical modification (silicone) is of no use and should not be included in further studies. Results of the physically and biochemically modified surfaces are promising. Further physical modification of the surface and coating with other extracellular matrix components should be tested with this method in vitro.¹⁷

A histologic sign for tight cell contact (for example, mucosa-tooth) is the existence of hemidesmosomes.¹⁸ Holgers et al could not find any hemidesmosomes in keratinocytes around nonmodified abutments.³ Further studies must be pursued to establish whether modified abutment surfaces form hemidesmosomes.

Moreover, it has to be verified in clinical tests that better adhesion of single cells in vitro leads to better adhesion of the abutments to skin and so to a reduced degree of inflammation in vivo.

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