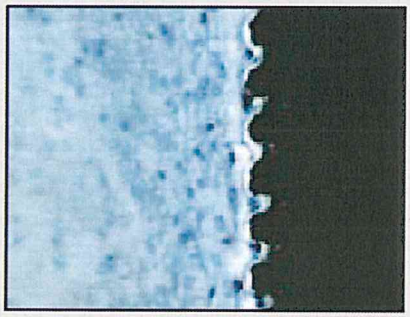



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Cell Response to Surfaces: A Concise Summary



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Surface nano- and microtexturing techniques have been used to enhance osseointegration, but how these surfaces work is not well understood. Using the knowledge gained from the cell and molecular biology fields, tissue engineering studies, and their own work, the authors and other researchers have developed surfaces for in vitro and in vivo control of the function of cells and tissues. In the present article, the authors summarize what they know about the process of cell response to surfaces, and what they have done and can do to develop surfaces that control hard- and soft-tissue formation and integration of implants. This article is intended to add to the clinician's understanding of cell and surface interactions, explain why certain surfaces are currently used, and describe what surfaces clinicians may see in the future. Int J Periodontics Restorative Dent 2016;36(suppl):s39-s46. doi: 10.11607/prd.2745

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Most researchers and clinicians who have worked with implantable medical devices, in particular within the dental, craniofacial, and orthopedic fields, have observed the effects of surface roughness (or lack thereof) on soft- and hard-tissue response.[1] In the authors' experience, surface roughening techniques have been used for years to enhance osseointegration. Conversely, smooth surfaces do not osseointegrate and are often used to avoid osseointegration; for instance, in fracture fixation devices, the devices are not meant to osseointegrate but rather to be removed. Although these techniques have been proven effective in clinical practice, the mechanisms involved are not well understood.

It is not the purpose of this review to compare surfaces. Instead, the authors will concentrate on the mechanisms that allow cells to respond to surface topography, ways in which clinicians and researchers can use this information to tissue engineer surfaces, and examples of surfaces that have been engineered for cell response. The present article reviews literature primarily from the cell biology and tissue engineering fields, because in recent years, those areas have provided many clues as to how cells respond to surfaces.

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The implant as extracellular matrix

The most accurate way to view cell response to surfaces is to view any surface that interfaces with cells as an extracellular matrix (ECM). Normally, an ECM is viewed as the tissue matrix within which cells reside. However, implant surfaces should also be considered a form of ECM. Cells never interact directly with any synthetic surface (in vivo or in vitro) as long as proteins are present, which they routinely are. In surgical sites, implants immediately come in contact with blood proteins, and many of these proteins adhere with these surfaces, as virtually all tissue-integrating implant surfaces are relatively hydrophilic and bind proteins immediately.[2] In addition, over time, as the cell-surface interface matures, the nature of the protein interface changes and cells change their attachment proteins.[2] In vitro, cell culture plates are hydrophilic, and most cell culture media contain proteins as well, so in most cases, this condition applies to cells cultured in vitro. Thus, proteins always mediate the interface between cells and surfaces. And so the interface between cells and artificial surfaces is analogous to the interface between that of cells and the ECM. For the remainder of the article, when cells and either ECMs or implant surfaces are discussed, it should be assumed that a proteinaceous interface exists between them.

Cell attachment

Cells form direct attachments to ECMs and implant surfaces. The first cells that reach the surface after implantation are macrophages and polymorphonuclear leukocytes, which are immune system cells. And although they play an important role in the healing process, they are mostly transient at the interface. The cells that produce structural tissue at the interface (in the case of dental implants) are mostly fibroblasts, osteoblasts, and epithelial cells.

The attachments that cells form are discontinuous and are analogous to spot welds between the cell and the surface. These attachments, which are collections of cell attachment proteins, are referred to as cell attachment plaques, or hemidesmosomes, in the case of epithelial cells attached to dental implants and teeth. These collections of proteins are cell-signaling molecules (ligands) that interact with clusters of integrins, which are transmembrane molecules that internalize the cell attachment signal into the cellular machinery, including the cell cytoskeleton and its associated cellular machinery.[3,4]

The combination of cell attachment plaques, integrins, and the cell cytoskeleton represent the mechanism by which the cell internalizes information from the ECM or implant surface. It is the three-dimensional arrangement of these cell attachments that determines cell shape, and cell shape determines growth control.[5]

Cell behavior on smooth surfaces

Extensive literature has been published on cell behavior on flat surfaces such as culture plates. For instance, a typical mammalian cell such as a fibroblast, in a medium containing significant amounts of protein, will attach, spread extensively, form stress fibers (organized collections of cytoskeleton components), and proliferate at very high rates until the cells reach monolayer (confluent) conditions. Considerable historical evidence suggests an inverse relationship between proliferation and ECM production, suggesting that extensive proliferation causes dedifferentiation.[6]

It is now clear that proliferation is partly based on extensive cell spreading and cell attachment plaque formation, as these have been shown to have a stimulatory effect on cell proliferation.[4] Early landmark studies of cell attachment showed that, if surfaces were modified to limit cell spreading, cell proliferation was also limited.[7] A significant determinant of this behavior, in vitro, is probably based on the three-dimensional configuration of the cell attachment plaques on the flat surface. Because these cell attachments are arranged in a planar array, they internalize this information and control the arrangement of the cytoskeleton accordingly. The normal ECM these cells live in, in vivo, almost never exists as a perfectly flat surface; therefore, this flat arrangement of cell attachments is an unusual arrangement for these cells, and the cells respond by

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spreading extensively, proliferating at abnormally high rates, and dedifferentiating.[6]

Cell behavior on three-dimensional and roughened surfaces

Cells behave much differently in ECM, on roughened surfaces, and on tissue-engineered surfaces. This difference in behavior is based on a concept called *contact guidance*. Contact guidance refers to the way in which cells respond to surface micro- and nanostructure. Ross G. Harrison, a pioneer in cell culture techniques and the first scientist to grow neurons in culture,[8] was supposedly the first to observe this phenomenon in 1907. He purportedly noted the way in which growing neurons followed structural defects in glass culture plates. Harrison mentored Paul Weiss in the 1930s; Weiss coined the term *contact guidance* and studied this phenomenon extensively. Weiss and Garber published a landmark paper in 1952[9] that examined the morphologic characteristics of cells cultured in three-dimensional fibrin clots of different concentrations and orientations. They found that cells in disorganized fibrin were more stellate in appearance and lacked significant orientation, whereas cells in organized fibrin became organized parallel to the fibrin and became more spindle-shaped. This publication was the first to demonstrate how an organized ECM caused cells to orient and change shape. It is widely recognized as the first tissue engineering publication and examination of cell response to three-dimensional scaffolds. Although the mechanisms of contact guidance were unknown at the time, we now know that contact guidance is based on the way cells extend filopodia to explore the ECM and then form cell attachment plaques with the ECM. The three-dimensional arrangement of these cell attachment plaques is the key to cell response to ECMs.

On complex surfaces, the arrangement of cell attachment plaques follows the three-dimensional arrangement of the surface, with cell attachment plaques forming on micro- and nanostructural peaks rather than in valleys. Cell attachments do not form in the small valleys of these surfaces because of the dynamics of the cell cytoskeleton. Therefore, a key factor in cell response may be that the actual amount of available surface area for cell attachment on these surfaces is limited relative to flat surfaces. Cell attachment processes contain cytoskeleton elements, which are tensile and compressive in nature and form in linear arrays. Thus, they cannot form effectively around sharp corners. This characteristic makes them incapable of conforming to small nano- and microindentations, so they attach to the crests of the structures rather than the depressions.[10] Stem cells have been shown to respond to roughened surfaces by changing differentiation, which is partially mediated by RhoA, which is a small, guanosine triphosphate (GTP)ase signaling molecule.[11]

In addition, because the surfaces are three-dimensionally complex, the arrangements of cell attachments are three-dimensionally complex as determined by the surface. The authors suggest that the results of this change in attachment configuration are *(1)* smaller numbers or areas of cell attachment resulting in less signaling for proliferation; and *(2)* a three-dimensional cytoskeletal arrangement more like that normally seen in ECMs, resulting in changes in cell differentiation.

Mechanisms involved with translation of cell configuration to differentiation

An extremely large body of literature addresses the mechanisms involved with translation of cell attachment configuration with cell differentiation. As with most biologic processes, multiple pathways and significant redundancy are built into the system. One particular system is very well documented and can be summarized as follows:

- The signals from the cell attachments, mediated by integrin transmembrane proteins, are internalized into the cellular machinery including the cytoskeleton.
- The configuration of the cytoskeleton controls many cellular signaling events including cellular response to growth and differentiation factors.[4]

This process is at least partially controlled by a system of small GTPase proteins such as Ras (rat sarcoma) family proteins,[12] Rho

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family proteins, and subfamily proteins, such as Rac and Rac1, and proteins such as Rho-associated coiled-coil-containing protein kinase 1, which is a downstream effector of RhoA.[13] These signaling molecules are one of the systems that control cell spreading, proliferation, and differentiation. Knocking out the Ras signaling system has been shown to stop cell migration and proliferation, effectively paralyzing the cell.[12] Supplemental data to this article, in the form of time-lapse videos of cell migration and proliferation, dramatically show the effect on cells when these Ras proteins are knocked out. This action effectively stops cell response to surfaces by interrupting cell cytoskeleton function.

Signaling molecules, such as these GTPase signaling molecules, have been shown to be one of the major pathways by which the signals form cell attachments, and their three-dimensional patterns are translated into changes in the cell cytoskeleton and modulation of cell function and gene expression.[4] The effects of these signaling molecules are complex, and it is not entirely clear how they are related, as they respond differently to different surfaces and in different cell types. A detailed study of this system is beyond the scope of this review. It is, however, important to note that these molecules represent an important and well-documented intermediate signaling system between the ECM, cell attachment plaques, the cell cytoskeleton, and overall cell function (migration, proliferation, differentiation, and apoptosis).

Using controlled surface configuration to control cell function—tissue engineering surfaces

The use of controlled surfaces to control cell function has only recently begun, with two different methods being the most successful: *(1)* control of surface chemistry (protein and surface chemistry patterning); and *(2)* control of surface micro- and nanogeometry.

Protein and surface chemistry patterning usually employs controlled microstructure stamps that are produced using soft lithography. These stamps are then used to apply cell attachment proteins to surfaces (like stamping ink on a surface) in very controlled patterns where cell attachment is desired. Other surface chemistries are also used in conjunction with these methods to produce hydrophobic surfaces to prevent cell attachment where it is not wanted. Some of the best examples of the effects of the use of micro- and nanofabricated surfaces to control cell spreading, growth, and migration have been produced using these methods.[14,15] In fact, supplemental data from the article by Doyle et al,[15] in the form of time-lapse videos of cell migration, show exactly how controlling cell attachment can control cell spreading and migration. The videos by Doyle et al show how cells respond when they are confined to channel-like regions, how they respond to flat surfaces, and how they respond to cross-hatched patterns (a concept similar to roughening). These methods have worked well in vitro, where the presence of other proteins in the environment can be controlled. However, in vivo, these protein-patterned surfaces face interference from additional attached proteins and have not been found to be effective.

Most current roughened surfaces are randomly roughened, meaning that they consist of a variety of surface structures depending on the method of processing. This approach has been shown empirically to work well for osseointegration and to strongly influence cell spreading and shape,[16] but optimization of these surfaces has been elusive, as their structure is not controlled. The authors' laboratory, as well as many investigators in the tissue-engineering field, has developed controlled micro- and nanotextured surfaces that demonstrate how specific surface configurations can be used to control cell function in vitro.[17–19] For example, Guillemette et al[20] used cell culture substrates containing cell-sized microgrooves to organize cells and the ECM produced by these cells to engineer multilayered tissue in vitro. Their results indicated that layers of cells and ECM, when cultured on these controlled surfaces, self-assembled into organized tissues. Multiple cell types were examined and, in many cases, when multiple layers of cells and ECM were used, additional layers aligned with the initial layers on the controlled surfaces. In examining the response of bone cells to oriented surfaces, Calzado-Martin et al[21] found that the cellular response was mediated at least in part by the RhoA/

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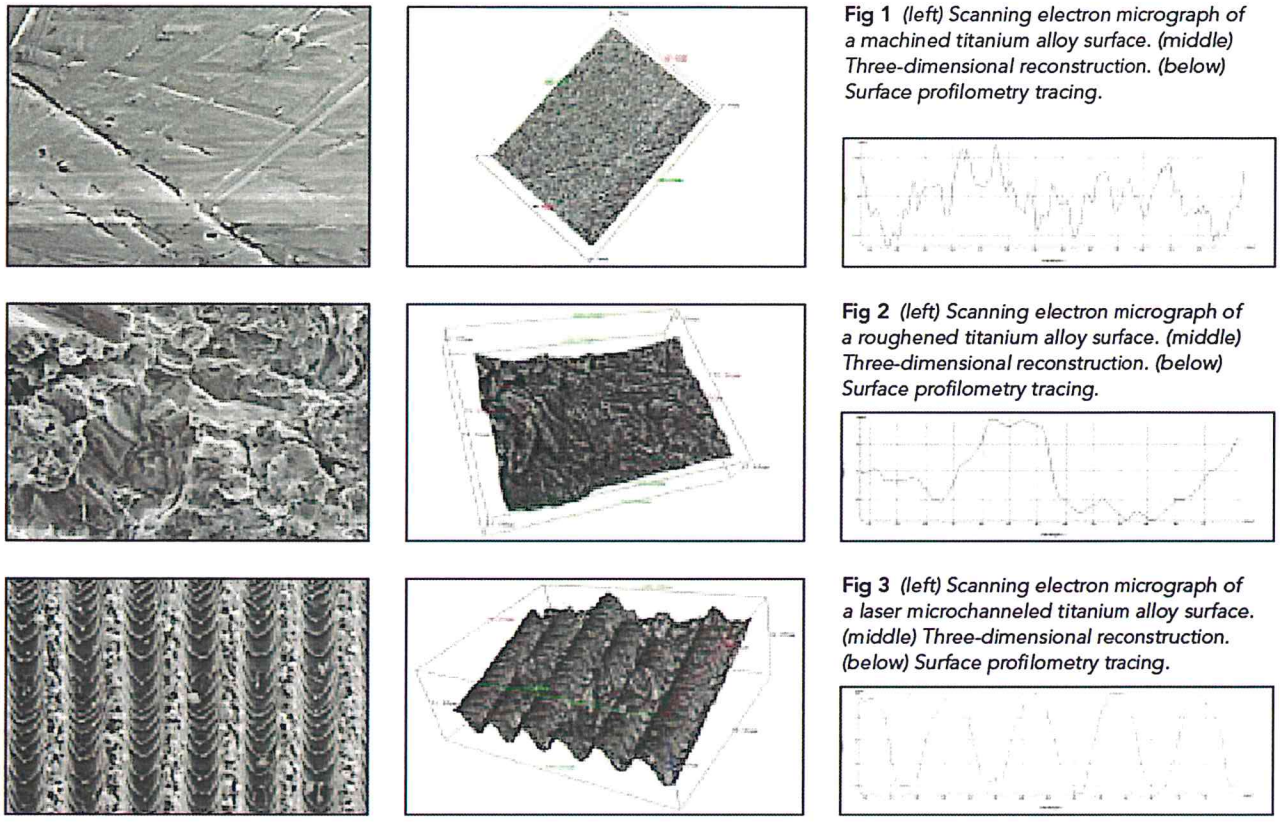

Fig 1 *(left) Scanning electron micrograph of a machined titanium alloy surface. (middle) Three-dimensional reconstruction. (below) Surface profilometry tracing.*

Fig 2 *(left) Scanning electron micrograph of a roughened titanium alloy surface. (middle) Three-dimensional reconstruction. (below) Surface profilometry tracing.*

Fig 3 *(left) Scanning electron micrograph of a laser microchanneled titanium alloy surface. (middle) Three-dimensional reconstruction. (below) Surface profilometry tracing.*

Rho-associated coiled-coil-containing protein kinase pathway.

The authors' laboratory has worked with similar microgrooved surfaces for a different purpose. On the basis of the previously noted data on the effects of cell spreading on proliferation, the authors hypothesized that microgrooved surfaces, if produced in the proper sizes relative to cell size, could be used to inhibit cell spreading and thus inhibit proliferation. We conducted a systematic study of microgroove dimensions, ranging from 2 μm to 12 μm (ridge and groove dimension as well as depth), and their effects on both fibroblasts and bone marrow–derived osteoblast-like cells. These surfaces, which were produced using silicon wafer technology similar to that used in the microelectronics industry, had profound effects on cell cytoskeleton configuration, cell orientation, spreading, and proliferation.[18,19] Microgrooves and ridges ranging from 6 to 12 μm (12–24 μm repeat spacing) and having similar depths were shown to reduce cell proliferation up to 50% while causing orientation and migration of the cells parallel to the microgrooves and preventing orientation and migration perpendicular to the microgrooves.

The authors also developed a method for producing similar microgroove patterns on titanium alloy surfaces using an ultraviolet laser micromachining method. These surfaces were characterized using specialized software (MeX 6.1, Alicona Imaging) and scanning electron microscopy (Zeiss EVO50 Environmental Scanning Electron Microscope, Carl Zeiss) and compared with machined surfaces and a successful and widely used random roughened surface known as the resorbable blast textured surface (Figs 1–3). The results indicated that the machined surface had roughness height and profilometry feature height values of 0.459 μm and 0.802 μm, respectively, and a developed surface

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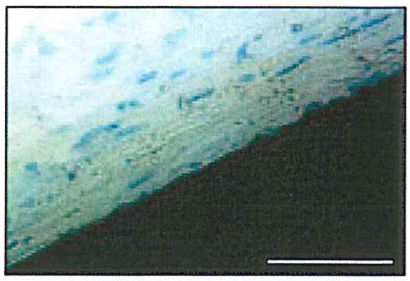

Fig 4 *Interference transmitted light photomicrograph of soft tissue adjacent to a machined dental implant collar in a mechanically loaded canine model at 6 months after implantation. The collagenous tissue and fibroblasts are organized parallel to the implant surface. Bar = 200 µm.*

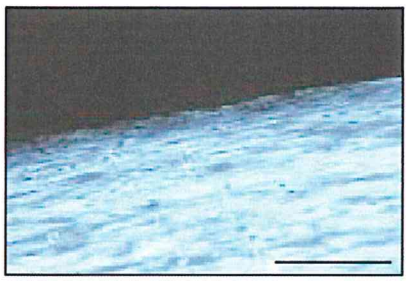

Fig 5 *Transmitted light photomicrograph of soft tissue adjacent to a roughened dental implant collar in a mechanically loaded canine model at 6 months after implantation. The collagenous tissue and fibroblasts are organized parallel to the implant surface. Bar = 500 µm.*

area, or area percent in excess of a flat surface of 13.09%. The roughened surface had values of 3.07 µm, 7.80 µm, and 30.51%, and the laser microchanneled (8 µm microchannel) surfaces had values of 2.94 µm, 7.93 µm, and 66.74% for roughness height, profilometry feature height, and developed surface area, respectively. In addition, the spacing between major features of the laser microchanneled surface was 16.18 µm. The profilometry feature height and spacing between major features were precisely at specification for the 8-µm microchanneled surface, demonstrating the precision of manufacturing of this surface. Other than the organization of this surface, the large increase in the developed area of this surface may also be one of the reasons for its success. Figures 1 to 3 show the scanning electron micrographs, three-dimensional reconstructions, and profilometry tracings of these surfaces.

The authors initially tested surfaces with 8-µm and 12-µm microchannels (16 µm and 24 µm repeat spacing), with similar depths, in an implantable chamber model placed in the canine femur.[22,23] The results indicated that these surfaces developed more extensive bone ingrowth and contact than either machined surfaces or roughened surfaces made of the same alloy and, in the case of the microchannel surfaces, the bone organized parallel to the microchannels. During mechanical tensile testing of the interface between new tissue and these surfaces, the authors found significant bone adhesion to the microchannels. Areas of fibrous tissue interface, such as those seen in marrow spaces, also showed attachment to these laser micromachined surfaces. Significant soft- or hard-tissue tensile attachment to any other metal surface was not observed.

These 8-µm and 12-µm surfaces were then applied to the collars of dental implants, and tissue response to them was examined in a canine, functionally loaded study.[24] When these microchanneled implant collars were placed in bone, the bone was observed to attach and remain at crestal levels and, in a significant number of cases, grow slightly up the collars (referred to as bone upgrowth). When portions of the collars were left supracrestal, we observed that the microchannels inhibited epithelial downgrowth and that these surfaces developed a significant area of fibrous tissue attachment. Perhaps the most significant observation, and one that was not fully appreciated at the time, was that the fibrous tissue in these areas was not oriented parallel to the implant surface—as is usually observed in machined and random roughened surfaces (Figs 4 and 5)—but rather was oriented tangentially to the surface (Fig 6). Fibrous tissue cells were also observed to be oriented within the microchannels (Fig 7). This result was unexpected but clinically significant, as these fibers, while not Sharpey fibers (no cementum is observed), could be interpreted to function like the supracrestal fibers that are part of biologic width around natural teeth. Equivalent results were found using either the 8-µm or 12-µm surfaces. Human histologic studies have confirmed these results,[25] which, in addition to the authors' observations from microchanneled abutments retrieved from human patients, have shown that the epithelial and fibrous attachments to these surfaces are extremely robust (Fig 8). In human and animal studies of microchanneled implants and abutments,[25,26] and in the authors' retrieved specimens, when mechanical separation

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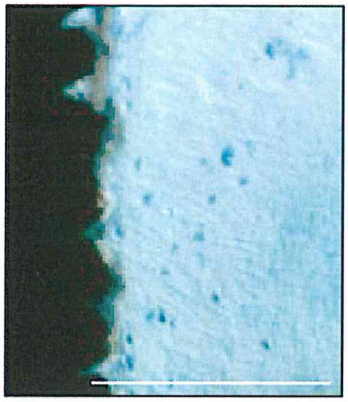

Fig 6 *Interference transmitted light photomicrograph of soft tissue adjacent to a laser microchanneled (12-μm ridge and groove) dental implant collar in a mechanically loaded canine model at 6 months after implantation. The plane of section is perpendicular to the microchannels. The collagenous tissue is organized tangentially relative to the implant surface. Bar = 50 μm.*

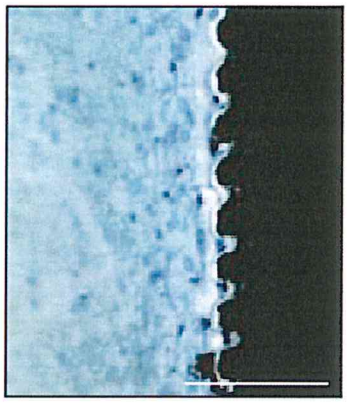

Fig 7 *Transmitted light photomicrograph of soft tissue adjacent to a laser microchanneled (12-μm ridge and groove) dental implant collar in a mechanically loaded canine model at 6 months after implantation. The plane of section is perpendicular to the microchannels. Many of the dark blue–stained fibroblasts at the implant interface are visible within the microchannels and appear to be oriented perpendicular to the plane of section. Bar = 50 μm.*

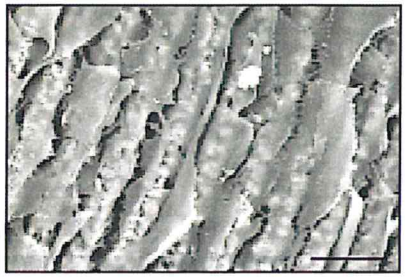

Fig 8 *Scanning electron micrograph of an 8-μm laser microchanneled abutment that had been removed from a human patient after 2 months. No attempt was made to preserve the tissue attachment upon removal, and the only attached tissue was observed on the microchannels. The rows of cells in and on the microchannels appear to be gingival epithelial cells. In other areas of this abutment (not shown), areas of fibrous tissue attachment were present. Bar = 30 μm.*

of the soft tissue from the surface is attempted, layers of epithelial cells, fibrous tissue, or both are observed to remain attached to the microchanneled surfaces, and failure is observed within the soft tissue rather than at the tissue-implant interface. This finding suggests that the soft-tissue attachment to these surfaces is stronger than the tissue itself.

These surfaces are now used on the BioHorizons Laser-Lok implants (BioHorizons), and a large body of literature has shown their efficacy, as summarized by Ketabi and Deporter[27] Newer references are also available through the BioHorizons website.

Conclusions

Cell and tissue response to surfaces can be controlled through chemical modification, microgeometric modification, or both. However, the chemical modification approach, while effective in vitro, has not shown such impressive results in vivo, probably because of interference by absorbed proteins from body fluids and other cellular processes at the interface. Microgeometric modification, however, shows every indication of being a long-term solution in vivo, and the mechanisms involved are now clear. And so optimization of surface microgeometric modification has begun. The results of the in vitro and in vivo studies noted previously have established a direct link between the three-dimensional organization of cell attachments to ECM and implant surfaces, their influence on the organization of the cell cytoskeleton, the resultant effects on the GTPase signaling system, and ultimately the effects on cell differentiation and cell function. A knowledge gap between how cells attach to surfaces and the related mechanisms that significantly control cell function no longer exists. In a notable position paper on tissue engineering from 2009, it was suggested that the way to control cell response was through the use of micro- and nanotextured surfaces.[28]

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This approach is now being used to tissue engineer implant surfaces to mimic the effects of ECMs, and the first generation of these surfaces has been very successful. This approach is only the beginning of tissue-engineered surfaces that will revolutionize tissue response to all types of implantable medical devices.

This has now had, and will have in the future, substantial impact on the clinical use of precision microtextured surfaces, from dental implants and orthopedic implants to surfaces for intraocular lenses, catheters, and many other applications. On the basis of current knowledge of cell-surface interactions, surfaces can be designed for a range of clinical applications, the efficacy of which has already been demonstrated in dental implants.

Acknowledgments

The authors reported no conflicts of interest related to this study.

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Optimizing Connective Tissue Integration on Laser-Ablated Implant Abutments

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Focused Clinical Question: The integration of connective tissue (CT) to a laser-ablated abutment with a microgrooved surface at the apical millimeter (LL) has been documented in both animal and human studies. How should the healing be influenced to optimize the CT integration on abutment surfaces?

Summary: When smooth machined titanium (ST) abutments were placed, epithelial attachment was found on abutments, but no CT integration was noted. On LL abutments, a zone of epithelial attachment and CT integration was noted. When an ST abutment was switched for an LL abutment, the soft tissue was inconsistent; however, when an LL abutment was placed after the removal of a prior LL abutment, CT integration was observed.

Conclusions: Consistent CT integration was observed on LL abutment surfaces after implant placement. Switching LL abutments with new LL abutments consistently led to CT integration. This was not consistent when an ST abutment was switched for an LL abutment. Plaque and gingival indices were comparable between teeth and abutments. Probing depth (PD) was lower around teeth. There was no difference in PD between abutments. CT integration on LL abutments was optimized by initial healing occurring on an LL abutment or by creation of a CT wound before insertion of the abutment. *Clin Adv Periodontics* 2016;6:153-159.

Key Words: Connective tissue; dental implants; epithelium; histology; microscopy, electron, scanning; wound healing.

Background

Modern endosseous implants are designed to be surgically embedded into alveolar bone, in which once osseointegration has occurred, they serve as long-term artificial replacements for roots of missing teeth or as anchors to stabilize and support full- or partial-arch prostheses.[1-5] Establishing soft tissue peri-implant attachment is essential to peri-implant health and long-term esthetics. Epithelial and connective tissue (CT) attachment to the tooth serves to protect the tooth from periodontal bone loss. The natural attachment apparatus of a tooth includes anchorage of gingival CT into the tooth root surface through fiber insertion. Attachment to a smooth machined surface titanium–implant abutment occurs through hemidesmosomes. This attachment is not as robust as a natural tooth, leading the scientific community to attempt to create an attachment of soft tissue to the abutment of an implant that mimics attachment to a natural tooth.

Innovation and technology, based on sound scientific knowledge, have melded with device design to develop implant body and abutment surfaces that encourage early cellular events in soft and bony tissue healing that may foster long-term tissue health and osseointegration.[6-9] Cell culture experiments,[6,7] animal studies,[10] and preliminary human trials[9] have illustrated the potential of implants and implant abutment surfaces to interact with cells and create a more stable tissue interface. In general, microtextured surfaces are thought to promote faster formation of new bone via osteogenesis, osteoconduction, and osteoinduction processes[6] and may have an effect on adhesion of the fibrin clot, adhesion of extracellular matrix components, and long-term interaction with cells to create a stable tissue interface.[11-13] CT integration has been reported on surfaces of implants retrieved in humans.[14] Integration of CT to the abutment surface has also been documented in both animal[10] and human[15,16] studies. However, how to manage the clinical environment to promote CT attachment has not been well established. The present study discusses requirements for wound creation and management of soft tissue healing phase to create CT attachment on abutment surfaces.

Decision Process

The clinical parameters for establishment of a zone of CT integration on an abutment need to be defined in clear protocols. The decision matrix presented here is based on findings of a trial conducted at the University of Alabama at Birmingham, Birmingham, Alabama. Western Institutional Review Boards, Puyallup, Washington, approved this study, and it was registered at ClinicalTrials.gov as NCT01822223. All participants provided written informed consent prior to treatment. The study was conducted from January 5, 2011 to November 29, 2011. Twenty patients were enrolled (five males and 15 females, aged 54 to 68 years [mean age: 57.60 years] and 36 to 69 years [mean age: 50.93 years], respectively). The

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Submitted October 27, 2015; accepted for publication December 23, 2015

doi: 10.1902/cap.2016.150068

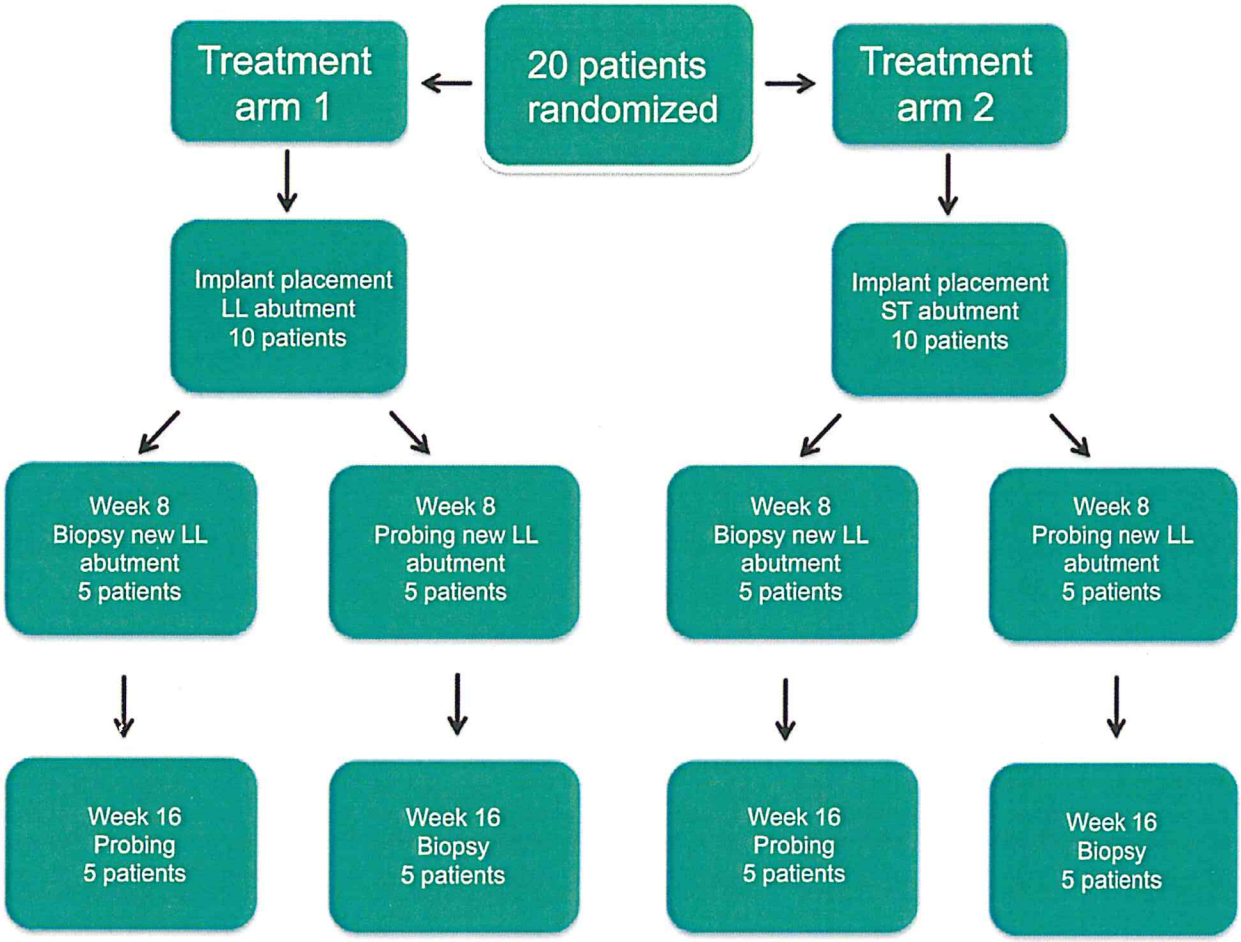

FIGURE 1 Study design.

study compared histologic and clinical results between two different implant abutments: 1) a laser-ablated abutment with a microgrooved surface at the apical millimeter (LL)[‡] and 2) a smooth machined titanium (ST) abutment.[§] Both devices were approved by the Food and Drug Administration. The abutments were analogous in design and material, except the test device had a laser-ablated surface. Both abutment types had a two-piece design and used an abutment screw to attach to the implant. A schematic representation of the study is presented in Figure 1.

A comprehensive treatment plan was developed through consultation between prosthodontic (SJO) and periodontal (NCG, MLG, PJV, and MSR) investigators. Participants received full-mouth oral prophylaxis before implant surgery. Implants were placed using a standard drilling technique. After thorough site irrigation, the implant was inserted directly into the prepared osteotomy. Following the computerized randomization scheme (Fig. 1), either LL or ST abutments were placed. At week 8, all abutments were harvested and replaced by LL abutments.

Five abutments per group were harvested for histologic analysis. Abutments were removed carefully with peri-abutment tissue attached using a soft tissue trephine. Harvesting soft tissue surrounding the abutments proved to be a challenging procedure. Soft tissue remained attached on the abutment surface on only five of 20 abutments harvested. For the other abutments, soft tissue was replaced on the abutment in correct tissue orientation. The shift in tissue location was taken into consideration during interpretation of histologic specimens.

The tissue–abutment construct was placed immediately in fixative, and vials were labeled appropriately. All biopsies were processed and analyzed by a research laboratory scientist, masked to abutment group assignment. Non-demineralized specimens were embedded in resin and polymerized. Blocks were sectioned in an axial direction. Slices were reduced by microgrinding and polishing to a final thickness of 30 to 40 µm. Sections were stained with toluidine blue/azure II stain.

The other five abutments per group were analyzed by scanning electron microscopy (SEM), prepared by dehydration through a graded series of acetones, and dried using carbon dioxide as a transitory fluid.

[‡] Laser-Lok, BioHorizons Implant Systems, Birmingham, AL.
[§] Laser-Lok, BioHorizons Implant Systems.

An adjacent tooth was compared to the implant-supported abutment in each patient. When comparing teeth to the two abutment types, there was no significant difference in mean plaque index (PI) ($P = 0.22$) or gingival index (GI) ($P = 0.29$) values between groups. Overall, all sites showed good mucosal health. There was a significant difference in mean probing depth (PD) values ($P < 0.001$) between groups. The least squares mean (lsmean) for teeth was significantly lower (lsmean = 2.4295) than that for LL (lsmean = 3.3419, $P < 0.001$) and ST (lsmean = 3.0789, $P < 0.001$) abutments. The difference in PDs between ST and LL abutments was not significant.

Clinical Scenario

Healing of CT Around ST Abutments

Figure 2a illustrates SEM and Figure 2b the histology of specimens harvested 8 weeks after placement of ST abutments and are representative of the healing of the soft tissue. Soft tissue appears to have shifted apically during harvesting or processing, and only a zone of epithelium was noted on the abutment surface; no zone of CT was noted on the abutment surface. No CT integration with the abutment surface was noted on 100% of the samples.

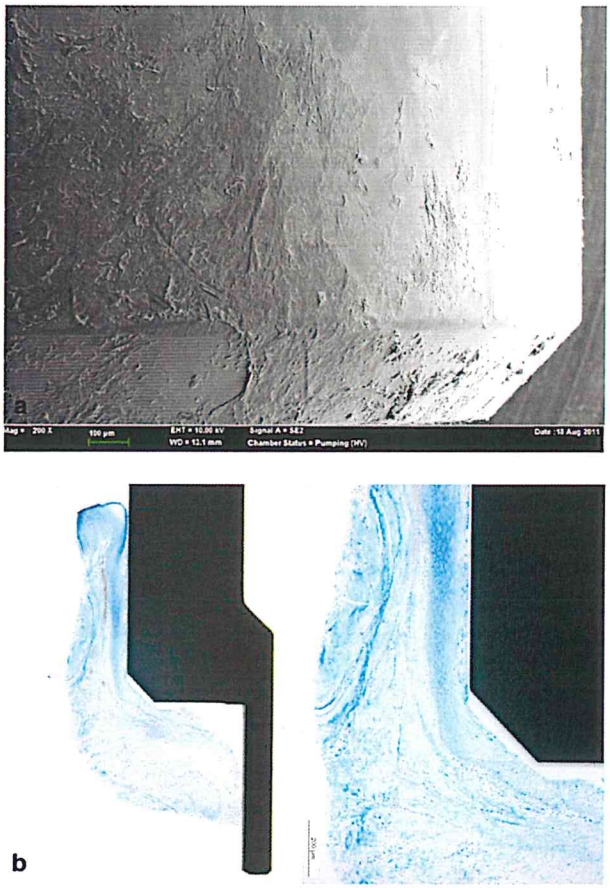

FIGURE 2a SEM of ST abutments harvested at 8 weeks. No CT integration with the abutment surface was noted. **2b** Histology of ST abutments harvested at 8 weeks. The soft tissue appears to have shifted apically during harvesting or processing. Only an epithelial zone is noted in tissue adjacent to the abutment (toluidine blue/azure II staining; original magnification ×200).

Healing of CT on LL Abutments

Healing 8 weeks after implant placement with an LL abutment is illustrated in Figure 3. A zone of epithelium adjacent to the abutment surface and a zone of CT on the abutment surface were apparent. Soft tissue attachment observed was a combination of epithelial attachment and CT attachment to the laser-ablated microgrooved surface. This finding was consistent in 100% of samples and showed CT integration.

Healing of CT on LL Abutments Inserted After the Removal of ST Abutments

Figure 4 illustrates SEM and histology of a specimen harvested 8 weeks after an abutment switch from ST to LL. CT integration is not observed, and oral biofilm is noted on the microgrooved surface. Figure 5 illustrates another

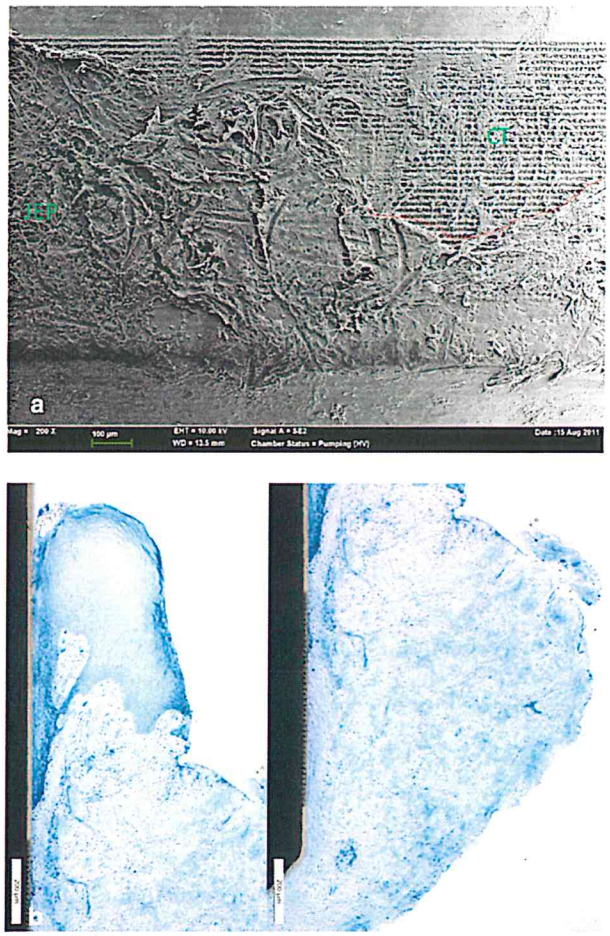

FIGURE 3a SEM of LL abutment harvested after 8 weeks. Soft tissue attachment observed was a combination of epithelial attachment and CT attachment to the laser-ablated microgrooved surface. JEP = junctional epithelium. **3b** Histology of LL abutment harvested after 8 weeks. A zone of epithelium adjacent to the abutment surface and a zone of CT on the abutment surface were apparent (toluidine blue/azure II staining; original magnification ×200).

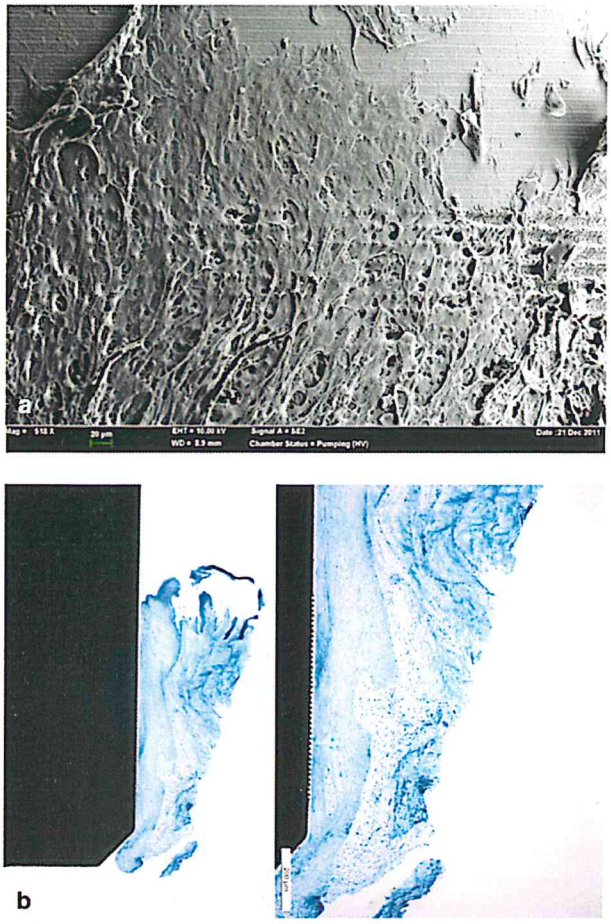

FIGURE 4a SEM of LL abutment 8 weeks after replacing ST abutment. Epithelium can be observed covering the LL surface. **4b** Histology of LL abutment 8 weeks after replacing ST abutment. Histology shows an epithelial zone adjacent to the abutment (toluidine blue/azure II staining; original magnification ×200).

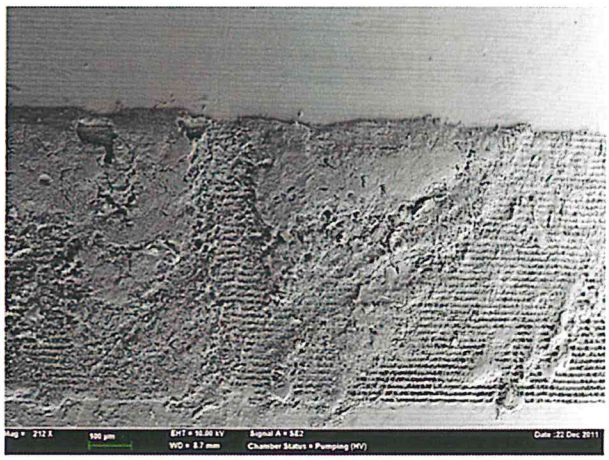

FIGURE 5 SEM specimen of LL abutment 8 weeks after replacing ST abutment. Biofilm can be noted on the LL surface (toluidine blue/azure II staining; original magnification ×200).

SEM of a specimen harvested 8 weeks after an abutment switch from ST to LL and indicates areas of CT integration observed histologically. CT integration when the ST abutment was switched to an LL abutment was inconsistent, with few specimens demonstrating CT integration. When ST abutments were removed with a biopsy that removed soft tissue adjacent to the abutment, healing on the new LL abutment was similar to healing observed when LL was placed at the time of implant placement.

Healing of CT on LL Abutments Inserted After the Removal of LL Abutments

SEM and histology of a specimen harvested 8 weeks after switching from an LL to a new LL abutment is shown in Figure 6. A zone of epithelium adjacent to the abutment surface and a zone of CT on the abutment surface were evident. CT integration was consistently observed on 100% of

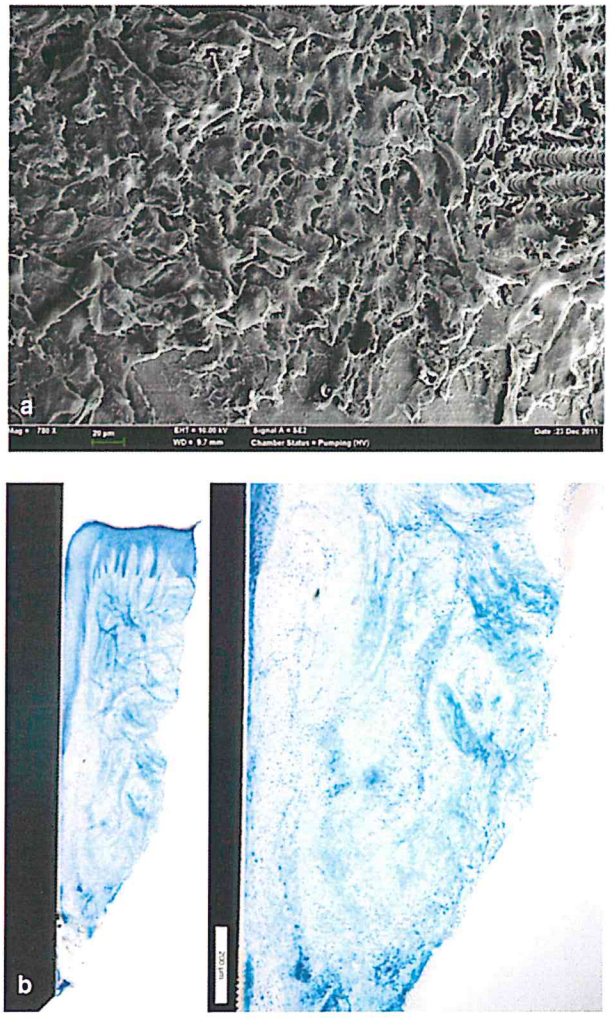

FIGURE 6a SEM of LL abutment 8 weeks after being switched from a previous LL abutment. Soft tissue integration is noted. **6b** Histology of LL abutment 8 weeks after being switched from a previous LL abutment. An epithelial zone and a CT zone on the abutment surface was observed (toluidine blue/azure II staining; original magnification ×200).

abutment surfaces. This was also observed in abutments in which soft tissue adjacent to the abutment was removed.

Discussion

Figure 7 presents the decision matrix to optimize CT healing on LL abutment surfaces. Healing of four different clinical scenarios was described. LL abutments were placed after implant placement and flap closure around the abutment. After an 8-week healing period, CT integration was observed in all specimens. CT integration on LL abutments was markedly different from healing that occurred around ST abutments placed in the same clinical scenario. Around ST abutments, an epithelial zone was noted, but no CT was found to be in contact with the implant surface.

CT integration found on LL abutment surfaces has been consistent with findings in previous studies in animals and humans.[10,15,16] Formation of the CT zone is proposed to be facilitated by inhibition of epithelial migration on the LL surface, providing an opportunity for CT to integrate with the LL surface.[7]

When ST abutments were removed and replaced with LL abutments, healing did not result in consistent formation of CT integration. It appears that when an epithelial zone was formed around an ST abutment, the LL surface did not have an opportunity to interact with CT. Healing was dictated by epithelium, and an epithelial zone was reestablished on the newly placed LL abutment.

When LL abutments replaced a previously placed LL abutment, CT integration was found consistently. It appears that disrupting integration that initially occurred allowed for CT to reintegrate with the abutment surface.

These findings have clinical significance in management of final abutment placement. When an ST abutment was placed at the time of implant placement and the final abutment was an LL abutment, epithelium needed to be removed to allow CT integration with a zone of CT on the abutment. Soft tissue facing the abutment may be de-epithelialized with either a diamond bur or surgical blade. With these techniques, care should be taken to prevent damage to the implant surface or surrounding soft tissue scaffold. A special tool could be developed for removal of the collar that would protect the implant surface and remove only the epithelial layer of the abutment sulcus.

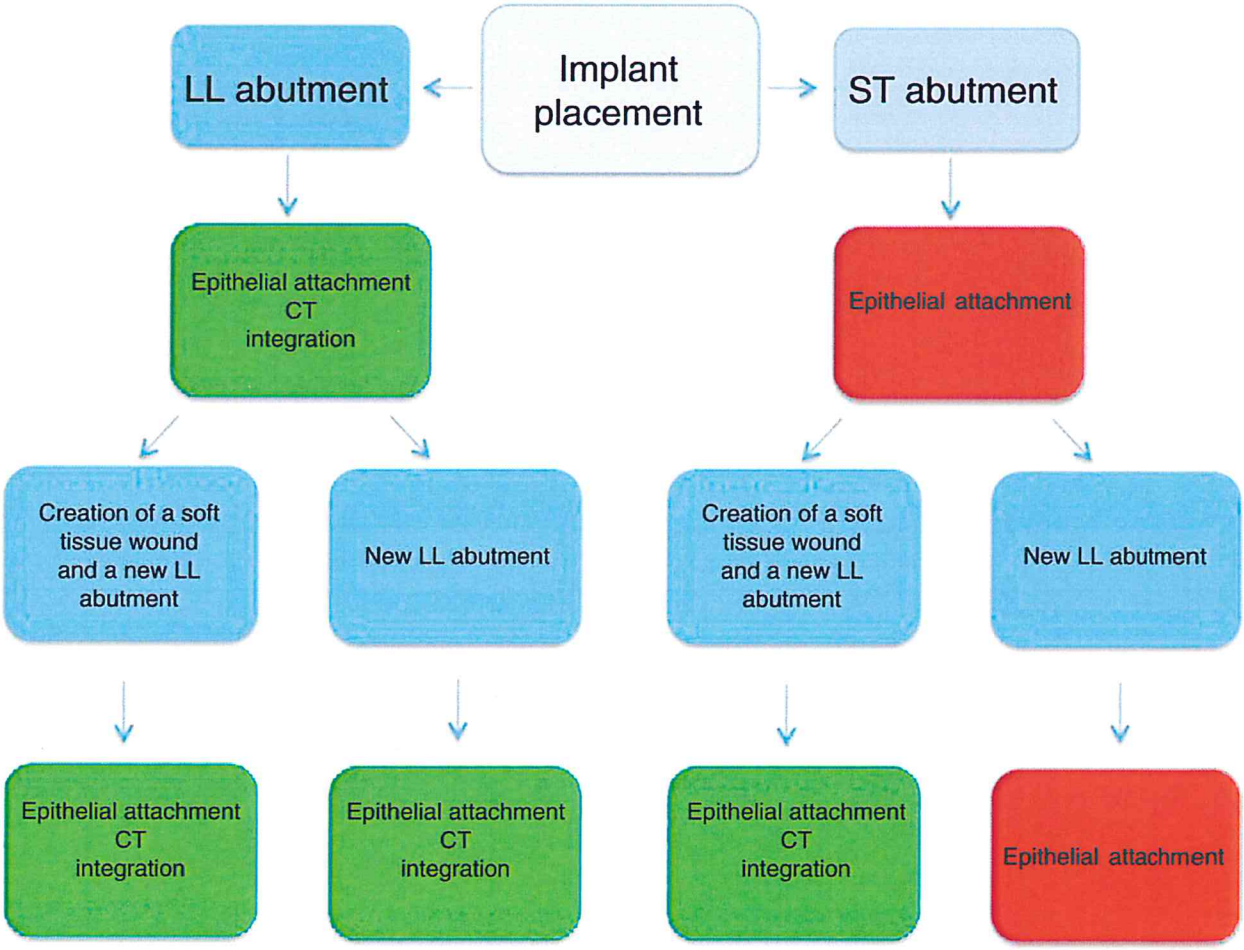

FIGURE 7 Decision matrix to optimize soft tissue integration onto LL abutments.

PDs around the natural adjacent teeth were less deep than measurements around implants with either type of abutment. This finding was supported by previous reports that found PD in general to be deeper around dental implants.[17,18]

PD measurements were similar for ST and LL abutments. Although a zone of CT integration was found surrounding LL abutments placed in the appropriate scenario, this was not reflected in PD data. The zone of laser-ablated surface on the LL abutments was only 0.7 mm and may have been too small to be measured given the inaccuracy of manual PD measurements around implants.[19] The CT zone may offer similar resistance to probing forces and therefore result in similar PD measurements.

No differences were found in plaque accumulation and inflammation around the teeth of the two abutment types. The healing period was short; during initial healing, patients were instructed to use chlorhexidine mouthrinse. Overall, teeth and implants were surrounded by healthy tissue, and patients were able to perform adequate oral hygiene measures. PI and GI are not very sensitive to small changes in plaque accumulation.

This was a short-term study of healing around abutments for two periods of 8 weeks. Long-term significance of the LL surface has not been established. In humans, implants with a laser-ablated microgrooved collar surface performed better than controls with a machined surface when comparing PDs and bone maintenance.[9,20-22] When combined with a beveled implant collar, bone healing and maintenance were found to be favorable in a canine study.[23]

Conclusions

Consistent CT integration was observed on LL abutment surfaces after implant placement. Switching LL abutments with new LL abutments consistently leads to CT integration. This was not consistent if an ST abutment was switched for an LL abutment. PI and GI were comparable between teeth and abutments. PD was lower around the teeth. There was no difference in PD between abutments.

The CT integration on LL abutments is optimized by initial healing occurring onto an LL abutment or creation of a CT wound before insertion of the abutment. Clinicians need to facilitate healing onto the LL surfaces by removing epithelium that would interface with the abutment surfaces. ■

Acknowledgments

This study was funded by BioHorizons Implant Systems (Birmingham, Alabama) for the authors to present the data at national meetings. Drs. Geurs and O'Neal have received lecture fees from BioHorizons. Dr. Geisinger has received salary and research support from BioHorizons, Straumann USA (Andover, Massachusetts), Zimmer Dental (Carlsbad, California), and DENTSPLY International (York, Pennsylvania). Ms. Haigh and Dr. Reddy report no conflicts of interest related to this study.

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