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The barrier between the keratinized mucosa and the dental implant

An experimental study in the dog

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Abstract. The present study was performed in order to examine the composition of the connective tissue that forms an attachment to a dental implant. 6 beagle dogs were used. All mandibular premolars were extracted. After 3 months of healing, 6 fixtures – 3 in each side of the mandible – (Astra Tech Implants, Dental System® TiO blast ; Astra Tech AB, Mölndal, Sweden) were installed. After another 3 months of healing, abutment (Uni-abutment® 45; Astra Tech AB, Mölndal, Sweden) connection was performed and a plaque control program was initiated. The animals were sacrificed and perfused with a fixative through the carotid arteries. Each implant site, including the implant and the soft and hard periimplant tissues, was dissected, decalcified in EDTA and further processed using a "fracture technique". The specimens were subsequently embedded in EPON, cut with the microtome set at 3 μm and the sections stained in PAS and toluidine blue. From the EPON-embedded blocks, ultra-thin sections were cut and electron micrographs were prepared. The detailed histologic and morphometrical examinations were restricted to a 200 μm wide zone of connective tissue interposed between the apical border of the junctional epithelium and the bone tissue. In the analysis, this zone was further subdivided into 2 different units; (i) one central, 40 μm wide unit (zone A) located immediately next to the implant surface, and (ii) one lateral, 160 μm wide unit (zone B) that was continuous with the central unit. The implant surface apical of the junctional epithelium and coronal of the bone crest appeared to be in direct contact with a connective tissue. Zone A of this connective tissue was characterized by its (i) absence of blood vessels and (ii) abundance of fibroblasts which were interposed between thin collagen fibers. The more lateral zone B contained comparatively fewer fibroblasts, but more collagen fibers and blood vessels. There are reasons to assume that the fibroblast rich barrier tissue next to the titanium surface plays a rôle in the maintenance of a proper seal between the oral environment and the peri-implant bone.

Key words: dogs; dental implants; fibroblasts; morphometry; peri-implant mucosa

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Current knowledge of the interface between the surface of a dental implant (made of c.p. titanium) and periimplant mucosa indicates that this particular boundary is composed of two portions; a marginal junctional epithelium (about 2 mm long) which is continuous with a zone of connective tissue attachment (about 1–1.5 mm high). This transmucosal passage which is established early during soft tissue healing following implant surgery constitutes an effective barrier between the oral environment and the periimplant bone. While the epithelial-implant interface has many features in common with the epithelial-tooth interface (James & Schultz 1973, Listgarten & Lai 1975, Gould et al. 1981, 1984, Hansson et al. 1983), marked differences seem to exist between the connective tissue attachment at teeth and the corresponding attachment tissues at implants (for review see Lindhe & Berglundh 1998). In the supra-alveolar compartment, the connective tissue attachment at teeth is characterized of a root cementum into which fibers (Sharpey's fibers) invest at an oblique angle to the tooth surface. This tissue is comprised of collagen

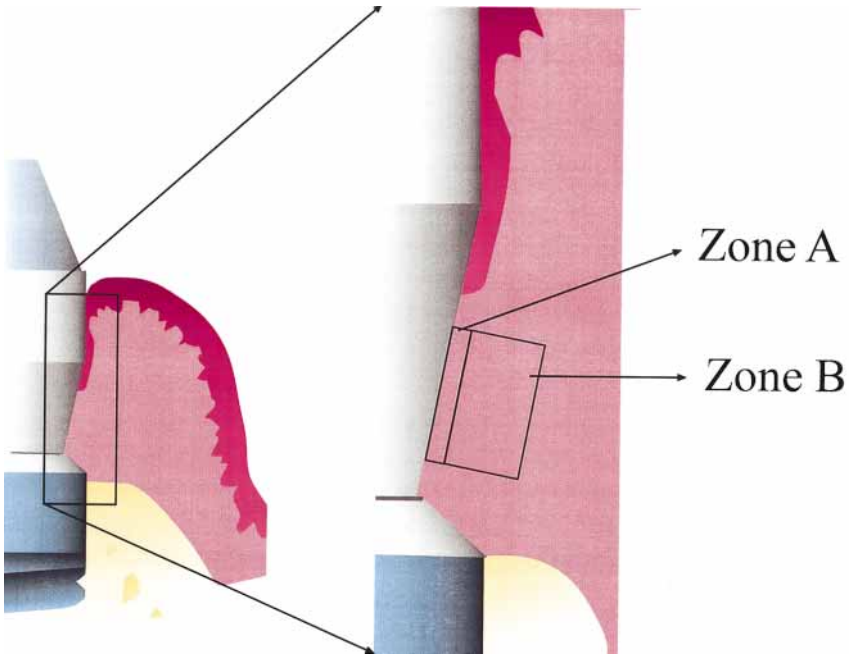

Fig. 1. Schematic drawing illustrating the peri-implant connective tissue zones analyzed.

(=70%), fibroblasts (=20%), vascular units (=5%), matrix and unidentified structures. The implant surface is devoid of a root cementum, and hence the collagen fibers in the supra-alveolar region invest in the ridge of the periimplant bone and run a course more or less parallel with the abutment portion of the titanium body (Berglundh et al. 1991, Buser et al. 1992, Ruggeri et al. 1992, 1994). The "inner zone" (100–200 μm) of this attachment tissue has been described by several authors (Berglundh et al. 1991, Buser et al. 1992) as having the character of a collagen rich but cell poor, scar tissue like, structure. Buser et al. (1992) in a publication describing soft tissues at non-submerged titanium implants stated "The implant surface was surrounded by a narrow, approximately 50 to 100 μm thick zone of connective tissue without blood vessels". From a recent experiment in the dog (Abrahamsson et al. 1996), details regarding the composition of the non-inflamed mucosa at different implant

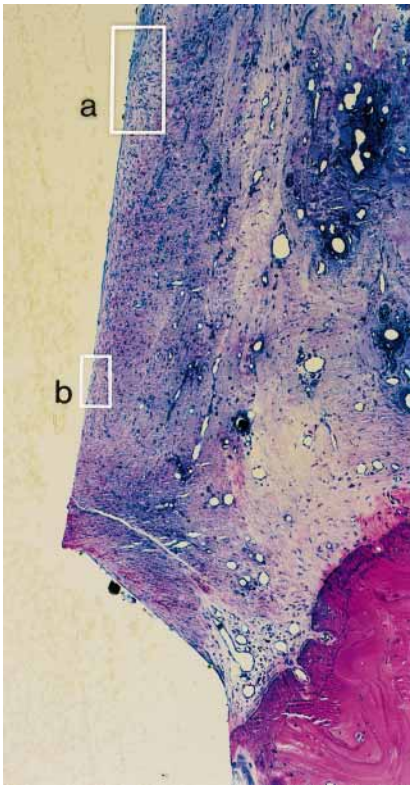

Fig. 2. Cross-section of the connective tissue interface portion of the peri-implant mucosa. Squares indicate areas shown in Fig. 3a and Fig. 4b. Original magnification×100.

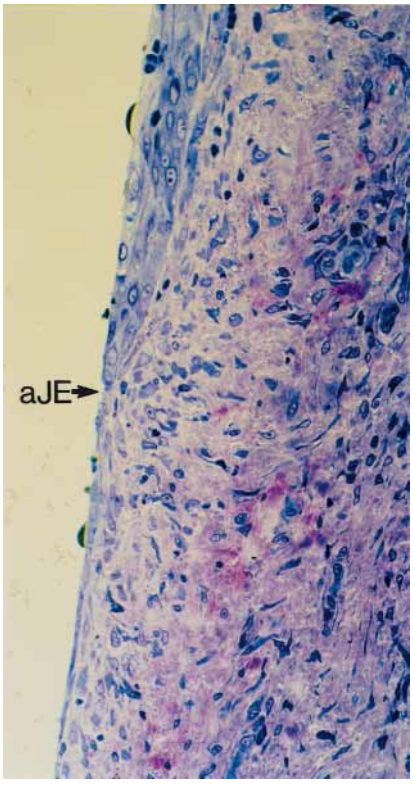

Fig. 3. Higher magnification of the upper square (a) of Fig. 2 illustrating the apical part of the junctional epithelium (aJE) and the peri-implant connective tissue. Original magnification×400.

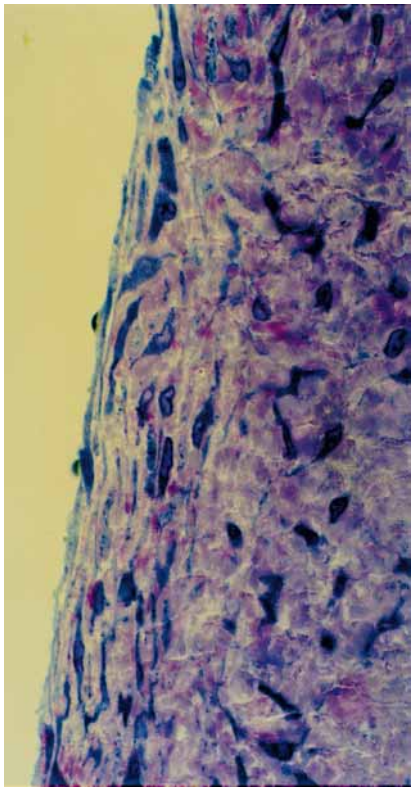

Fig. 4. Higher magnification of the lower square (b) of Fig. 2 illustrating the peri-implant connective tissue interface. Original magnification×1000.

systems were reported. The authors concluded that the mucosal barrier that formed at the various titanium surfaces following "1 – stage and 2 – stage implant installations had similar composition". Thus, the connective tissue in a 300–600 μm wide zone next to the titanium surface was rich in collagen (>85%), but poor in cells (7–8%) and vascular structures (2–3%). A further analysis of the material presented by Abrahamsson et al. (1996) seemed to indicate, however, that the tissue composition in the 300–600 μm wide zone of connective tissue was not homogenous. Thus, while density of collagen emerged to be high in more peripheral layers of this zone, a narrow region close to the implant surface appeared to be more rich in cells.

The aim of the present study was to further examine the composition of the connective tissue next to the surface of a dental implant made of c.p. titanium.

Material and Methods

6 beagle dogs, about 1 year of age, were included in the study. In order to establish recipient sites for implants, all mandibular premolars were extracted. After 3 months of healing, 6 fixtures – 3 in each side of the mandible – (Astra Tech Implants, Dental System® TiO blast ; Astra Tech AB, Mölndal, Sweden) were installed. After another 3 months of healing, abutment (Uni – abutment® 45°; Astra Tech AB, Mölndal, Sweden) connection was performed and a plaque control program initiated. This called for tooth and abutment cleaning once a day, 5 days a week, and was maintained for 6 months.

The animals were sacrificed with an overdose of Sodium-Pentothal and perfused with a fixative (Karnovsky 1965) through the carotid arteries. The mandibles were removed. Each implant site, including the implant and the soft and hard periimplant tissues, was dissected, decalcified in EDTA and further processed using a "fracture technique" described in detail by Berglundh et al. (1991, 1994). Before the hard tissue was fully decalcified, incisions – parallel with the long axis of the implant – were made through the periimplant tissues and 4 different blocks (mesio-buccal, disto-buccal, mesio-lingual, disto-lingual) hereby obtained. Decalcification was completed in EDTA. The specimens were subsequently embedded in EPON, cut with the microtome set at 3 μm and the sections stained in PAS and toluidine blue (Schroeder 1969).

The histological examination was restricted to a 200 μm wide zone of connective tissue interposed between the apical border of the junctional epithelium and the bone tissue (Fig. 1). This zone was further subdivided into 2 different units; (i) one central ("inner"), 40 μm wide unit (zone A) located immediately next to the implant surface, and (ii) one lateral ("outer"), 160 μm wide unit (zone B) that was continuous with the central unit. The connective tissue in these zones was analyzed using a morphometric technique (for details see Berglundh et al. 1991). The relative proportions occupied by collagen (Co), fibroblasts (Fi), vascular structures (V) and residual tissue (e.g., leukocytes, nerves, matrix components) were determined. The examinations were performed in a Leica DM-RBE® microscope (×1000) (Leica, Germany) equipped with an image system (Q-500 MC®; Leica, Germany) and a lattice comprising 100 light points (Schroeder & Münzel-Pedrazzoli 1973).

From each of the EPON-embedded blocks, ultra thin sections (about 500 Å) representing the connective tissue identified above were cut in an Ultrothome® (LKB, Sweden). The sections were placed on a 200 mesh grid and contrasted with uranyle acetate and lead citrate. Electron micrographs were obtained from 2 equally wide regions; one *Inner region* – representing a 30 μm wide segment next to the implant surface and one *Outer region* – representing a second 30 μm wide segment located about 150 μm lateral to the implant. The proportion of fibroblasts present in these 2 regions was assessed in the electron micrographs using a point counting procedure and a 42-point lattice (Weibel 1969).

Statistical analysis

Mean values for the different variables examined were calculated for each implant and animal. Differences between various zones and regions were analyzed using the Student *t*-test for paired observations. The null hypothesis was rejected at $p<0.05$.

Results

The interface between the implant and the mucosa was comprised of a junctional epithelium and a connective tissue. The junctional epithelium was about 2 mm long and about 40 μm wide. The implant surface apical of the junctional epithelium and coronal to the bone crest seemed to be in direct contact with a connective tissue (Figs. 2–4).

The 40 μm wide, "inner" (zone A; Table 1) portion of this connective tissue was characterized by its (i) absence of blood vessels and (ii) abundance of fibroblasts which were interposed between thin collagen fibers. The fibroblasts in this portion were oriented with their long axis parallel with the adjacent collagen fibers and with the implant surface (Fig. 5). The fibers extended from the periosteum of the bone crest in vertical direction towards the oral epithelium of the periimplant mucosa. Collagen fibers that contacted the implant surface in perpendicular direction were not seen. Zone A was in lateral direction continuous with an "outer" portion (zone B; Table 1) of mesenchymal tissue which seemed to contain comparatively fewer fibroblasts, but more and larger collagen fibers which extended in different directions (Fig. 6). In addition, zone B appeared to contain a substantial number of vascular structures.

The connective tissue in zones A+B (Table 1) was comprised of 80.61% collagen, 12.98% fibroblasts, 3.42% vascular structures and 3.0% residual tissue.

A more detailed analysis of the connective tissue within the attachment zone demonstrated that there were marked differences between the connective tissue of zones A and B. Thus, while zone B was characterized by its high collagen (82.36%) and low fibroblast (11.5%) density, zone A was rich in cells (fibroblasts=32.32%) and had a relatively low proportion of collagen (66.47%).

Measurements made in the electron micrographs demonstrated that fibroblasts occupied 28% of the barrier tissue in the *Inner region* but only about 10% of the *Outer region* (Table 2).

Discussion

The connective tissue in a 200 μm wide zone lateral to the implant surface was characterized by its large amount of collagen fibers, its relatively low number of fibroblasts and vascular units. The overall composition of the peri-implant connective tissue examined in the present study, was similar to that previously

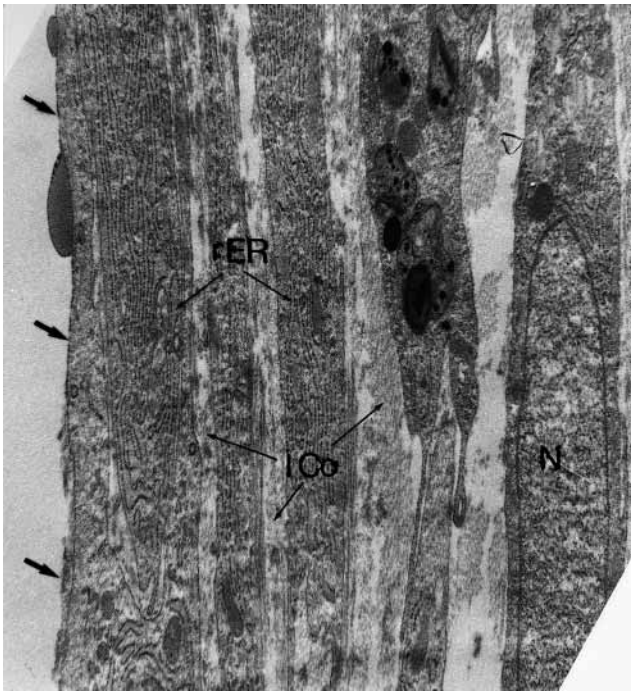

Fig. 5. Electron micrograph of the inner region of the peri-implant connective tissue. The fibroblasts are orientated with their long axis parallel with the longitudinally sectioned collagen fibrils and with the implant surface. Arrows indicate the implant/connective tissue interface. rER: the rough endoplasmic reticulum; lCo: the longitudinally sectioned collagen fibrils; N: the nucleus. Original magnification×24000.

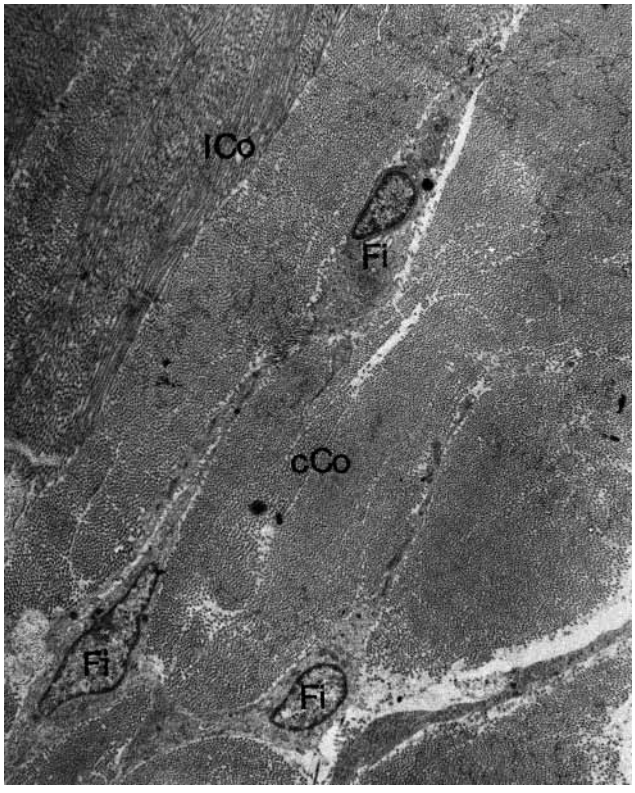

Fig. 6. Electron micrograph of the outer region of the peri-implant connective tissue illustrating fewer fibroblasts and more collagen fibers. Fi: Fibroblast; cCo: the cross sectioned collagen fibrils; lCo: the longitudinally sectioned collagen fibrils. Original magnification×7000.

reported from experiments in the dog (Berglundh et al. 1991, Buser et al. 1992, Abrahamsson et al. 1996; for review see Lindhe & Berglundh 1998). The corresponding region of the supraalveolar gingival tissue, i.e., a 100 μm wide zone of tissue lateral to the acellular root cementum included about 76% collagen, 5% fibroblasts and 2.5% vascular structures (Berglundh et al. 1991). The above findings, thus, demonstrate that the periimplant mucosa from a structural point of view is different from gingiva. It is suggested, therefore, that the term gingiva should not be used to describe the soft tissue that surrounds dental implants.

Berglundh et al. (1991) implied that the paucity of cells in the zone of connective tissue attachment "may indicate that the tissue turn-over of the periimplant mucosa is less rapid than that of the gingiva". This hypothesis was supported by findings of Abrahamsson et al. (1996). They studied the attachment zone of the periimplant mucosa at 3 different implant systems and stated that "The tissue in this zone had the composition of a scar, i.e. a high density of collagen and a low density of cells and vascular structures". It was argued that the attachment, because of its limited content of cells and vascular structures had a poor regenerative potential (Lindhe & Berglundh 1998).

In the present study, a more detailed analysis of the connective tissue within the attachment zone was carried out. The findings from the morphometric analysis performed in the light microscope (Table 1) indicated that there were marked differences between the central (0–40 μm) and a more laterally (40–200 μm) positioned region of this connective tissue. Thus, while the lateral region was characterized by its high collagen (82.36%) and low fibroblast (11.5%) density, the "inner" region was rich in cells (fibroblasts=32.32%) and had a relatively low proportion of collagen

Table 1. Results from the morphometric measurements; mean (SD)

	Zone A+B	Zone A	Zone B
Co	80.61 (2.60)*	66.47 (2.73)	82.36 (4.45)*
V	3.42 (0.64)*	0.25 (0.35)	3.27 (1.21)*
Fi	12.98 (2.19)*	32.32 (2.92)	11.50 (2.92)*#
R	3.00 (1.04)*	1.07 (0.43)	2.89 (1.00)*

Zone A: a 40-μm wide zone of connective tissue immediately lateral to the implant surface. Zone B: a 160-μm wide zone lateral to but continuous with zone A; mean (SD).
The volume % of the connective tissue occupied by collagen (Co), vascular structures (V), fibroblast (Fi) and residual tissue (R). Light microscopic measurements.
* Indicates a statistically significant difference from zone A ($p<0.05$).
# Indicates a statistically significant difference from zone A+B ($p<0.05$).

Table 2. Results of the electron microscopic measurements in the inner and outer regions; mean (SD)

Inner region		Outer region
28.12 (5.97)	*	11.59 (5.82)

The volume % of fibroblasts in the connective tissue.
* Indicates a statistically significant difference from inner zone ($p<0.05$).

(66.47%). The above findings were confirmed by measurements made in the electron micrographs showing that fibroblasts occupied 28% of the barrier tissue in the Inner region but only about 10% of the *Outer region* (Table 2).

As stated above, findings from previous experiments have been interpreted to demonstrate that the barrier between the titanium surface of a dental implant and the periimplant mucosa is maintained through a delicate scar with a low tissue turn-over. The current observations seem to contradict this concept. Thus, there are reasons to assume that the fibroblast rich barrier tissue next to the titanium surface has a high turn-over and that fibroblasts, indeed, may play an important role in establishment and maintenance of a proper mucosal seal.

Acknowledgements

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Zusammenfassung

Die Barriere zwischen keratinisierter Mukosa und dentalen Implantaten. Eine experimentelle Studie beim Hund

Diese Studie untersuchte den Aufbau des Bindegewebes, das eine Anheftung an dentale Implantate herstellt. Dazu wurden bei 6 Beagle-Hunden alle Unterkiefer-Prämolaren entfernt. Nach einer Heilungsphase von 3 Monaten wurden 6 Implantate gesetzt – 3 auf jeder Seite des Kiefers. Nach weiteren 3 Monaten der Heilung wurden die Aufbauten montiert und die Plaquekontrolle begonnen. 6 Monate später wurden die Tiere geopfert und mit einem Fixierungsmittel durch die Halsschlagader perfundiert. Jede Implantatstelle einschließlich des Implantats und der umgebenden periimplantären Hart- und Weichgewebe wurde herausgetrennt, mit EDTA demineralisiert und nach der "Bruch-Technik" weiterbearbeitet. Die Präparate wurden in EPON eingebettet, mit einem Mikrotom in 3 μm dicke Schichten geschnitten und mit PAS sowie Toluidin-Blau gefärbt. Von den in EPON eingebetteten Blöcken wurden Ultradünnschnitte hergestellt und elektronenmikroskopisch untersucht. Die detaillierte histologische und morphometrische Auswertung wurde auf eine Zone von 200 μm Breite apikal des Epithelansatzes und koronal des Alveolarknochens beschränkt. In der Auswertung wurde diese Zone in 2 Bereiche weiter unterteilt: (i) eine zentrale, 40 μm breite Einheit (Zone A) unmittelbar auf der Implantatoberfläche und (ii) eine seitliche, 160 μm breite Einheit (Zone B), die sich der zentralen Einheit anschloß. Die Implantatoberfläche zwischen Epithelansatz und Knochen schien in unmittelbarem Kontakt mit dem Bindegewebe zu sein. Zone A des Bindegewebes zeichnete sich (i) durch Abwesenheit von Blutgefäßen und (ii) eine große Zahl von Fibroblasten aus, die zwischen den Fasern lagen. Die mehr lateral gelegene Zone B enthielt vergleichsweise weniger Fibroblasten, aber mehr Kollagenfasern und Blutgefäße. Es gibt Hinweise darauf, daß das fibroblastenreiche Barrieregewebe unmittelbar auf der Titanoberfläche eine Rolle in der Aufrechterhaltung der Abdichtung zwischen Mundhöhle und periimplantärem Knochen spielt.

Résumé

La barrière entre la muqueuse kératinisée et l'implant dentaire. Étude expérimentale chez le chien

La présente étude a été entreprise pour examiner la composition du tissu conjonctif qui forme une attache sur un implant dentaire. Nous avons utilisé 6 chiens beagle. Toutes les prémolaires mandibulaires ont été extraites. Après 3 mois de cicatrisationn, 6 fixtures ont été posées – 3 de chaque côté de la mandibule – (Astra Tech Implants, Dental System® TiO blast; Astra Tech AB, Mölndal, Suède). Après ultérieurement 3 mois de cicatrisation, la connexion des piliers (Uniabutment® 45; Astra Tech AB, Mölndal, Suède) a été faite et un programme de contrôle de la plaque a été mis en route. Les animaux ont été sacrifiés et une perfusion de fixateur a été faite par les artères carotides. Chacun des sites implantaires, comprenant l'implant et les tissus péri-implantaires mous et durs, a été disséqué, décalcifié dans l'EDTA et préparé ultérieurement à l'aide d'une "technique de fracture". Les spécimens ont ensuite été inclus dans l'EPON, taillés au microtome réglé à 3 μm et les coupes ont été colorées par le P.A.S. et le bleu de toluidine. Des coupes ultra-fines ont été coupées à partir des blocs inclus dans l'EPON, et des microphotographies électroniques ont été préparées. Les examens histologiques et morphométriques détaillés ont été limités à une zone de 200 μm de largeur de tissu conjonctif interposé entre la limite apicale de l'épithélium de jonction et le tissu osseux. Dans l'analyse, cette zone a été ultérieurement subdivisée en deux portions différentes: (i) une portion centrale de 40 μm de largeur (zone A) située au contact immédiat de la surface de l'implant, et (ii) une portion latérale de 160 μm de largeur (zone B) continuant la portion centrale. On a constaté que la surface de l'implant en apical de l'épithélium de jonction et en coronaire de la crête osseuse, était en contact direct avec un tissu conjonctif. La zone A de ce tissu conjonctif était caractérisée par (i) l'absence de vaisseux sanguins et (ii) l'abondance de fibroblastes, qui étaient interposés entre de minces fibres collagènes. La zone B, plus latérale, contenait relativement moins de fibroblastes, mais plus de fibres collagènes et de vaisseaux sanguins. Il y a lieu de supposer que le tissu barrière riche en fibroblastes et situé au contact de la surface de titane joue un rôle pour maintenir un joint adéquat entre le milieu buccal et l'os péri-implantaire.

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