

Histological Analysis of the Ankylos Peri-implant Soft Tissues in a Dog Model

Henri Tenenbaum, DDS, PhD,* Jean-François Schaaf, DDS,** and Frédéric J. G. Cuisinier, DDS, PhD***

Osteointegration is a recognized means of anchoring implants in bone. However, few studies have investigated the relationship between the implant/abutment device and the soft tissues. Supracrestal tissues around implants seem to be similar to gingiva around teeth in many ways.¹ This tissue has a dense, collagenous lamina propria and is covered with stratified squamous, attached oral epithelium. The apical part of the gingival sulcus is lined by a junctional epithelium of typical tooth morphology. In the connective tissue, the collagen fibers originate from the bone crest, adjacent teeth, and epithelial papillae and they converge on the implant to form circular fibers around the implant. It is clear that there is no insertion of fibers on the implant surface as it is in the cementum of a tooth. However, it may be important to notice if the majority of fibers are oriented perpendicular or parallel to the implant surface, especially regarding the resistance of the tissues to external aggression. Resistance to probing is greater around teeth than around implants, so probing depths are significantly deeper around implants.² Histologically, the probe tip (which is about 0.5 mm in diameter) is coronal to the apical extension of the junctional epithelium around teeth, whereas around implants the probe tip is always apical to

Purpose: The importance of the soft tissue–implant interface is enhanced by the presence of a microgap between the implant and the abutment, which represents a contamination site for bacteria. The aim of this study was to investigate the interface between the Ankylos gap-free implant system and the surrounding soft tissues in a dog model. **Materials and Methods:** Six Labrador dogs were included in the study and two Ankylos implants were inserted per dog. The dogs were killed 6 months after abutment placement without functional loading and without plaque control. The implants were analysed histologically by scanning electron microscopy, light microscopy, and histomorphometry. **Results:** Some sections exhibited histologic signs of a mild inflammation. The connective tissue between the most apical epithelial cells of the

junctional epithelium and the alveolar crest was characterized by collagen fibers running from the periosteum and the alveolar crest toward the oral epithelium and, in front of the cone-shaped abutment, by a narrow zone of extracellular matrix with a few collagen fibers. **Conclusion:** Compared with results obtained in other studies using different types of implant (Astra, Bränemark, ITI), the Ankylos implant showed a higher length and a larger width of connective tissue contact as well as a shorter epithelial downgrowth. The absence of a microgap in the Ankylos system could explain the histologic mild inflammation in the connective tissue. (Implant Dent 2003;12:259–265) **Key Words:** junctional epithelium, connective tissues, histomorphometry, microgap

the junctional epithelium and close to the bone crest.² This may be related to the orientation of the collagen fibers because the major connective tissue fibers are parallel to the long axis of the implant. The importance of the soft tissue–implant interface is enhanced by the presence of a microgap between the implant and the abutment. This microgap represents a potential contamination site for plaque and bacteria.³ In nonsubmerged implants, the microgap can be kept away from the gingival tissues.⁴ However, in the case of submerged implants, the microgap is positioned just over the bone crest, in front of collagen fibers mostly oriented parallel to the abutment surface, so that it provides an opportunity for bacterial infection.

Accordingly, two-phase implant systems require new approaches to fulfil the requirements of being gap-free and bacteria resistant if the connection point between the implant and the abutment is to be positioned subgingivally. In the Ankylos system, the abutment is screwed into the inner cone of the implant element and the microgap is small (1 μ m) (Fig. 1). The aim of this study was to examine the peri-implant soft tissues around the Ankylos implant in a dog model.

MATERIALS AND METHODS

Six adult (age 9 ± 2 years) female Labrador dogs were included in the study. Animal selection and manage-

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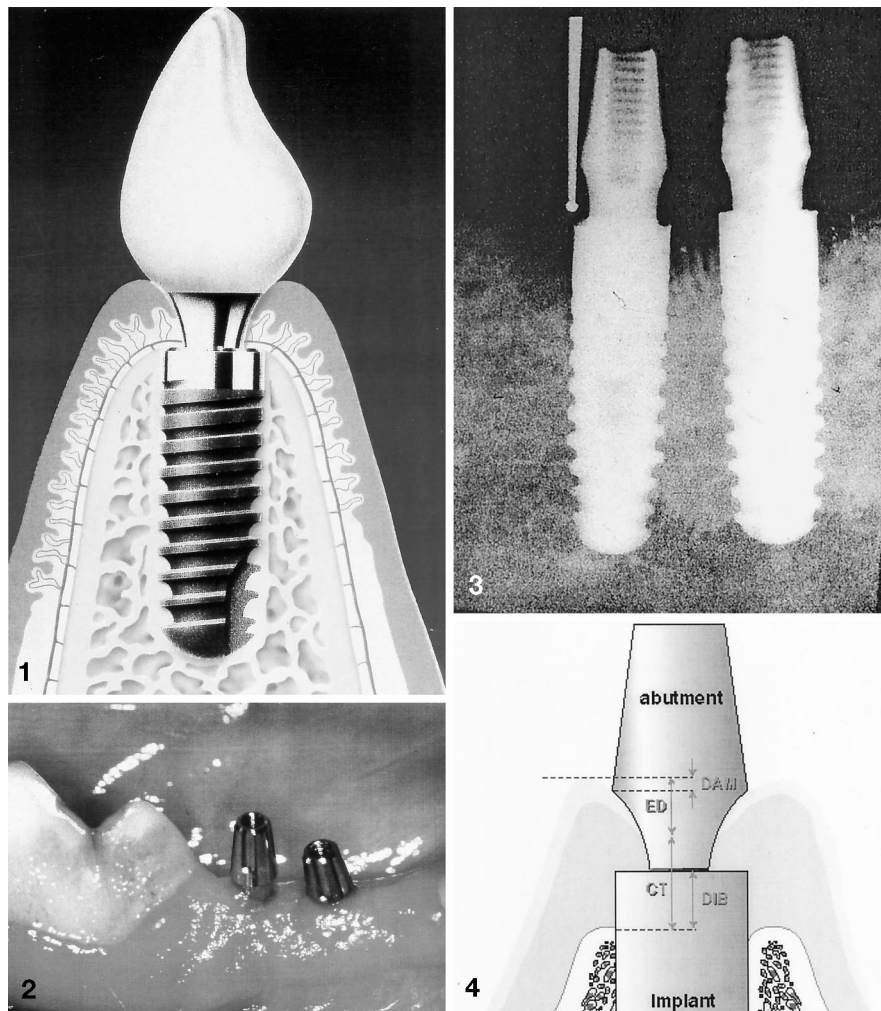


Fig. 1. Schematic drawing of the Ankylos system. The abutment is screwed into the inner cone of the implant.

Fig. 2. Clinical view of the abutments after 6 months without functional loading or plaque control.

Fig. 3. Radiograph at the time of death with a pressure-sensitive periodontal probe (Vivacare) placed in the peri-implant sulcus.

Fig. 4. Schematic drawing illustrating the landmarks used for the histometric measurements: distance between the top of the cone-shaped part of the abutment and the gingival margin (DAM), extent of epithelial downgrowth (ED), length of connective tissue contact with the abutment and the implant (CT), and distance between the top of the implant and crestal bone-implant contact (DIB).

ment and all surgical procedures followed routines approved by Institut National de la Santé et de la Recherche Médicale (INSERM, France) according to European Community guidelines for the care and use of laboratory animals (DE 86/809/CEE). Surgical procedures were performed with propofol general anaesthesia (Diprivan, Zeneca Pharma, France; 6 mg·kg⁻¹·h⁻¹). A broad-spectrum antibiotic was used for immediate postsurgical infection control (Augmentin, SmithKline Beecham, France; 2 g iv). During all operative times, the dogs

breathed under ventilator support via an endotracheal tube. Ketoprofen (Profenid, Specia-Rhone Poulenc Rorer, France; 100 mg) was used for immediate postsurgical pain control.

During the preparatory period, two premolars were extracted in the right mandibular quadrant. After 3 months of healing, two Ankylos implants (Ankylos; Friadent, Mannheim, Germany) were inserted per dog and the flaps were sutured to cover the fixtures. Abutments were placed on the implants 3 months later and the dogs were sacrificed 6 months after

abutment placement without functional loading and without plaque control (Fig. 2). The dogs were killed using an overdose of sodium pentobarbital (Dolethal, Vetopharma, France) under general anesthesia induced by an intramuscular injection of 200 mg of ketamine chlorohydrate (Ketalac, Parke-Davies, France).

Radiographs were taken at the time of death. At that time, a pressure-sensitive periodontal probe (Rigid Metal Tactile Sensor Vivacare TPS Probe, Vivadent, Schaan, Liechtenstein) with a force of 0.2 N was placed in the peri-implant sulcus to show the position of the probe's tip relative to the implant design (Fig. 3). Each specimen containing one implant with the surrounding tissues was prepared to produce undecalcified sections, which were analyzed by scanning electron microscopy, conventional light microscopy, and histomorphometry. After 7 days of fixation in a 2% glutaraldehyde and 2% paraformaldehyde solution in 0.1M sodium cacodylate buffer (pH 7.4), the specimens were embedded in Epon. After hardening, blocks were cut with a diamond saw (Escil, France) at thickness of approximately 10 µm. After grinding and polishing, the sections were glued to Plexiglas plates with cyanoacrylate glue (Superbond, DuPont, Wilmington, DE) and colored with Goldner-Masson trichrome.

The histometric analysis (Fig. 4) included the assessment of the distance between the top of the cone-shaped part of the abutment and the gingival margin (DAM), extent of epithelial downgrowth (ED), length of connective tissue contact with the abutment and the implant (CT), and distance between the top of the implant and crestal bone-implant contact (DIB). The distances between the landmarks were determined using a microscope (Zeiss, Iena, Germany) equipped with a color digital camera (Sony, Pitman, NJ) connected via a frame-grabber board (Miro DC 30, Pinnacle system, Mountain View, CA) to a Pentium-based PC (ABC, Strasbourg, France). The numerical images were obtained from the NIH image software (Scion Corporation, Frederick, MD). Means and standard deviations for buccal and lingual aspects

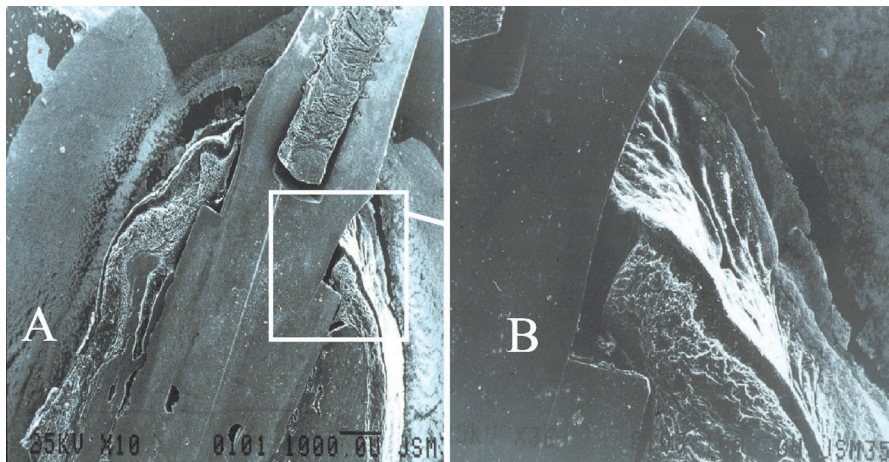


Fig. 5. Scanning electron microscope images of the Ankylos implant. **a**, Overview of a transverse section through implant, bone, and soft tissues. **b**, Enlarged view of the soft tissue-implant interface. Notice the dark aspect of oral and peri-implant epithelium.

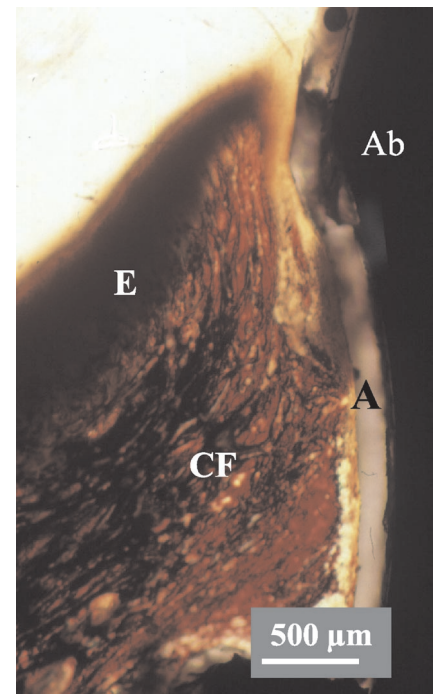


Fig. 6. Light microscope view showing a transverse section through the abutment, implant, and soft tissues. AB, abutment; E, epithelium; CF, connective fibers; A, artifact space between connective fibers and abutment.

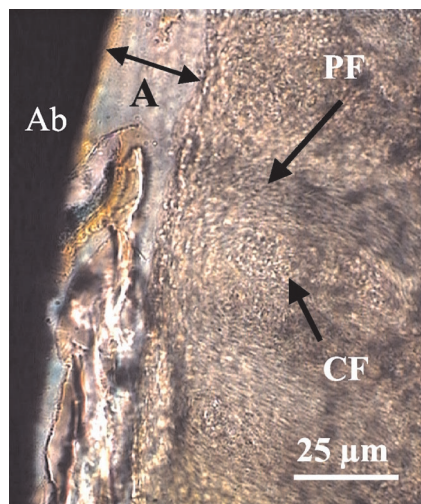


Fig. 7. Light microscope view showing the connective tissue-abutment interface. AB, abutment; CF, circular fibers; PF, perpendicular fibers; A, artifact space between collagen fibers and the abutment. The space between the abutment and the connective tissue was due to specimen preparation.

a keratinized epithelium consisting of numerous layers of epithelial cells forming rete pegs. The peri-implant sulcus was lined with nonkeratinized epithelial cells (Fig. 5). The most apical epithelial cells of the junctional epithelium were always located clearly above the alveolar crest so that the implant and abutment surface between the most apical epithelial cells and the alveolar bone crest showed a direct connective tissue contact (Fig. 6). This connective tissue was characterized by collagen fibers running in different directions. Vertical fibers ran from the periosteum and the alveolar crest toward the oral epithelium, whereas perpendicular fibers that directly contacted the implant and abutment surface were observed in a few sections (Fig. 7). A narrow zone of extracellular matrix with a few collagen fibers characterized the connective tissue close to the implant and abutment surface, especially in front of the cone-shaped abutment. Some sections exhibited histologic signs of a mild inflammation with transmigrating polymorphonuclear leukocytes and lymphocytes in the sulcular epithelium.

Histometric Observations

The results of the histometric measurements are reported in Table 1. The distances between the top of the cone-shaped part of the abutment and the gingival margin were similar on the buccal and lingual aspects. The mean DAM was 0.68 ± 0.94 mm on buccal sides and 0.46 ± 0.41 mm on lingual sides.

The mean extent of ED was longer on buccal sides (1.31 ± 0.63 mm) compared with the lingual sides (0.84 ± 0.53 mm). In contrast, the mean CT was shorter on buccal sides (2.01 ± 1.17 mm) than on lingual sides (3.62 ± 0.67 mm). The mean DIB was shorter on buccal sides (1.17 ± 0.95 mm) than on lingual sides (2.14 ± 0.28 mm).

DISCUSSION

The shape of the peri-implant mucosa found in our study (ie, a belly-shaped anatomy on the buccal side compared with the lingual mucosa, which tapers off coronally to a knife-edge shape) is consistent with that reported in other dog studies.⁵⁻⁷ In this study, the vertical dimension of the peri-implant mucosa (distance between the gingival margin to the first bone-implant contact – DAM + ED + CT) seemed to be 4.00 mm on the buccal side and 4.92 mm on the lingual aspect. Various results were found in other studies.⁵⁻⁸ Using a non-submerged approach with different types of implants, Buser et al⁵ observed values between 2.39 and 2.70

were calculated for each dog that completed the study.

RESULTS

After the surgical procedure, complication-free healing of the soft tissues was observed for all implants. After healing, all implants were firmly anchored in the jaws. None of the radiographs showed signs of a continuous peri-implant radiolucency. All 12 implants achieved osseointegration demonstrating direct bone contact. The peri-implant oral mucosa revealed

Table 1. Detailed Results of the Histometric Measurements

Measurement (mm)	DAM	ED	CT	DIB
buccal	0.68 (0.94)	1.31 (0.63)	2.01 (1.17)	1.17 (0.95)
lingual	0.46 (0.41)	0.84 (0.53)	3.62 (0.67)	2.14 (0.28)

Data are mean (SD).

DAM, distance between the top of the cone-shaped part of the abutment and the gingival margin; ED, extent of epithelial downgrowth; CT, length of connective tissue contact with the abutment and the implant; DIB, distance between the top of the implant and crestal bone implant contact.

mm on the buccal side and between 1.58 and 2.00 mm on the lingual side. Comparing submerged and nonsubmerged ITI implants, Weber et al⁶ measured the vertical dimension of the peri-implant mucosa and found 2.49 mm and 2.53 mm, respectively.

In a study using Bränemark fixtures, Berglundh and Lindhe⁷ noticed that the distance between the outer surface of the oral epithelium (gingival margin) and the bone crest was on average 3.65 mm. They did not measure the bone saucerization that was found to vary from 0.08 to 0.39 mm in the Buser et al⁵ study. The results of Berglundh and Lindhe⁷ seems to be consistent with a previous study by Ericsson and Lindhe² in which the same distance between the gingival margin and the bone crest was an average 3.3 mm using Bränemark fixtures. In a comparative study using three types of implants (Astra, Bränemark, and ITI) placed using a nonsubmerged approach,⁸ the distance from the gingival margin to the marginal level of bone-to-implant contact was 3.10, 3.15, and 3.03 mm, respectively, even without any plaque control protocol during a 5-month period.

The difference between our results and those of previous studies seems to be related mainly to a higher length of connective tissue contact with the abutment and the implant, because the epithelial downgrowth is shorter. The 1.31 mm of epithelial downgrowth found on the buccal side is less than that found in the Ericsson and Lindhe² study (1.7 mm), whereas the length of connective tissue contact found in this study (ie, more than 2.0 mm) exceeds that measured by Ericsson and Lindhe² (1.6 mm). Previous studies^{5,6} demonstrated that the extent of epithelial downgrowth was significantly more apical adjacent to submerged implants compared with nonsubmerged implants, and that the

height of connective tissue contact was greater in nonsubmerged implants. In contrast, Abrahamsson et al⁹ demonstrated that the extent of epithelial downgrowth was about 2 mm long in both the submerged and nonsubmerged implants, matching the length of epithelium lining in the submerged group of Weber et al⁶. The Ankylos implant, which used a submerged approach, revealed a shorter epithelial downgrowth than that observed with other nonsubmerged implants.

In previous studies,^{10–12} plaque formation on titanium implants resulted in the establishment of inflammatory lesions in the adjacent mucosa. Nevertheless, the infiltrated tissue occupied a small portion of the connective tissue lateral to the junctional epithelium. Ericsson et al¹³ reported that the distance between the mucosal margin and the apical border of the infiltrated connective tissue following 9 months of plaque accumulation in Labrador dogs was 0.84 mm; the same distance was 1.6 mm after 5 months of plaque accumulation in beagle dogs.⁹ Our histologic findings of a mild inflammation in the connective tissue underneath the sulcular and junctional epithelium are probably more in agreement with those of Ericsson et al¹³ in the same Labrador dogs. If the difference in the infiltrated connective tissue extension was due to the animals used in the study (Labrador versus beagle dogs), this would have to be confirmed in other experiments. It is interesting to note that Isidor¹⁴ showed (in monkeys) a dense inflammatory infiltrate in the supracrestal peri-implant mucosa after 18 months of plaque accumulation enhanced by cotton cords twisted around the abutments. Another explanation of the histologic mild inflammation in the connective tissue could be the absence of a microgap in the Ankylos system.

Weber et al⁶ showed a slight ery-

thema around several two-stage implants despite an oral hygiene regimen, and speculated that the consequent presence of a microgap between implant and transmucosal abutment could have contributed to the finding. Quirynen and Van Steenberghe³ confirmed the possibility of microbial leakage through the transmucosal abutment and the microgap between implant and abutment, particularly when the gap exceeded 30 μ m. In the Ankylos system, especially the implant–abutment connection, the connective tissue is wider and longer and the microgap is virtually absent, which could help protect against microbial invasion.

CONCLUSIONS

The soft tissue–implant interface is the key to the long-term success of an implant. The significance of that interface is enhanced by the fact that the presence of a microgap between the implant and the abutment represents a contamination site for bacteria. In the Ankylos system, the absence of the microgap, combined with a larger width and a higher length of connective tissue, could be more protective against microbial leakage and soft tissue inflammation.

Disclosure

The authors claim to have no financial interest in any company or product mentioned in this article.

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Abstract Translations [German, Spanish, Portuguese, Japanese]

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An Hunden durchgeführte Versuchsreihe zur histologischen Untersuchung der Ankylos-Implantate umlagernden Weichgewebsteile

ZUSAMMENFASSUNG: Zielsetzung: Zunehmend wird bei Implantationsbehandlungen das Augenmerk auf die Berührungsfläche zwischen dem das Implantat umlagernden Weichgewebe und dem Implantat selbst gerichtet, da man inzwischen um die Existenz von Minimalspalten zwischen Implantat und Stützzahn weiß, die eine ideale Brutstätte zur Bakterienbildung darstellen. Die vorliegende Studie zielte darauf ab, diese Berührungsfläche bei Verwendung des Ankylos Implantationssystems, das sich durch absoluten Schluss zwischen Implantat und Stützzahn ohne Spaltenbildung auszeichnet, anhand einer an Hunden durchgeführten Versuchsreihe zu untersuchen. **Materialien und Methoden:** Jedem der sechs zur Studie herangezogenen Labrador-Hunden wurden je zwei Ankylos Implantate eingepflanzt. Sechs Monate nach Stützzahnsetzung erfolgte die Einschläferung der Tiere. Es wurde weder eine funktionelle Belastung des Implantats noch eine untersuchende Kontrolle hinsichtlich Plaque-Bildung durchgeführt. Die histologische Untersuchung der Implantate wurde mittels Rasterelektronenmikroskop, Lichtmikroskop sowie durch histomorphologische Messungen vorgenommen. **Ergebnisse:** Manche der untersuchten Bereiche wiesen eine geringfügige Entzündungsneigung auf. Das Verbundgewebe zwischen den äußersten Epithelzellen des verbindenden Epithels und dem Alveolarkamm wurde von kollagenen Fasern durchzogen, die sich vom Periosteum und dem Alveolarkamm zum Epithel der Mundschleimhaut erstreckten. Das Gewebe war außerdem im vorderen Bereich des konusförmigen Stützzahns von einem schmalen Streifen extrazellulären Muttergewebes mit einigen durchlaufenden Kollagenfasern geprägt. **Schlussfolgerung:** Im direkten Vergleich zu den in anderen Studien unter Verwendung verschiedener Implantatsysteme (Astra, Bränemark, ITI) erzielten Ergebnissen wies das Ankylos System eine größere Kontaktlänge und -breite des verbindenden Gewebes sowie ein kürzeres Epitheltiefenwachstum auf. Die im verbindenden Gewebe der Berührungsfläche bei histologischer Untersuchung entdeckte geringfügige Entzündung erklärt sich voraussichtlich durch das Fehlen des Minimalspalts zwischen Implantat und Stützzahn durch Einsatz eines Implantats der Ankylos Serie.

SCHLÜSSELWÖRTER: Verbindungsepithel, verbindende Gewebsteile, histomorphologische Messungen, Minimalspalt

Análisis histológico de los tejidos suaves periimplantes Ankylos® en un modelo en perros.

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ABSTRACTO: PROPÓSITO: La importancia de la interfaz de tejido suave-implante es mejorada por la presencia de un microespacio entre el implante y el pilar que representa un lugar de contaminación con bacteria. El propósito de este estudio fue investigar la interfaz entre el Ankylos, un sistema de implante sin espacio, y los tejidos suaves que lo rodean en un modelo en perros. **MATERIALES Y MÉTODOS:** Seis perros labradores se incluyeron en el estudio y se insertaron 2 implantes Ankylos por perro. Los perros fueron sacrificados 6 meses después de la colocación del pilar sin carga funcional y sin control del sarro. Los implantes fueron analizados histológicamente con un microscopio de barrido electrónico, microscopio de luz y histomorfometría. **RESULTADOS:** Algunas secciones exhibieron señales histológicas de una suave inflamación. El tejido conectivo entre las células epiteliales más apical del epitelio de la unión y la cresta alveolar se caracterizó por fibras de colágeno que corrían desde el periostio y la cresta alveolar hacia el epitelio oral y, al frente del pilar con forma de cono, en una zona delgada de una matriz extracelular con algunas fibras de colágeno. **CONCLUSIÓN:** Comparados con los resultados obtenidos en otros estudios usando distintos tipos de implantes (Astra, Bränemark, ITI), el Ankylos demostró una longitud más alta y un ancho más grande de contacto con el tejido conectivo así como un crecimiento epitelial más corto. La ausencia del microespacio en el sistema Ankylos podría explicar la suave inflamación histológica en el tejido conectivo.

PALABRAS CLAVES: epitelio de la unión, tejidos conectivos, histomorfometría, microespacio

Análise histológica dos tecidos moles em um peri-implante Ankylos em um cachorro como modelo.

SUMÁRIO: PROPÓSITO: a importância da interface do implante de tecido mole é incrementada pela presença de uma micro abertura entre o implante e o suporte o que representa um local de contaminação para bactérias. O objetivo deste estudo foi investigar a interface entre o Ankylos, um sistema de implante sem abertura, e os tecidos moles circundantes em um cachorro como modelo. **MATERIAIS E MÉTODOS:** 6 cães raça Labrador foram incluídos no estudo e 2 implantes Ankylos foram inseridos por cachorro. Os cães foram sacrificados 6 meses depois da colocação do suporte sem a carga funcional e sem controle de placa. Os implantes foram analisados histologicamente utilizando-se um microscópio de elétrons, um microscópio leve e histomorfometria. **RESULTADOS:** algumas seções exibiram sinais histológicos de uma inflamação leve. O tecido conjuntivo entre as células epiteliais mais apicais do epitélio de conexão e a crista alveolar foi caracterizado por fibras de colágeno crescidas desde o periósteo e a crista alveolar em direção ao epitélio oral e, em frente do suporte em forma de cone, por uma zona estreita de matriz extracelular com algumas poucas fibras de colágeno. **CONCLUSÃO:** comparado aos resultados obtidos em outros estudos usando diferentes tipos de implante (Astra, Bränemark, ITI) o Ankylos mostrou um comprimento e largura maiores de contato de tecido conjuntivo bem como um encolhimento menor epitelial. A ausência de uma micro abertura no sistema Ankylos poderia explicar a leve inflamação histológica do tecido conjuntivo.

PALAVRAS CHAVE: epitélio de conexão, tecidos conjuntivos, histomorfometria, micro abertura

Ankylos®のインプラント周辺部軟部組織のイヌ・モデルにおける組織学的分析

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要約：

目的：軟部組織とインプラントの境界面が細菌汚染する問題は、インプラントとアパットメントの間に微細なギャップがある場合により深刻化する。本研究の目的は、ギャップ・フリーのインプラント・システムであるAnkylos®と周辺組織の境界面についてイヌ・モデルで調査することにあった。

素材と方法：6匹のラブラドル犬が使われ、各犬に2つのAnkylos®インプラントが挿入された。イヌは、機能的loadingとブラーク・コントロールを伴わないアパットメントの設置6か月後に殺された。各インプラントは走査電子顕微鏡、光学顕微鏡、組織形態計測による組織学的分析を受けた。

結果：断面標本の一部には、軽度の炎症の組織学的形跡が見られた。上皮接合部の歯根上皮細胞と歯槽陵の間の結合組織には、ほとんどの場合、骨膜と歯槽陵から口腔上皮にかけてのコラーゲン繊維と、さらに紡錘型アパットメントの前部ではコラーゲン繊維を少量伴う細胞外基質の細い带状部分とが、顕著に視測された。

結論：異なるインプラント材料（Astra、Bränemark、ITI）を使った他の研究結果との比較では、Ankylos®において結合組織接合部の幅・長さがより大きく、上皮downgrowthがより短いことがわかった。断面標本の一部には、軽度の炎症の形跡が見られた。結合組織の炎症がAnkylos®において組織学的に軽度であったことには、微細なギャップがなかったことが貢献している可能性が考えられる。

キーワード：上皮接合部、結合組織、組織形態計測、微細ギャップ

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