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Organization of the connective tissue barrier around long-term loaded implant abutments in man

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Abstract: The study aimed to investigate the connective tissue seal and the spatial organization of collagen fibers around long-term loaded implants in man. Block specimens containing smooth titanium implant abutments and the surrounding supracrestal connective tissue were obtained from patients rehabilitated for at least 1 year with implant-retained overdentures or implant-supported fixed prostheses and were histologically investigated. The histological features of the connective tissue around long-term loaded titanium abutments were specific: the tissue was rich in collagen fibers, organized in bundles, presenting a constant spatial arrangement, similar to that reported in animal studies. Circular fibers, the most numerous, were located externally, and longitudinal fibers internally. Radial fibers inserted on the abutment surface, similar to those of the periodontal system, were not observed in any case. No histological differences were found between tissue sampled around implants supporting a fixed restoration and those anchoring an overdenture.

It is now accepted that the biological seal around oral implants consists of two principal layers, the epithelial attachment and the underlying connective tissue barrier. Current knowledge of the histological and cytological aspects of these tissues is based on studies in dogs or nonhuman primates. The epithelial component of the implant–mucosa interface (IMI) has been demonstrated to form a cuff–like barrier which adheres to the surface of the titanium abutment (Abrahamsson et al. 1998; Berglundh et al. 1991; Berglundh et al. 1992; Berglundh et al. 1994; Ericsson et al. 1992). Histological studies suggest that peri-implant epithelium is differentiated in a similar way to the junctional epithelium (JE) around teeth and provides direct attachment of the epithelium to the titanium via hemidesmosomes (Arvidson et al. 1996; Berglundh et al. 1991; Lindhe & Berglundh, 1998).

The morphology and structure of the connective tissue barrier has also been investigated in animals (Berglundh & Lindhe, 1996; Moon et al. 1999). The connective tissue immediately next to the implant surface is characterized by an absence of blood vessels and abundant fibroblasts, which are interposed between thin collagen fibers. The connective tissue at a distance from the implant contains more abundant fibers, which may run in a variety of directions and appear to be functionally organized.

It is accepted that implant–mucosal interface-connective tissue (IMI-CT) is not identical to periodontal mucosal connective tissue (Romanos et al. 1995), and that IMI-CT may be formed as a scar-like response to implant surgery (Buser et al. 1992; Cochran et al. 1997). Regarding collagen fiber orientation, circular fibers have frequently been reported around the abut-

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ment. In a study on beagle dogs, Berglundh and coworkers (Berglundh et al. 1991) also reported collagen fibers running in an apico-coronal direction. Similar findings have been reported by Listgarten (Listgarten et al. 1991) and Chavrier (Chavrier et al. 1994). Experiments in monkeys (Schroeder et al. 1981) suggest that when the abutment surface contains porosities, for example around titanium plasma-sprayed abutments, radially oriented fibers resembling dentogingival fibers will also form.

Few studies (Arvidson et al. 1996) have investigated human peri-implant soft tissue, in normal clinical condition of functional loading, and they have not described the organization of the collagen fibers.

The major objective of the present study was to histologically evaluate the human connective tissue around smooth titanium abutments, using clinically healthy implants that had been in function for at least 12 months. Special consideration was given to the spatial orientation of the collagen bundles. A secondary aim was to evaluate whether the type of loading (overdenture or fixed prosthesis) influenced the characteristics of the implant–mucosal interface.

Material and methods

Seven adult patients (six women and one man, age range 52–75 years, mean age 62 years) were included in the study. Five were totally edentulous patients rehabilitated with mandibular overdenture (OVD) retained by two Brånemark™ () implants with ball attachments, and two patients presented partial bilateral mandibular edentulism in the molar region rehabilitated with mandibular implants supporting fixed prostheses. Tissue surrounding nine implant abutments was investigated on one side only of the five edentulous patients and bilaterally in the fixed prosthesis group. Seven out of nine implant abutments were surrounded completely by keratinized mucosa, while two implants showed buccally mobile mucosa. All patients gave written informed consent to the procedure, for which ethical approval had been obtained.

At the time of biopsy, the clinical and radiographic appearances of all implants were normal with regard to osseointegration and correct prosthetic fit. Clinical evaluation, by evaluating pocket depth, bleeding and plaque index (Silness et al. 1964), and morphology of peri-implant mucosa revealed no signs of inflammation or peri-implantitis in any case included in the study.

With patients under local anesthesia (Carbocaine™ 3% without adrenalin) (a cut, using a number 15 scalpel, was made through the tissue down to the periosteal layer all around the abutment at 2 mm; the abutment screw was removed; the abutment plus mucosa was carefully removed en bloc using abutment pincers, an identical abutment was inserted to the implant for continued prosthetic treatment. Local anesthetic was administered in all cases at least 1 cm from the biopsy site so that results would not be compromised. Specimens were fixed in 10% buffered formaldehyde, then washed in phosphate buffer, dehydrated in alcohol and included in acrylic resin (Kulzer, Germany). Ground sections (100 μm) were obtained by using of a cutting-grinding apparatus (Exakt, , Germany), oriented either transversally (4 specimens, 2 OVD + 2 fixed prosthesis) or longitudinally (5 specimens, 3 OVD + 2 fixed prosthesis) to the implant axis. Sections were examined on a phase-contrast microscope for evaluation of collagen bundles, then stained with hematoxylin–eosin for routine microscopic observation.

Observation were performed on a Leitz Dialux microscope (Leitz,) connected through a CCD camera to a PC-based image analyzer.

The localization and spatial orientation of collagen fiber bundles were analyzed on the two clearest sections of each specimens. For each section the evaluation began from the abutment surface along 1000 μm for longitudinal cuts, and along 1200 μm for transversal cuts, using a grid 200 × 200 μm in size. The count was done twice for each section, on opposite sides of

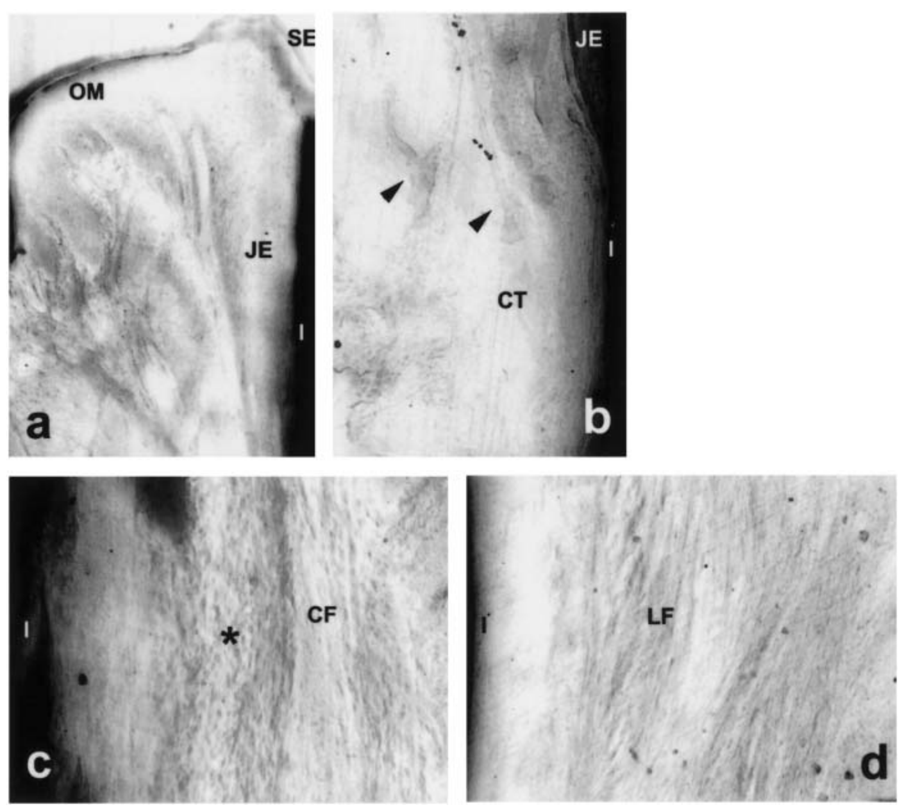

Fig. 1. (a) Normal keratinized epithelium (OM), a thin sulcular epithelium (SE) and inflammation-free junctional epithelium (JE) around the titanium abutment (I). Phase contrast microscope, original magnification ×10. (b) Connective tissue barrier (CT) below the level of the junctional epithelium (JE). Some vessels are present (arrows). Phase contrast microscope, original magnification ×15. (c) In transversal sections, a dense network of circular fibers (CF), the most frequently found fibers in all samples, may be observed 200–800 μm from the titanium surface. A cell-rich connective tissue (*) is present closer to the abutment surface. (d) Longitudinal fibers (LF), in smaller numbers, observed in longitudinal sections in the first 200 μm from the abutment surface. Phase contrast microscope, original magnification ×25.

the abutment. The density of the structures identified was expressed by on semi-quantitative scale (absence of fibers [−]; very occasional fibers [±]; occasional fibers [+]; frequent fibers [++]; abundant fibers[+++]).

Information on the spatial orientation of collagen fibers bundles was used to construct a 3D graphic model on a CAD system (IBM-Intelli Station, Pro-Engineer 2000i program,). The model reproduced the localization and the relative distance from the titanium surface of the variously orientated collagen fibers bundles on the basis of microscopic observations, while the size of each collagen bundle was set arbitrarily for optimal model visualization.

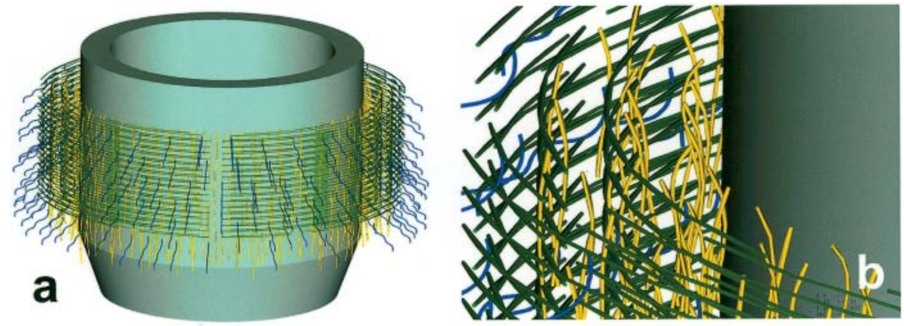

Fig. 2. (a) Three-dimensional graphic representation of the microscopic data relative to collagen fiber distribution: (I) longitudinal fibers are observed in the inner 200 μm (yellow), (II) circular fibers between 200 and 800 μm (green), and (III) oblique bundles externally (blue). (b) Detail from Fig. 2a showing the speculative disposition of different collagen fiber systems.

Results

A normal keratinized mucosa continuing in a thin sulcular epithelium was observed around the titanium abutment in all the cases considered (Fig. 1a). No specimen presented histological signs of peri-implantitis or gingival pocket formation (Fig. 1a,b).

The spatial distribution of collagen bundles in the longitudinal and transverse sections is reported in Table 1.

Inflammatory cells were occasionally observed, in small numbers below the epithelium, inside the connective tissue around the abutment. In all the specimens, connective tissue (Fig. 1b) presented a high density of cells in the first 200 μm adjacent to the abutment and a lower density thereafter, while collagen bundles were abundant all around the implant, with a maximum density of between 200 and 800 μm from the abutment surface. A small number of blood vessels could be observed both very close to the abutment surface and distant from the implant, but not in the area dominated by collagen bundles.

Phase-contrast microscopy revealed that the collagen bundles were not randomly oriented but rather organized in three major systems (Fig. 1c,d; Table 1):

1. circular fibers, the commonest type in all samples, which were usually observed in transversal sections 200–800 μm from the titanium surface, and only in some cases in close proximity to the abutment surface;
2. longitudinal fibers, in smaller numbers, observed in longitudinal sections in the first 200 μm from the abutment surface;
3. oblique fibers, in small separate bundles, which could be observed externally to the above systems, with variable orientation and connecting the inner fibers with the submucosa and periosteum.

Radially oriented fibres were never seen in the inner area close to the abutment surface. The computer graphic representation of collagen bundles around the titanium abutment is shown in Fig. 2 (a,b).

No histological difference was detected between abutments supporting fixed prostheses and those supporting MIR-OVD, nor was there any difference related to the presence or absence of attached gingiva.

Table 1. *Spatial distribution of collagen fibers. Distances are expressed from the abutment surface. The longitudinal and oblique fibers were observed only up to 1000 μm (*). Absence of fibers (–); very occasional fibers (±); occasional fibers (+); frequent fibers (++); abundant fibers (+++)*

	0–200 μm	200–400 μm	400–600 μm	600–800 μm	800–1000 μm	1000–1200 μm
Circular fibers	+–	++	+++	++	–	–
Longitudinal fibers	+++	++	+	–	–	*
Oblique fibers	–	–	–	+	++	*

Discussion

This study found that in healthy clinical conditions of human beings, collagen fibers of the peri-implant connective tissue barrier are spatially oriented, with an inner system dominated by longitudinal fibers and a more extensive external circular system. This collagen 'cuff' appears to be linked to the periosteum and the submucosa by means of oblique bundles. The morphology is substantially constant, and was similar in most tissue specimens; it did not appear to depend on the type of prosthetic rehabilitation, nor on the presence of attached gingiva. The histological organization is strictly comparable to that observed in chronic beagle dog models (Berglundh et al. 1991; Lindhe & Berglundh, 1998).

The presence of a dominant circular system of collagen fibers around the abutment is in accordance with the concept of peri-implant 'circular ligament' proposed by Ruggeri (Ruggeri et al. 1992), and confirmed by Piattelli (Piattelli et al. 1997). The latter study reported a limited number of circular fibers, located coronally along the abutment, with only longitudinal fibers in lower strata. We observed a prevalence of circular fibers at all levels, from the bone crest up to the JE. As far as we have been able to determine, the type of prosthetic rehabilitation, and thus of functional loading on the implant itself and on the surrounding mucosa, has never been taken into consideration in investigating the connective tissue differentiation around the implant. Nor has the time of functional loading been evaluated, although it may be an important determinant. Indeed, the organization of collagen fibers that we observed may be related to the long period of functioning of the implants. Thus differences between this and other observations could be time-dependent; the type of prosthesis does not seem to be relevant.

The three-dimensional graphic representation of the microscopic data, though arbitrary and observer-dependent, suggests the presence around titanium implant abutments of a differentiated network of fibers, which might be of clinical relevance as a mechanical protection for the underlying bone–implant interface. The organization differs from that of the periodontal connective tissue, as radial fibers appear to be lacking. The histological methods used may not precisely detect the interface between the titanium and the surrounding soft tissues, nor may they detect small amounts of collagen fibers. Thus the presence of some collagen fibers radiating out from the abutment surface cannot be excluded; if they exist, however, their numbers must be very low, as such bundles were never observed. The presence of radial fibers has been described (Schroeder et al. 1981) as specific to rough titanium surfaces. The present results do not exclude this hypothesis, as only smooth titanium abutments were considered.

Résumé

Cette étude a eu pour but de vérifier le scellement du tissu conjonctif et l'organisation spatiale des fibres collagène autour des implants chargés depuis longtemps chez l'être humain. Les spécimens contenant le pilier en titane lisse et le tissu conjonctif sus-crestal avoisinant ont été prélevés chez des patients qui avaient été traités depuis au moins une année pour des prothèses amovibles sur implants ou des prothèses fixées sur implants, et analysés histologiquement. Les caractéristiques histologiques du tissu conjonctif le long des piliers en titane étaient spécifiques : le tissu était riche en fibres collagène, organisé en bandes, présentant un arrangement spatial constant semblable à celui rapporté dans les études animales. Les fibres circulaires, les plus nombreuses, étaient présentes à l'extérieur et les fibres longitudinales à l'intérieur. Des fibres radiales insérées sur la surface de l'implant, semblables à celles présentes dans le système parodontal, n'ont jamais été observées. Aucune différence histologique n'a été trouvée entre le tissu échantillonné autour des implants avec prothèse amovible et avec prothèse fixée.

Zusammenfassung

Es war das Ziel dieser Studie, das Bindegewebssiegel und die räumliche Organisation der Kollagenfasern um langzeitbelastete Ïmplantate bei Menschen zu untersuchen. Blockbiopsien, welche glatte Ïmplantataufbauzylinder aus Titan und das umgebende suprakrestale Bindegewebe enthielten, wurden bei Patienten entnommen, welche vor mindestens 1 Jahr mit implantatgetragenen Hybridprothesen oder mit implantatgetragenen festsitzenden Rekonstruktionen versorgt worden waren. Bie Biopsien wurden histologisch untersucht.
Die histologischen Charakteristiken des Bindegewebes um langzeitbelastete Titanaufbauzylinder waren spezifisch: das Gewebe war reich an kollagenen Fasern, organisiert in Bündeln, welche eine konstante räumliche Anordnung zeigten, ähnlich der in Tierstudien berichteten Anordnung. Zirkuläre Fasern, der Hauptanteil der Fasern, waren extern lokalisiert. Longitudinale Fasern befanden sich intern. Radiale Fasern, welche ähnlich wie Fasern des parodontalen Systems an der Oberfläche der aufbauzylinder ansetzen, konnten in keinem Fall beobachtet werden. Zwischen den Gewebeproben von Ïmplantaten mit fixen Rekonstruktionen und von Ïmplantaten, auf welchen Hybridprothesen verankert waren, konnten keine histologischen Unterschiede gefunden werden.

Resumen

El studio intentó investigar el sellado de tejido conectivo y la organización espacial de fibras colágenas alrededor de implantes cargados a largo plazo en el hombre. Se obtuvieron especimenes en bloque confeniendo pifares de titanio liso de implantes y el tejido conectivo circundante supracrestal de pacientes rehabilitados por lo menos un año con sobredentaduras implantorretenidas o prótesis fijas implantosoportadas y se investigaron histológicamente.
Las caracteristicas histológicas del tejido conectivo alrededor de pilares de titanio cargados a largo plazo fueron especificas: el tejido fue rico en fibras colágenas, organizada en recimos, presentando una distribución espacial constante, similar a la reportada estudios animales. Se localizaron fibras circulares, las más numerosas, externamente y las fibras longitudinales internamente. No se observaron en ningúin caso fibras radiales insertadas en la superficie del pilar, similares a aquellas del sistema periodontal. No se encontraron diferencias histológicas entre el tejido muestreado alrededor de implantes soportando una restauración fija y aquellos anciando una sobredentadura.

要旨

本研究は、ヒトにおいて長期に荷重を受けていたインプラント周囲の結合組織による封鎖とコラーゲン繊維の空間的配列を調べた。インプラント・オーバーデンチャーあるいはインプラント義歯を少なくとも1年間装着してきた患者から、滑沢なチタン製インプラント・アバットメントとその周囲の歯槽頂線上の結合組織を含むブロック標本を採取して、組織学的検査を行った。長期に荷重を受けてきたチタン製アバットメント周囲の結合組織は明らかな組織学的特徴を示した：組織はコラーゲン繊維が豊富で、束状に配列し、一定の空間的配置は、動物研究で報告されているそれと類似していた。環状繊維が最も多数であり外側に位置しており、縦走繊維は内側に配置していた。歯周組織の場合と類似した、アバットメント表面に付着する放射状繊維は、全く観察されなかった。
固定式補綴物を支持するインプラントとオーバーデンチャーを支持するインプラントの間には、組織標本に組織学的な差異はなかった。

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