Soft Tissue Attachment to Titanium Implants Coated with Growth Factors

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[Correction added after online publication 6 December 2012: author name corrected to P. Mark Bartold]

ABSTRACT

Background: Enhancing the connective tissue seal around dental implants may be an important factor in implant survival.

Purpose: The objective of the study was to investigate the effect of implant surface modification with either platelet-derived growth factor (PDGF) or enamel matrix derivative (EMD) on connective tissue attachment to titanium implants.

Materials and Methods: Eighteen implants (Branemark® Mk III Groovy NP (3.3 mmØ × 10 mm, Nobel Biocare) were implanted subcutaneously into 12 rats. Six implants each were coated with either PDGF or EMD immediately prior to implantation and six implants were left uncoated. Implants were retrieved at 4 and 8 weeks and assessed histologically to compare the soft tissue adaptation to the implant surfaces.

Results: Ingrowth by soft connective tissue into the threads of all implants was noted at 4 and 8 weeks. Coating with growth factors did not alter the orientation of fibroblasts and collagen fibers. The depth of connective tissue penetration into the implant grooves was significantly greater for the implants coated with PDGF at 4 weeks. The thickness of the connective tissue in growth was significantly less for the implants coated with PDGF at 8 weeks.

Conclusion: Coating of the implant surface with rhPDGF-BB or EMD can increase the speed and quantity of soft tissue healing around the implant surface.

KEY WORDS: connective tissue, growth factors, tissue seal

INTRODUCTION

The peri-implant soft tissues form a crucial seal between the oral environment, the bone, and the implant surface.[1,2] This seal is fragile because of the absence of supracrestal connective tissue attachment (as seen with teeth) and presence of an epithelial attachment of variable length. When subjected to bacterial or mechanical challenge, the destruction of peri-implant soft connective tissues can be a faster and more devastating process than in periodontal tissues.[3,4] Thus, enhancing the seal formed by the peri-implant soft tissues, especially that of the titanium/connective tissue interface may be an important factor in implant survival.

Surface modification of titanium implants may improve the ability of connective tissue components in the peri-implant mucosa to attach to the implants. Currently, most dental implant types incorporate a "roughened" surface as part of their macro-design. Many of these surfaces are able to absorb proteins and thus act as a reservoir, or carrier, for attachment proteins, growth factors, and other biological agents which may be of assistance for soft or hard tissue integration. In vitro studies have shown that epithelial cell adhesion to titanium surfaces coated with biological agents such as fibronectin, laminin, and collagen was enhanced in comparison with uncoated titanium[5–9].

Platelet-derived growth factor (PDGF) is secreted locally during clotting by blood platelets at the site of

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DOI 10.1111/j.1708-8208.2010.00327.x

soft- or hard-tissue injury and it stimulates a cascade of events that leads to the wound-healing response. PDGF has also been shown to influence periodontal ligament fibroblast migration, proliferation, and synthetic activity.[10–12] Improvement in periodontal wound healing has been observed after applying PGDF-BB, leading to significant bone, cementum, and periodontal ligament regeneration.[13–15] These results clearly illustrate the beneficial effect of PDGF on both soft and hard tissue healing and regeneration.

Enamel matrix derivative (EMD) proteins, of which 90% are amelogenins, are secreted during tooth development and play a crucial role in the formation of acellular root cementum[16–18]. These proteins are thought to induce the formation of the periodontal attachment during tooth formation, and it is believed that EMD used in treatment of periodontal defects mimic the development of the tooth-supporting apparatus.[19] Apart from its original use as an agent to enhance and promote periodontal regeneration, EMD has also been reported to be effective in the management of recession defects by enhancing soft tissue adherence to exposed root surfaces.[20–24]

A number of studies have examined the changes in soft tissue level after implant placement.[25–27] Despite significant differences in experimental designs, the majority of studies conclude that gingival recession between 0.6 mm to 1.5 mm is unavoidable. While multiple factors can influence gingival recession around transmucosal dental implants, there is little doubt that the amount of connective tissue attachment to implant surfaces is important.[28] Various methods have been proposed to improve the quality of the soft tissue interface, including micro and macro design features of the transmucosal portion of the implant.[29,30]

In light of the above, we have hypothesized that surface modification of roughened surface (TiUnite) titanium implants surface with PDGF or EMD results in improved connective tissue bioactivity of the implant surface, thereby promoting cell attachment and connective tissue formation, which is expected to result in an improved soft tissue attachment to the implant surface. Accordingly, the aim of this study was to investigate if surface modification of roughened surface (TiUnite) titanium implants with PDGF or EMD has the potential to enhance connective tissue attachment to titanium implants.

MATERIALS AND METHODS

Animals

Twelve female Dark Agouti (DA) rats between 6 to 8 weeks old were used. These were acquired through the Animal Services Division, Institute of Medical and Veterinary Science (IMVS), Adelaide. The research protocol related to the use of animals in this study was approved by the Animal Ethics Committees of both the University of Adelaide and the Institute of Medical and Veterinary Science.

Implants

Eighteen Branemark System® Mk III Groovy NP (3.3 mmØ × 10 mm) (Nobel Biocare AB, Göteborg, Sweden) implants were used. Six test implants were coated with enamel matrix protein derivative (Emdogain®, Straumann, Malmö, Sweden), six test implants were coated with reconstituted recombinant human PDGF-BB (rhPDGF-BB, Pepro Tech, Rocky Hill, New Jersey, USA), and six control implants were uncoated.

Preparation of Growth Factors and Coating of Implants

A commercially available enamel matrix protein derivative (Emdogain® (Lot no. E1636A), Straumann, Malmö, Sweden) with a concentration of 30 mg/mL in a propylene glycol alginate carrier was purchased. Recombinant human PDGF-BB (rhPDGF-BB) was purchased from Pepro Tech (Rocky Hill, New Jersey, USA) and 500 μg was reconstituted in 1.67 mL of sterile saline in accordance to the manufacturer's instruction to produce a rhPDGF-BB concentration of 0.3 mg/mL and stored at 4°C until ready for use. This concentration is the same as GEM 21S® (Osteohealth, Shirley, New York, USA), a commercially available rhPDGF-BB used in conjunction with β-TCP in periodontal regenerative therapy. Immediately prior to implantation, the implants were immersed in Emdogain® (30 mg/mL), PDGF (0.3 mg/mL), or sterile saline for 30 seconds. This resulted in a clearly visible wet coating of the implant surface. The amount of PDGF-BB or Emdogain bound to the implant surface could not be quantitated. The coated implants were then placed into the surgical sites.

Experimental Groups

Three experimental groups were studied (EMD, PDGF, and Saline) at two time points (4 and 8 weeks). In each

group and for the two time points selected, one animal received two implants with the remaining animal receiving a single implant. A total of 18 implants were used.

Surgical Procedures

All surgical procedures were performed using inhalation anesthesia induced with 2% v/v isofluorane with O_2 flow rates set at 2 L/minute. Following the administration of anesthesia, a subcutaneous incision measuring approximately 20 mm was made along the dorsal midline between the left and right shoulders. A subcutaneous pouch below either the right or left shoulder was created for the placement of the implants. If two implants were to be placed, then pouches were created below the left and right shoulder. After the implants were secured in their positions, the incision was closed using wound autoclips and swabbed with Betadine. Postoperatively, the rats were administered 0.3 mg/mL enrofloxacin (Baytril ® 25, Bayer AG, Leverkusen, Germany) orally for 1 week. The autoclips were removed 2 weeks after implant placement and the rats were monitored daily and weighed weekly during the healing period.

Implant Retrieval

The rats were euthanized by CO_2 asphyxiation and the implants were located through implantation records and palpation. For implant retrieval, a similar but larger dorsal incision was made and the implant retrieved with a sample of surrounding tissue. The retrieved samples were placed in a fixative (10% phosphate buffered saline buffered formalin) for 48 hours prior to processing into resin blocks.

Resin Embedding

The retrieved implant/tissue biopsies were transferred from the fixative and dehydrated in serial steps of alcohol concentrations and subsequently embedded in a methyl-methacrylate resin (Merck Schuchardt OHG, Hohenbrunn, Germany). The resulting resin embedded implant/tissue blocks were cut using an Isomet slow-speed diamond saw (Beuhler, IL, USA) along the long axis of the implant and maximizing the volume of surrounding tissue to obtain two central sections. The distal portions of the implant/tissue blocks were cut along the same axis to create a resin block with parallel surfaces.

Thin Sections

Six implant/tissue resin embedded block sections representative of each coated and control group were selected for thin sectioning. These were further sectioned using an Isomet slow-speed diamond saw to 100-μm sections and mounted with an adhesive on a clear Perspex slide. The sections were polished with progressively finer silicon carbide abrasive discs mounted on a Abramin micro-grinding system (Struers, Denmark) with the final polish using diamond paste to achieve a specimen thickness of 14–18 μm as previously described[31] and measured by a micrometer (Moore & Wright, Sheffield, England). The sections were imaged on confocal microscopy and then stained with hematoxylin and eosin (H&E).

Confocal Laser Scanning Microscopy Analysis

Confocal laser scanning microscopy of the sectioned implant/tissue resin embedded blocks was carried out using a Leica TCS SP5 Confocal Microscope System (Leica Microsystems, Heidelberg, Germany).[32] The implant/tissue block sections were viewed using a ×20 IMM objective lens (magnification of ×200) on a Leica DMI6000B inverted microscope (Leica Microsystems, Heidelberg, Germany), using the argon-neon laser set at a power setting of 40% and emitting at a wavelength of 458 nm, allowing confocal laser scanning microscopic analyses of collagen autofluorescence. The filter cube on the microscope was set for blue fluorescent light for excitation of the green fluorophores. The threads and groves for the length of the implant to the edge of the fibrous in growth were imaged and were captured using the LAS AF software (Leica Microsystems, Heidelberg, Germany).

Histological Assessment

The sections were stained with hematoxylin and eosin and viewed under a light microscope (Olympus BH-2 Research microscope, Olympus Australia, Mount Waverly, VIC, Australia) connected to a 2 megapixel digital CMOS color camera (Altra20, Soft Imaging System, Gulfview Heights, SA, Australia). The length of each implant was viewed histologically to assess a number of parameters to determine the tissue reaction around each implant. This part of the study was qualitative only and no attempt was made to quantitate the general histological observations. Inflammation was recorded when inflammatory cells were found within the immediate vicinity of the implanted materials. Acute inflammation was recorded if a predominantly polymorphonuclear leucocyte cell infiltrate was detected

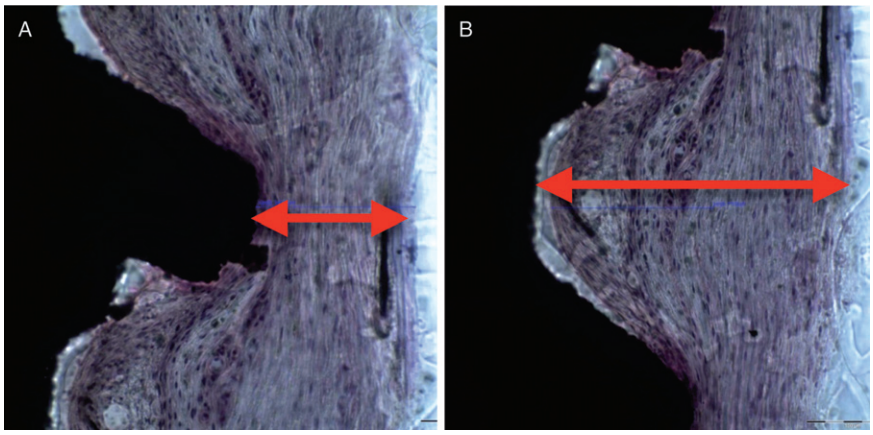

Figure 1 Measurement of (A) thickness of fibrous tissue and (B) depth of connective tissue penetration.

around the implanted material. Chronic inflammation was recorded when the cell infiltrate consisted predominantly of plasma cells, monocytes/macrophages, or lymphocytes. The distribution and density of fibrous tissue around the implant and within the implant threads was recorded and subjected to histomorphometric analyses (see next section). The distribution of vasculature was noted by the presence of blood vessels around the implants and the presence of adipose tissue surrounding the implants was also noted.

Histomorphometric Measurements

The measurement of the thickness of the connective tissue layer was taken from the apex of the implant threads of the first 10 threads from the implant collar on both the left and right hand sides. The measurement of the depth of connective tissue penetration into the implant grooves were taken from the first 10 grooves from the implant collar on both the left and right hand sides. Measurement of the thickness of fibrous tissue in growth was taken at the apex of the implant threads (Figure 1A) and the depth of connective tissue penetration into the implant grooves (Figure 1B). The depth of penetration was measured perpendicularly from an imaginary line connecting the apices of two adjacent threads to the maximum depth of tissue penetration into the implant groove. The measurements were carried out at a magnification of ×200 in an Olympus BH-2 bright-field microscope (Olympus Optical Co. Ltd, Tokyo, Japan) equipped with an image system Altra 20 color camera (Olympus Soft Imaging Solutions, Munster, Germany) and ANALYSIS imaging software package.

Statistical Analysis

Mean values for each variable were calculated for each implant unit. The differences within the 4- and 8-week groups of control and coated implants were analyzed using a one-way analysis of variance (ANOVA) and only proceeded with post hoc comparisons when the one-way ANOVA was found to be statistically significant ($p < .05$). Statistical analysis was carried out using a Graph Pad Prism 5 for Windows statistical software package (Graph Pad Software Inc., La Jolla, CA, USA).

RESULTS

Histological Assessment – Qualitative Analysis at 4 Weeks

Fibrous tissue ingrowth into the threads of the control and coated implants was evident after 4 weeks. The connective tissue layer adherent to the implants and the surrounding tissues were well-organized with little indication of any residual inflammation. The cellular and fibrous arrangement around the implants seen under confocal microscopy was identical when viewed under light microscopy using H&E stained sections. In all sections, there was some separation (considered to be artifactual as a result of the tissue sectioning) between the connective tissue layer and a thin cellular layer on the implant surfaces.

For the control uncoated implants viewed under confocal microscopy (Figure 2A), collagen autofluorescence indicated that the collagen fibers were aligned parallel with the long axis of the implant. The same thin sections, when stained with H&E, showed a dense

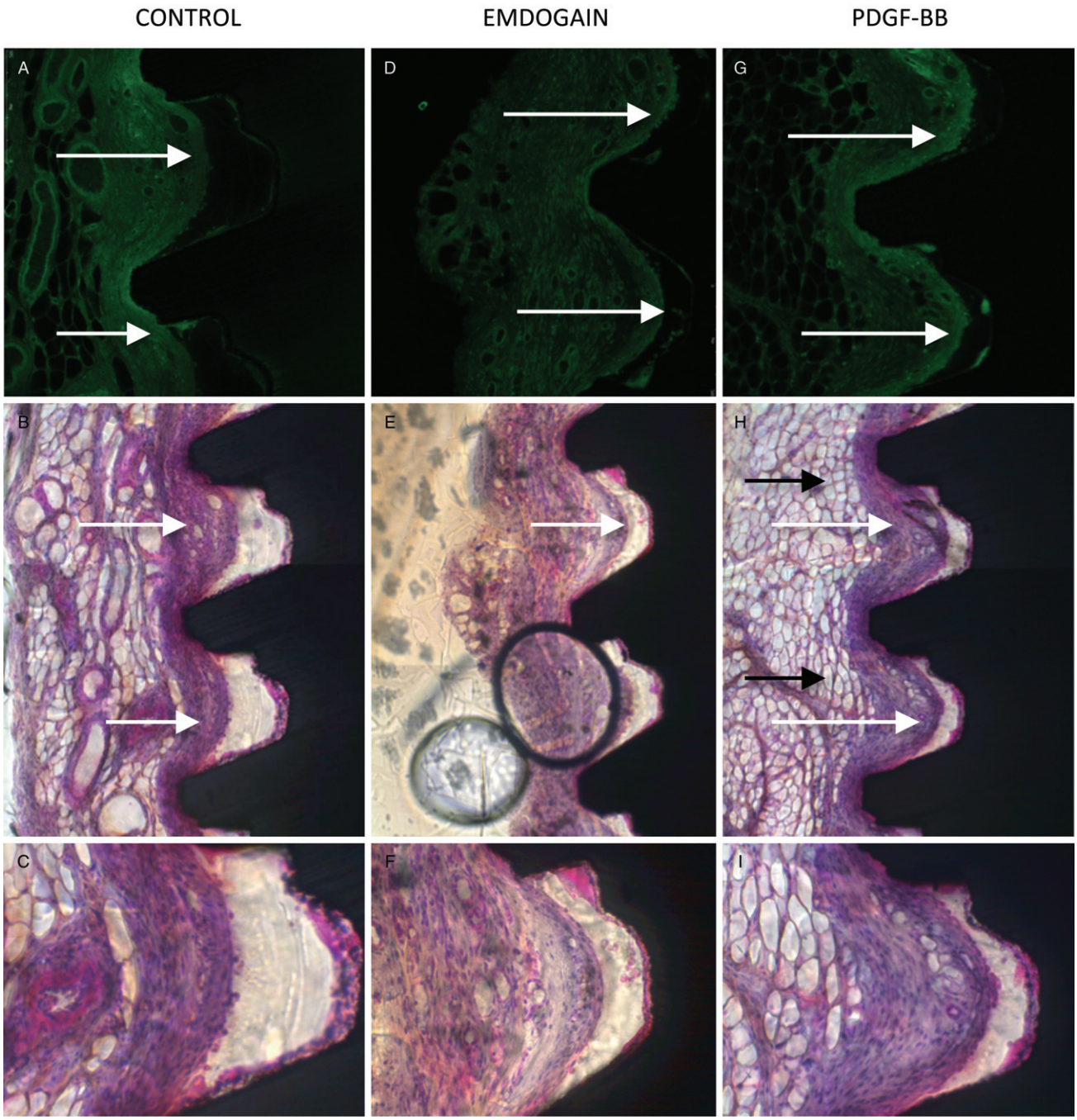

Figure 2 Histologic appearance of the implants and surrounding tissues at 4 weeks. (A) Control (uncoated) implant. Thin section (16 µm/unstained), viewed under confocal microscopy. Autofluoresnce of collagen fibers indicated by arrows. Original magnification ×200. (B) Control (uncoated) implant. Thin sections (16 µm/H&E stained), light microscopy, fibrous capsule shown by white arrows. Original magnification ×200 (composite image). (C) Control (uncoated) implant. Thin section (16 µm/H&E stained), light microscopy, original magnification ×200 (enlarged image). (D) Emdogain®-coated implant. Thin section (16 µm/unstained), viewed under confocal microscopy. Autofluoresnce of collagen fibers indicated by arrows. Original magnification ×200. (E) Emdogain®-coated implant. Thin sections (16 µm/H&E stained), light microscopy. Fibrous capsule shown by white arrow. Original magnification ×200 (composite image). (F) Emdogain®-coated implant. Thin section (16 µm/H&E stained), light microscopy, original magnification ×200 (enlarged image). (G) rhPDGF–BB-coated implant at 4 weeks of healing. Thin section (16 µm/unstained), viewed under confocal microscopy. Autofluoresnce of collagen fibers indicated by arrows. Original magnification ×200. (H) rhPDGF–BB-coated implant. Thin sections (18 µm/H&E stained), light microscopy. Fibrous capsule shown by white arrows. Adipose tissue shown by black arrows. Original magnification ×200 (composite image). (I) rhPDGF–BB-coated implant. Thin section (18 µm/H&E stained), light microscopy, original magnification ×200 (enlarged image). PDGF = platelet-derived growth factor.

distinct layer of fibroblasts over the implant threads and suspended over the implant grooves, surrounded by less dense connective tissue (Figure 2B). The fibroblasts also were aligned parallel to the long axis of the implant. A thin (1–3 cells thick) but distinct cellular layer, in intimate contact with the surface of the implant grooves, that autofluoresced for collagen was evident (Figure 2A).

The collagen fiber orientation and fibroblast alignment observed in the coated implants at 4 weeks was no different to that reported for the uncoated implants when viewed under confocal and light microscopy. However, from this qualitative histological analysis, there was a greater depth of connective tissue penetration into the implant grooves with the Emdogain® and PDGF-coated implants and a thicker dense cellular/connective tissue layer with the Emdogain® coated implants (Figure 2, D–F).

The presence of an adipose-like cell layer almost devoid of other cell types surrounding the dense connective tissue layer was a distinctive feature of the rh-PDGF-BB coated implant at 4 weeks (Figure 2, G–I).

Histological Assessment – Qualitative Analysis at 8 Weeks

When viewed at 8 weeks, neither the controls nor the growth factor-coated implants showed any distinct change in collagen fiber orientation or fibroblast alignment when viewed under confocal and light microscopy. The collagen fibers and fibroblasts were still aligned parallel to the long axis of the implants (Figure 3).

For the control implant at 8 weeks, there was a greater depth of connective tissue penetration into the implant grooves than at 4 weeks; however, a variable thickness of the connective tissue layer was noted around the implant, with some areas having a thick dense connective tissue layer, but in other areas, the dense connective tissue layer remained thin (Figure 3, A–C). A layer of adipose tissue was noted adjacent to the fibrous tissue surrounding the control implants.

The Emdogain®-coated implant at 8 weeks also showed penetration of connective tissue into the implant grooves. There was also a dense connective tissue layer formed over the threads of the implants (Figure 3, D–F). The connective tissue layer was well organized with densely packed collagen fibers. Less adipose tissue (compared with the control and PDGF-coated implants was noted for the Emdogain®-coated implants.

The rhPDGF–BB-coated implant at 8 weeks also showed clear penetration of fibrous connective tissue into the implant grooves. In addition, rhPDGF–BB-coated implants, were also surrounded by adipose-like tissue which was more pronounced than for the controls (Figure 3, G–I).

Histomorphometric Measurements

The mean thicknesses of the connective tissue layer at implant threads 1–10 for the uncoated, Emdogain® and rhPDGF–BB-coated implants were (mean ± SD) 155.9 ± 102.0 μm, 103.7 ± 58.2 μm, and 139.2 ± 86.3 μm, respectively, at 4 weeks and 100.9 ± 98.7 μm, 110.6 ± 40.7 μm, and 37.5 ± 32.2 μm at 8 weeks (Figure 4).

One-way ANOVA found that there were no significant differences between the thicknesses of the connective tissue layer at the implant threads between the uncoated and coated implants at 4 weeks ($p = .1431$). However at 8 weeks, a significant difference in the thicknesses of the connective tissue layer ($p = .0012$) was detected. Further post hoc testing of the groups using Bonferroni's multiple comparison test statistically confirmed that the rhPDGF–BB-coated implant had a significantly thinner connective tissue layer at the implant threads than the uncoated ($p = .0029$) and Emdogain® coated ($p = .0007$) implants. There was no significant difference in the thickness of the connective tissue layer between the Emdogain®-coated and uncoated implants at 8 weeks ($p = .6356$).

There was no significant change in the thicknesses of the connective tissue layer for the uncoated and Emdogain®-coated implants between 4 and 8 weeks ($p = .6356$). This was not the case for the rhPDGF–BB-coated implants where the thickness of the connective tissue layer significantly decreased between 4 and 8 weeks ($p < .0001$).

The mean depth of connective tissue penetration into implant grooves 1–10 for the uncoated, Emdogain® and rhPDGF–BB-coated implants was 127.2 ± 24.5 μm, 131.5 ± 22.4 μm, and 156.9 ± 30.5 μm, respectively, at 4 weeks and 165.4 ± 25.4 μm, 174.0 ± 17.9 μm, and 170.1 ± 25.51 μm at 8 weeks (Figure 5).

One-way ANOVA detected a significant difference depth of connective tissue penetration into the implant grooves at 4 weeks ($p = .0014$). Once again, using Bonferroni's multiple comparison test as a post hoc test, the depth of connective tissue penetration into the implant grooves was found to be significantly greater for the

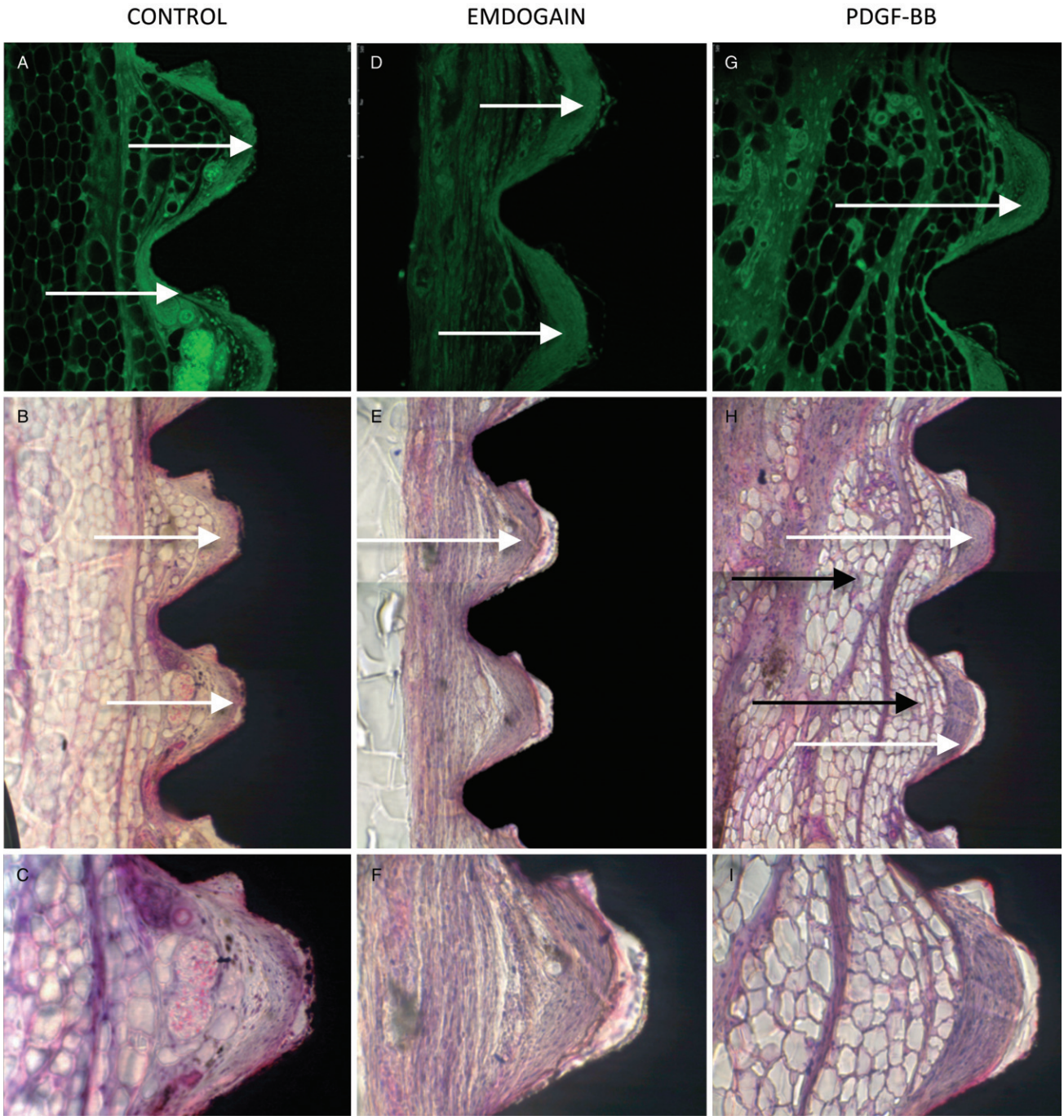

Figure 3 Histologic appearance of the implants and surrounding tissues at 8 weeks. (A) Control (uncoated) implant. Thin section (16 µm/unstained), viewed under confocal microscopy. Autofluoresnce of collagen fibers indicated by arrows. Original magnification ×200. (B) Control (uncoated) implant. Thin sections (16 µm/H&E stained), light microscopy, fibrous capsule shown by white arrows. Original magnification ×200 (composite image). (C) Control (uncoated) implant. Thin section (16 µm/H&E stained), light microscopy, original magnification ×200 (enlarged image). (D) Emdogain®-coated implant. Thin section (16 µm/unstained), viewed under confocal microscopy. Autofluoresnce of collagen fibers indicated by arrows. Original magnification ×200. (E) Emdogain®-coated implant. Thin sections (16 µm/H&E stained), light microscopy. Fibrous capsule shown by white arrow. Original magnification ×200 (composite image). (F) Emdogain®-coated implant. Thin section (16 µm/H&E stained), light microscopy, original magnification ×200 (enlarged image). (G) rhPDGF–BB-coated implant. Thin section (16 µm/unstained), viewed under confocal microscopy. Autofluoresnce of collagen fibers indicated by arrows. Original magnification ×200. (H) rhPDGF–BB-coated implant. Thin sections (18 µm/H&E stained), light microscopy. Fibrous capsule shown by white arrows. Adipose tissue shown by black arrows. Original magnification ×200 (composite image). *I*, rhPDGF–BB-coated implant. Thin section (18 µm/H&E stained), light microscopy, original magnification ×200 (enlarged image). PDGF = platelet-derived growth factor.

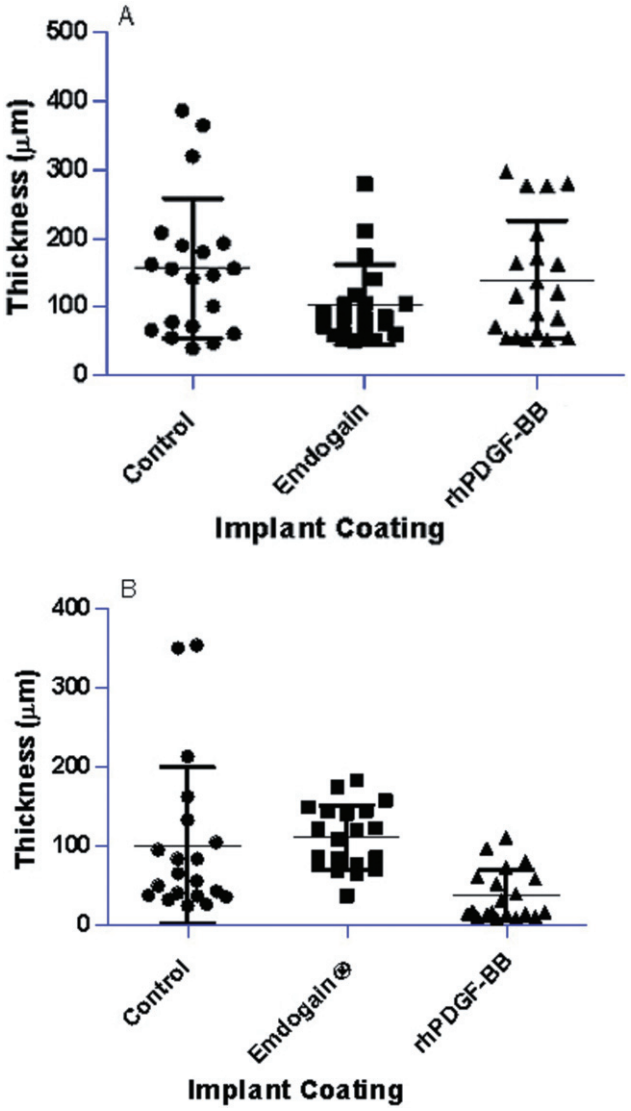

Figure 4 Vertical scatter plots illustrating the thickness of connective tissue layers on implant threads 1–10 (lefthand side and righthand side) of uncoated and coated implants at (A) 4 weeks and (B) 8 weeks. PDGF = platelet-derived growth factor. Mean ± SD.

implant coated with rhPDGF-BB than for the uncoated ($p = .0007$) and Emdogain®-coated ($p = .0035$) implants at 4 weeks. However, at 8 weeks, this difference was no longer significant ($p = .5092$). Coating the implant with Emdogain® was not found to significantly alter the depth of connective tissue penetration into the implant grooves over an uncoated implant, confirmed by the post hoc testing ($p = .5968$).

Post hoc tests indicate that the depth of connective tissue penetration into the implant grooves increased for the uncoated ($p < .0001$) and Emdogain®-coated ($p < .001$) implants between 4 and 8 weeks, but remained the same for the rhPDGF–BB-coated implants.

DISCUSSION

The nature of the connective tissue attachment around dental implants is becoming an increasingly important issue. In particular, the thickness and adherence of the connective tissue to implants has been determined to affect tissue remodeling, biologic width and bone resorption around newly placed implants.[25,33,34] From these studies, it has been concluded that with increasing emphasis on esthetics, the thickness of the peri-implant soft tissues is one important aspect for a satisfactory

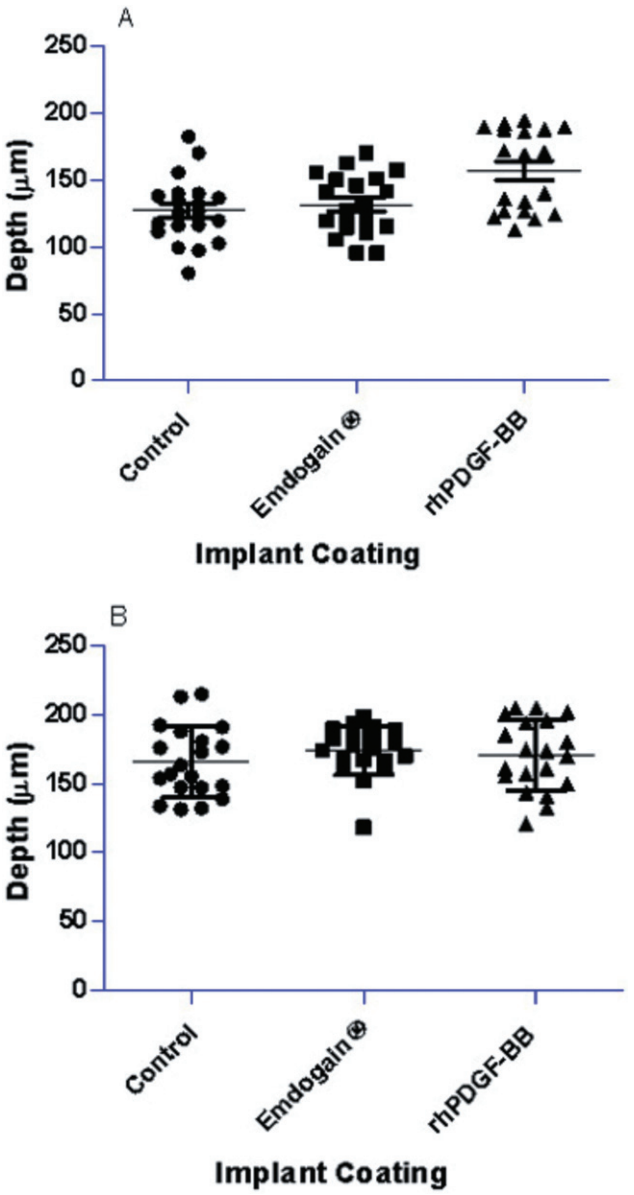

Figure 5 Vertical scatter plots illustrating the depth of connective tissue penetration at implant threads 1–10 (lefthand side and righthand side) of the coated and uncoated implants at (A) 4 weeks and (B) 8 weeks. PDGF = platelet-derived growth factor. Mean ± SD.

clinical outcome. In addition, the adherence of peri-implant soft tissues to the implant/abutment surface is crucial to its function as a barrier between the oral environment and the underlying bone and implant surfaces. Enhancing this adherence by implant surface modification with biological agents may serve to improve implant survival, by preventing soft tissue recession and aiding esthetic outcomes.

In this study, a distinct ingrowth of soft connective tissue into the threads of all the implants occurred regardless of whether the implants were coated or uncoated. This is similar to what occurs with an osseointegrated implant intraorally, whereby the connective tissue forms a nonvascularized, circular, scar-like structure around the transmucosal portion of the implant. The qualitative analysis and histomorphometric measurements of the uncoated implants indicated that resolution of inflammation and connective tissue formation was complete by 4 weeks. However, the tissue response around the implants, which included tissue maturation and organization, continued between 4 to 8 weeks, as evidenced by the significant change in depth of connective tissue penetration into the implant threads. These observations in a murine subcutaneous implant model are consistent with the healing patterns of soft tissues around transmucosal implants placed in dogs.[35] In the canine model, the peri-implant mucosa exhibited minor signs of inflammation during the first 2 weeks of healing, but from 4 weeks, the connective tissue of the mucosa was well organized and the soft tissue barrier adjacent to titanium implants placed in a nonsubmerged protocol was well established and stable after 6 to 8 weeks.[35]

In all sections there was some separation, which was considered to be artifactual as a result of the tissue sectioning, between the connective tissue layer and a thin cellular layer on the implant surfaces. Although not directly measured, it appeared that the degree of separation may have been less for the 8-week samples than the 4-week samples. To determine whether this reflects differences in the maturity and "strength" of the tissues would require further detailed tensile strength assessment of the tissue attachment to the implants, which was beyond the scope of this study.

A number of in vitro studies have investigated the effect of implant surface modification with biological agents on epithelial cell and fibroblast attachment to titanium surfaces. For example, coating machined, plasma-sprayed, and hydroxyapatite titanium surfaces in vitro with fibronectin and laminin-1, enhanced gingival fibroblast and epithelial cell attachment.[5] Similarly, coating titanium alloy with laminin-5 has been found to enhance gingival epithelial cell attachment and hemidesmosome assembly.[6] While type IV collagen can provide an excellent substrate for epithelial cell attachment to titanium surfaces,[7] in vitro studies have shown that cell adhesion to titanium discs coated with collagen was enhanced compared with uncoated titanium.[8,9]

In the present study, coating of the TiUnite surface of titanium implants with Emdogain® or rhPDGF coating did not affect the orientation of the fibroblasts or collagen fibers in the encapsulating connective tissue layer. The orientation of the fibroblasts and collagen fibers when viewed under bright field microscopy and confocal laser scanning microscopy, respectively, was parallel to the long axis of the implant. There was good deposition of connective tissue onto the TiUnite surface and implant grooves for both the uncoated and growth factor-coated implants at the end of the study period. This indicates a degree of soft tissue integration onto implant surface may be possible and is consistent with an in vivo study demonstrating that gingival connective tissue cell grafts onto implants in the presence or absence of EMD results in a fibrous connective tissue attachment.[36]

Two recent in vivo studies provide evidence that microtexturing of implant surfaces can influence the soft tissue response of peri-implant tissues around implants with a machined surface, acid-etched surface, or an oxidized and microporous TiO_2 layer.[29,37] A shorter epithelial attachment and a longer connective tissue seal was observed with the acid-etched and oxidized implants compared with the machined surface implants.[29] Moreover, with the microtopographically complex oxidized TiUnite implant surface, the attached connective tissue showed functionally oriented collagen fibrils oriented toward the implant surface.[37] Similarly, the microgrooved area of "Laser-Lok" implants (Biohorizons Implant Systems, Birmingham, AL, USA) has been noted to be covered with connective tissue with functionally oriented collagen fibers running toward and attaching to the grooves of the implant surface.[30]

In the present study, deposition of a connective tissue layer onto the TiUnite implant surface was observed for both uncoated and growth factor-coated implants. Compared with the control and EMD-coated implants, rhPDGF-BB coating significantly increased

the depth of connective tissue penetration into the implant grooves at 4 weeks. However, the depth of connective tissue penetration for the rhPDGF–BB-coated implants did not change after 4 weeks, while for the EMD-coated implants, the depth of tissue penetration into the implant threads continued to increase. Thus, by 8 weeks, the control and growth factor coated implants exhibited similar depths of connective tissue penetration. Therefore, coating the TiUnite implant surface with rhPDGF-BB seemed to increase the speed of connective tissue deposition, but ultimately the same degree of soft tissue integration occurs around the TiUnite implant surface regardless of whether it has been coated or not. These findings are consistent with the observation that growth factors such as rhPDGF-BB have short half-lives and so, after a sharp initial increase in clinical soft tissue attachment to treated tooth root surfaces, no significant gains are observed or maintained in the long-term.[38]

An incidental finding of this study was the absence of adipose tissue deposition around the Emdogain®-coated implants. While the reason for this response is unclear, it is possible that Emdogain® may inhibit either the differentiation or proliferation of preadipocytes in the immediate vicinity of the implant.

In conclusion, this study shows that good soft tissue integration can be achieved on a moderately roughened TiUnite surface. Surface modification of the TiUnite surface by coating with rhPDGF-BB could increase the speed of soft tissue healing around the implant surface. However, the increased speed of healing with rhPDGF-BB coating could result in a less robust titanium–connective tissue interface. A positive influence of implant surface modification with Emdogain® on soft tissue attachment and maturation around the implant surface was also noted. These observations indicate that growth factor modification may influence soft tissue adaptation to implants and the clinical implications of this need further investigation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the provision of the implant hardware by Nobel Biocare AB, Sweden. Technical assistance with the tissue embedding and sections was provided by V. Li and H. Tsangari from the Bone and Joint Research Laboratory, IMVS. Lyn Waterhouse from Adelaide Microscopy provided expert assistance with confocal microscopy.

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