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Periodontal tissue regeneration by recombinant human transforming growth factor-β3 in *Papio ursinus*

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Background and Objective: Osteogenic proteins of the transforming growth factor-β superfamily induce periodontal tissue regeneration in animal models, including primates. To our knowledge, no studies have been performed in periodontal regeneration using the transforming growth factor-β3 isoform. In the present study, recombinant human transforming growth factor-β3 was examined for its ability to induce periodontal tissue regeneration in the nonhuman primate, *Papio ursinus*.

Material and Methods: Class II furcation defects were surgically created bilaterally in the maxillary and mandibular molars of four adult baboons. Heterotopic ossicles, for transplantation to selected furcation defects, were induced within the *rectus abdominis* muscle by recombinant human transforming growth factor-β3. Forty days later, the periodontal defects were implanted with recombinant human transforming growth factor-β3 in Matrigel® as the delivery system, with recombinant human transforming growth factor-β3 plus minced muscle tissue in Matrigel®, or with the harvested recombinant human transforming growth factor-β3-induced ossicles. Sixty days after periodontal implantation, the animals were killed and the specimens harvested. Histological analysis on undecalcified sections measured the area and volume of new alveolar bone and the coronal extension of newly formed alveolar bone and cementum.

Results: Morphometric analyses showed pronounced periodontal regeneration in experimental defects compared with controls. Substantial regeneration was observed in defects implanted with fragments of heterotopically induced ossicles and with recombinant human transforming growth factor-β3 plus minced muscle tissue.

Conclusion: Recombinant human transforming growth factor-β3 in Matrigel® significantly enhanced periodontal tissue regeneration in the nonhuman primate, *P. ursinus*.

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The effects of transforming growth factor-β1 in periodontal repair in canine and sheep models have been unremarkable (1–3). However, the transforming growth factor-β3 isoform is considered to be far more potent, as a regulator of functions associated with osteogenesis and angiogenesis, than transforming growth factor-β1 or -β2

(4,5). When implanted in heterotopic extraskeletal sites of the nonhuman primate, *Papio ursinus*, the mammalian transforming growth factor-β3 isoform was reported to be a powerful inducer of endochondral bone formation (6). In the present study, recombinant human transforming growth factor-β3 was examined for its ability to induce periodontal tissue regeneration in the nonhuman primate, *P. ursinus*.

Material and methods

Animals

Four clinically healthy adult Chacma baboons (*P. ursinus*) were selected from the nonhuman primate colony of the University of the Witwatersrand, Johannesburg. Selection criteria, housing conditions and diet were as previously described (7).

Implant materials

To induce heterotopic bone, recombinant human transforming growth factor-β3 (Novartis, Basel, Switzerland) was used either alone or in synergistic combination with recombinant human osteogenic protein-1 (6,7) (Stryker Biotech, Hopkinton, MA, USA), with either Matrigel® matrix (BD Biosciences, San José, CA, USA) or baboon insoluble collagenous bone matrix as the carrier. The periodontal implants consisted of recombinant human transforming growth factor-β3 in Matrigel® matrix, recombinant human transforming growth factor-β3 combined with minced *rectus abdominis* muscle tissue, and the heterotopically induced ossicles harvested from the *rectus abdominis*.

Surgery

Pouches were prepared by sharp and blunt dissection in the *rectus abdominis* of each animal (7,8), and the following materials were implanted: 75 μg of recombinant human transforming growth factor-β3 in Matrigel® carrier; 2.5 μg of recombinant human transforming growth factor-β3 combined with 25 μg recombinant human osteogenic protein-1 in Matrigel® carrier; 2.5 μg of recombinant human transforming growth factor-β3 combined with 25 μg recombinant human osteogenic protein-1 in insoluble collagenous bone matrix carrier; 1.5 μg of recombinant human transforming growth factor-β3 combined with 25 μg of recombinant human osteogenic protein-1 in Matrigel® carrier; and 1.5 μg of recombinant human transforming growth factor-β3 combined with 25 μg of recombinant human osteogenic protein-1 in insoluble collagenous bone matrix carrier. Following heterotopic implantation, periodontal Class II furcation defects were surgically prepared, as previously described (9,10). Forty days later, it was noted that the following ossicles (previously determined by palpation of the abdomen), had been resorbed: 2.5 μg of recombinant human transforming growth factor-β3 + 25 μg of recombinant human osteogenic protein-1 in Matrigel®, and 1.5 μg of recombinant human transforming growth factor-β3 + recombinant human osteogenic protein-1 in Matrigel® ossicles. All remaining ossicles were harvested, fragmented and placed on ice, awaiting transplantation to the allocated periodontal sites. Mucoperiosteal flaps were raised to re-expose the alveolar bone and the furcation defects previously created. Exposed roots were root-planed and a small bur was used to make notches on each root to indicate the level of the residual bony housing. Periodontal furcation defects were implanted, according to a Latin square design, with one of the following: Matrigel® carrier alone (control); 75 μg of recombinant human transforming growth factor-β3 in Matrigel®; 75 μg of recombinant human transforming growth factor-β3 + minced muscle in Matrigel®; heterotopic bone ossicle induced by 75 μg of recombinant human transforming growth factor-β3 in Matrigel® (Fig. 1A); or heterotopic bone ossicle induced by 2.5 μg of recombinant human transforming growth factor-β3 + 25 μg of osteogenic protein-1 in insoluble collagenous bone matrix. Any remaining ossicles, or ossicle fragments not used for transplantation, were fixed in 10% buffered formalin and processed for histology in order to characterize morphologically the transplanted material. The periodontal defects were allowed to heal for 60 d, after which the animals were killed with an overdose of sodium pentobarbitone. Bilateral carotid 0.9% saline perfusion was followed by 10% formaldehyde perfusion. Maxillary and mandibular tissue blocks, together with surrounding bone and soft tissue, were harvested *en bloc and* fixed in 10% phosphate-buffered formalin.

Histology

Periodontal specimens and remnants of the heterotopic ossicles were processed for resin embedding (K-Plast resin; MEDIS-Weber, Buseck,

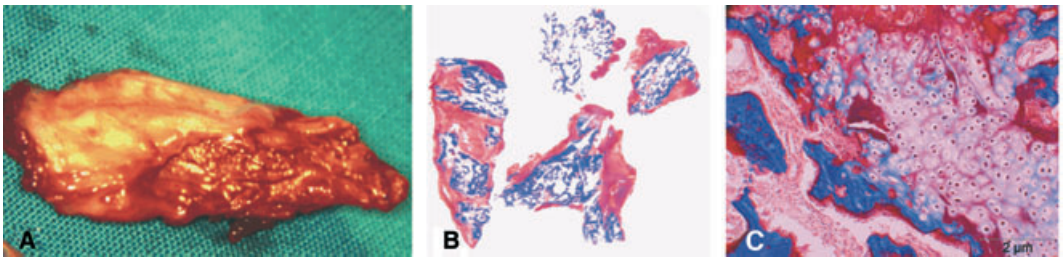

Fig. 1. (A) Harvested, heterotopically induced ossicle from the *rectus abdominus* muscle. (B) Undecalcified section of induced ossicle showing mineralized bone (in blue). Magnification ×1.25. Modified Goldner's stain. (C) Induced ossicle showing bone and cartilage. Magnification ×20. Modified Goldner's stain.

Germany) for the preparation of undecalcified sections. The polymerized blocks were trimmed and sectioned, as previously described (9–11). Briefly, serial sections were cut at 6 μm using a Leica SM2500E heavy-duty microtome (Leica Microsystems, Bensheim, Germany) and labelled levels 1 through 100. The sections were stained free-floating using a modified Goldner's trichrome method for undecalcified sections (9–11). Stained sections were examined using an Olympus Provis AX70 Research Microscope (Olympus Optical Company, Tokyo, Japan). Remnants of the ossicles were processed undecalcified, cut, stained and labelled levels 1 through 30.

Histomorphometry

Induced ossicles — Histological sections of the ossicle remnants at levels 1 and 30 were selected for histometric analysis. The fractional volume of bone (i.e. mineralized bone and osteoid), as well as cartilage and carrier matrix, was measured by the point counting technique (12). Using the Olympus research microscope at ×4 magnification with the 100 lattice point Zeiss Integration Platte II (Zeiss, Oberkochen, Germany), the graticule was superimposed over two sources (13), and 200 points per slide were measured (i.e. 400 points per ossicle). Using GraphPad Prism™ computer software (GraphPad Software Inc., San Diego, CA, USA) for statistical analyses, the mean values, standard deviation and standard error were calculated, and bar graphs were plotted.

Periodontal defects — Histological sections of the periodontal defects at levels 1, 50 and 100 were selected for histomorphometric analyses. The performance of all four variables in periodontal defect sites [i.e. Matrigel® carrier alone (control), 75 μg of recombinant human transforming growth factor-β3/Matrigel®, 75 μg of recombinant human transforming growth factor-β3/muscle/Matrigel® and induced ossicles] was assessed according to the following criteria: area of newly regenerated alveolar bone compared with the total defect area; fractional volume of alveolar bone (i.e. mineralized bone plus osteoid); linear measurement of newly regenerated alveolar bone and cementum compared with defect height; and regeneration of alveolar bone by individual animals.

Using the Olympus research microscope at ×2 magnification, and the analySIS® Imager imaging software system with a CC12 digital camera (Wirsam Scientific and Precision Equipment, Johannesburg, South Africa), the area of newly formed bone was measured from the apical border of the notch to the coronal area of newly formed bone and compared with the total defect size. Measurements were expressed as a percentage of the total defect size.

Fractional volume of bone (i.e. mineralized bone and osteoid) was calculated by the point counting technique (12). Using the Olympus research microscope at ×4 magnification, a 100-lattice point Zeiss Integration Platte II was superimposed over two sources, namely apical and coronal areas (13), and 200 points per slide (levels 1, 50 and 100) were measured (i.e. 600 points per furcation defect).

The height (in mm) of the new alveolar bone in relation to total defect height at the medial and distal aspects of each furcation defect, as well as the height of the new cementum, was measured.

Animal response comparison was performed by measuring the volume of alveolar bone regenerated within the periodontal defects by each animal, irrespective of implant type.

GraphPad Prism™ computer software for statistical analyses was used to compute the mean value, the standard error of the mean and bar graphs for all five criteria; *p*-values were generated by one-way analysis of variance.

Results

Induced ossicles: histology and histomorphometry

Seventy-five micrograms of recombinant human transforming growth factor-β3/Matrigel®-induced ossicles (Fig. 1B,C) — Sections showed multiple areas of osteogenesis and chondrogenesis with mineralized bone in blue lined by osteoid seams. Figure 2A shows the distribution of bone (i.e. mineralized bone plus osteoid), as well as cartilage, within the induced ossicles. Values, expressed as a percentage of the mean, show that the ossicles consisted of 40.9% bone (26.1% mineralized bone plus 14.8% osteoid). The mean percentage of cartilage present in the ossicles was 8.8%, and no residual Matrigel® carrier (0.0%) was detected.

2.5 micrograms of recombinant human transforming growth factor-β3/25 μg of recombinant human osteogenic protein-1/insoluble collagenous bone matrix-induced ossicles (image not shown) — Sections showed multiple areas of osteogenesis and limited chondrogenesis within the residual collagenous matrix. Values, expressed as a percentage of the mean, showed that the ossicles consisted of 30.1% bone (20.9% mineralized bone plus 9.1% osteoid). The mean percentage of cartilage present in the ossicles was 1.6%, with 12.8% residual insoluble collagenous bone matrix carrier (Fig. 2A).

The distribution of mineralized bone, osteoid, cartilage and carrier matrix within the harvested induced ossicles was similar and there was no significant difference between the two types of ossicles ($p > 0.05$), as demonstrated by Bonferroni's Multiple Comparison Test.

Periodontal implants: histology

Matrigel® control — Histological sections of control specimens demonstrated partial healing. There was an abundance of fibrous tissue that extended to the coronal area of the furcation defect with limited insertion of collagenous fibres. The periodontal ligament consisted of areas sparsely populated with collagenous fibres, predominantly organized parallel to the denuded dentinal surface (image not shown).

Recombinant human transforming growth factor-β3/Matrigel® (Fig. 3A,B) — Histological sections showed newly formed alveolar bone, new periodontal

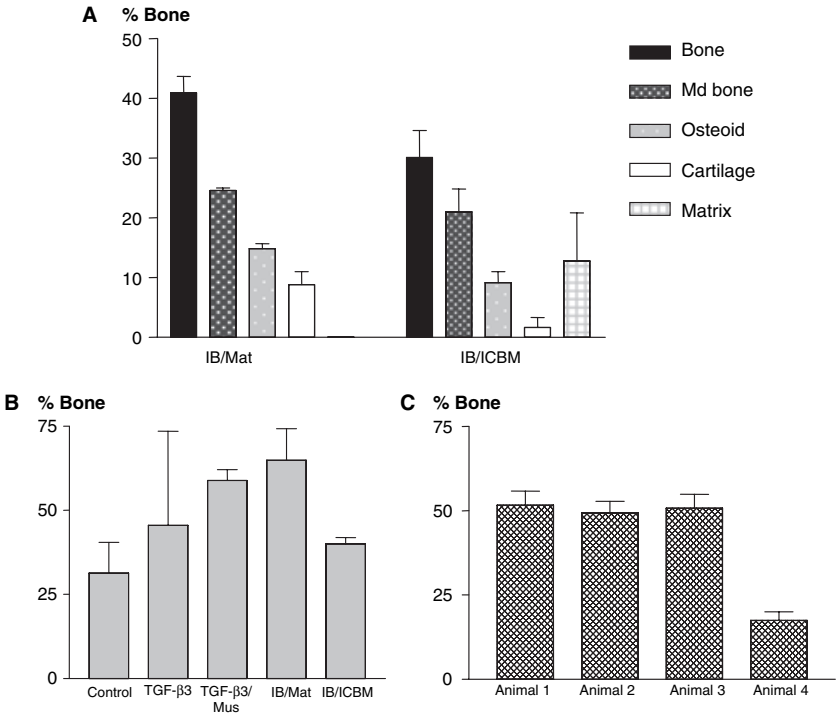

Fig. 2. (A) Distribution of bone (i.e. mineralized bone plus osteoid), as well as cartilage and matrix, within the induced ossicles. (B) Area of new alveolar bone compared with the total defect, as regenerated by the five implant variables. (C) Volume (%) of alveolar bone regenerated by each animal. Control, Matrigel® alone; IB/ICBM, bone ossicles induced by 2.5 μg of recombinant human transforming growth factor-β3/ 25 μg of recombinant human osteogenic protein-1/baboon insoluble collagenous bone matrix; IB/Mat, bone ossicles induced by 75 μg of recombinant human transforming growth factor-β3 in Matrigel® carrier; Md bone, mineralized bone; TGF-β3, recombinant human transforming growth factor-β3 in Matrigel® carrier; TGF-β3/Mus, recombinant human transforming growth factor-β3 and muscle tissue in Matrigel® carrier.

ligament and new cementum within the defect areas. The new periodontal ligament was highly vascularized, with well-demarcated Sharpey's fibres. High-power examination of the sections revealed a repetitive pattern of capillary sprouting in close contact with the periodontal ligament fibres originating from the newly formed alveolar bone. The arrangement of Sharpey's fibres appeared to be governed by the position of the capillaries, demonstrating a 'buttonhole' effect along the edge of the newly formed alveolar bone (Fig. 3B). This feature was particularly prominent in histological sections of periodontal regeneration by recombinant human transforming growth factor-β3 with Matrigel®.

Recombinant human transforming growth factor-β3/muscle/Matrigel® (Fig. 3C,D) —Histological sections showed the presence of newly formed alveolar bone with new cellular cementum and new, well-vascularized, periodontal ligament with Sharpey's fibres. The new alveolar bone appeared to be well distributed within the exposed furcation defects, extending from the apical notches to the coronal area of the defects. The new periodontal ligament exhibited highly organized collagenous fibres with functional insertion of Sharpey's fibres into both new alveolar bone and new cementum (Fig. 3D).

Seventy-five micrograms of recombinant human transforming growth factor-β3/ Matrigel®-induced bone (Fig. 3E,F) — Histological examination of periodontal defects showed new alveolar bone extending into the coronal area of the furcation defect. One furcation defect showed osteogenic invasion of the pulp cavity as a result of damage to the dentinal area during the surgical creation of periodontal defects (Fig. 3E). The new cementum appeared to be cellular with a newly formed collagenous matrix of cementoid into which the new collagenous fibres were inserted. The new periodontal ligament appeared to be well vascularized with an abundance of well-organized Sharpey's fibres (Fig. 3F).

2.5 micrograms of recombinant human transforming growth factor-β3/25 μg of recombinant human osteogenic protein-1/ insoluble collagenous bone matrix-induced bone — Histological examination of periodontal defects showed incomplete regeneration of new alveolar bone with new cementum and highly vascularized periodontal ligament with insertion of Sharpey's fibres (image not shown).

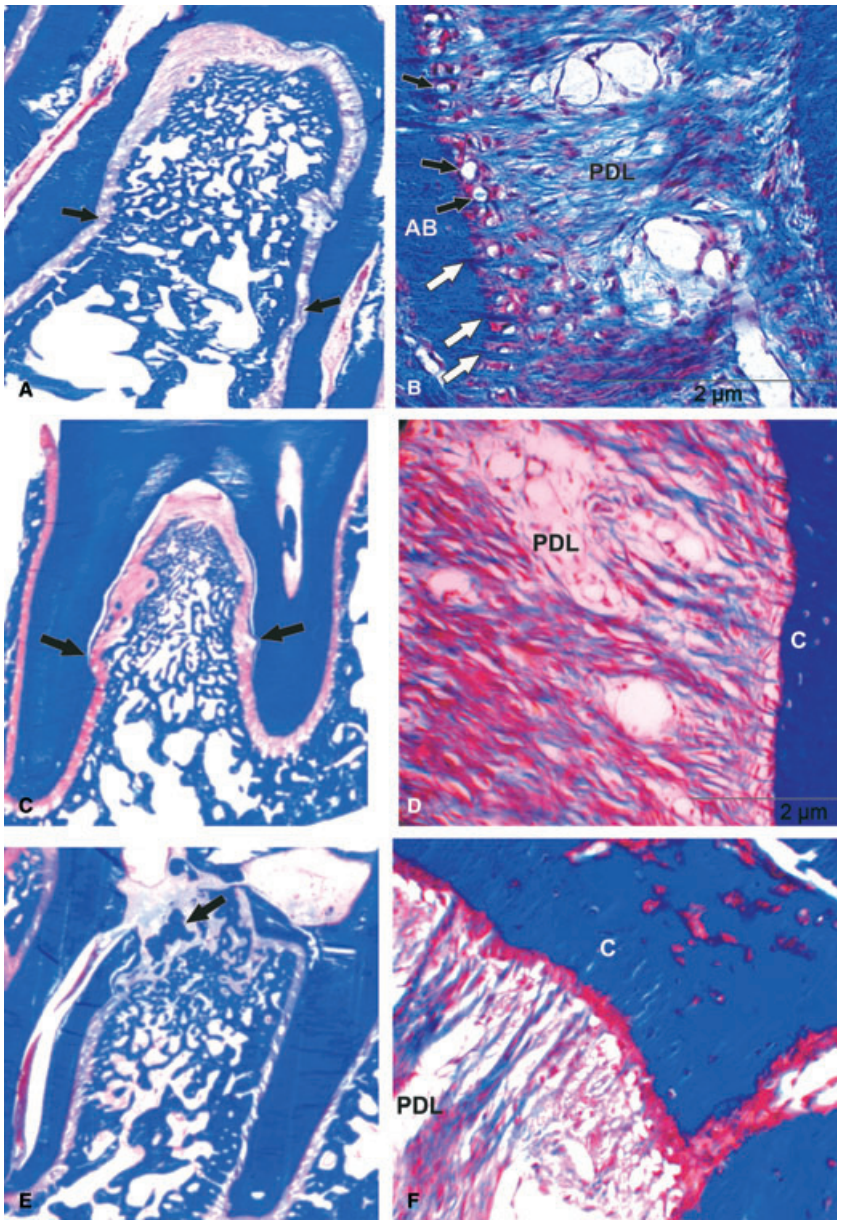

Fig. 3. Periodontal tissue regeneration by: (A) Recombinant human transforming growth factor-β3 in Matrigel® carrier. The arrows indicate the notches made at the time of implantation. Magnification ×1.25. (B) Recombinant human transforming growth factor-β3 in Matrigel® carrier. New periodontal ligament with repetitive pattern of capillaries along the edge of alveolar bone (black arrows) and well-defined insertion of Sharpey's fibres (white arrows).Magnification ×40. (C) Recombinant human transforming growth factor-β3 plus muscle tissue in Matrigel® carrier. Arrows indicate the notches made at the time of implantation.Magnification ×1.25. (D) Recombinant human transforming growth factor-β3 plus muscle tissue in Matrigel® carrier. New periodontal ligament with vascularization and insertion of Sharpey's fibres into new cellular cementum.Magnification ×40. (E) Periodontal tissue regeneration by heterotopically induced bone. Magnification ×1.25. The arrow indicates the osteogenic invasion of pulp cavity as a result of dentine disruption during the preparation of the defect. (F) Insertion of Sharpey's fibres into the cementoid matrix of cementum. Magnification ×40. Modified Goldner's stain. AB, alveolar bone; C, new cellular cementum; PDL, periodontal ligament.

Periodontal implants: histomorphometry

Area (%) — Figure 2B shows the area of regenerated alveolar bone by the five implant variables. Transforming growth factor-β3/muscle (58.9 ± 3.2%) and Matrigel®-based heterotopic bone (64.9 ± 9.4%) implants regenerated the most alveolar bone compared with the control (31.3 ± 9.1%).

Volume (%) — Alveolar bone regenerated by Matrigel®-based heterotopic bone (53.1 ± 4.9%) showed a significant difference ($p < 0.05$) compared with the control (30.3 ± 5.4%). There was no significant difference

($p > 0.05$) between the other variables, as demonstrated by Dunnett's Multiple Comparison Test, which compared the control group with the experimental groups.

Linear measurements (mm) — Table 1 shows the linear measurements (in mm) for the height of the defect from the notch to the fornix of the furcation, the height of the new cementum and new alveolar bone, as regenerated by each of the five implant types for both mesial and distal root surfaces.

Animal response comparison — Animal response comparison was performed by measuring the volume of alveolar bone regenerated within the periodontal defects by each animal, irrespective of implant type. Figure 2C shows very little variation between animals 1, 2 and 3 (51.6%. 49.3%, 50.7%, respectively). However, treated furcation defects in animal 4 (17.4%) showed a significant reduction of periodontal tissue regeneration in comparison with the treated furcation defects of the other animals.

Discussion

Osteogenic proteins of the transforming growth factor-β superfamily induce periodontal tissue regeneration in canine and nonhuman primate models (14). The present study evaluated, in nonhuman primates, periodontal tissue regeneration induced by recombinant human transforming growth factor-β3 when implanted in Class II furcation defects, either by direct application of the morphogen to the defects, or by the transplantation of recombinant human transforming growth factor-β3-induced heterotopic ossicles. Both methods have shown a remarkable potential for the regeneration of alveolar bone, periodontal ligament and cementum within the exposed furcations. To our knowledge, no studies have been published using transforming growth factor-β3 in periodontal regeneration, let alone in periodontal defects of the nonhuman primate, *P. ursinus*.

All treated furcation defects of animal 4 showed limited periodontal tissue regeneration (Fig. 2C). At the time of surgery it became apparent that animal 4 was older than the others. *In vitro* studies on rodent bone marrow stem cells, by Quarto *et al.* (15), showed that age is related to a deficit of osteoprogenitor cells. Fleet *et al.* (16) demonstrated that the activity of an osteoinductive protein is significantly compromised by advancing age but that this could be partially reversed by increasing the amount of growth factor at the implant site. With these observations in mind, it would appear that the enthusiastic response to transforming growth factor-β3 might be regulated by age.

Coelho *et al.* (17) maintained that transforming growth factor-β3, as the main isoform in canine intramembranous bone, such as the mandible, should be of significant benefit when employed in the repair of mandibular defects because it demonstrated a high capacity for mesenchymal cell stimulation of angiogenesis and/or osteogenesis. Merwin *et al.* (18), and Cox *et al.* (4,19), described the angiogenic potency of transforming growth factor-β3 more than a decade ago. Endochondral bone induction by transforming growth factor-β3 in heterotopic sites was noted more recently by Ripamonti *et al.* (6,20), highlighting the evidence that angiogenesis is a prerequisite for bone formation (21). The most prominent feature of periodontal tissue regeneration within the defects implanted with recombinant human transforming growth factor-β3/Matrigel® was the striking vascularization seen in the periodontal ligament (Fig. 3B). The formation of multiple capillaries along the edge of the alveolar bone appeared to preside over the arrangement of insertion of the Sharpey's fibres, emphasizing Trueta's concept that vascularization is a prerequisite for osteogenesis (21). In the present study, periodontal defects implanted with recombinant human transforming growth factor-β3/Matrigel® showed virtually complete healing (Fig. 3A) in two of the four implant sites. However,

Table 1. Histometric analysis of periodontal tissue regeneration in the furcation defects

	Control	SEM	*n*	TGF-β3	SEM	*n*	TGF-β3/ muscle	SEM	*n*	IB/ Matrigel	SEM	*n*	IB/ ICBM	SEM	*n*
Mesial															
N-F	6.0	± 0.3	8	5.9	± 0.1	4	6.2	± 0.1	8	5.5	± 0.4	8	5.2	± 0.4	4
N-C	4.5	± 0.7	8	3.8	± 0.9	4	6.1	± 0.2	8	5.3	± 0.4	8	5.1	± 0.4	4
N-AB	2.9	± 0.6	8	2.8	± 0.9	4	4.8	± 0.3	8	4.6	± 0.4	8	2.6	± 0.9	4
Distal															
N-F	6.0	± 0.2	8	5.9	± 0.3	4	6.5	± 0.1	8	5.8	± 0.4	8	5.9	± 0.4	4
N-C	3.7	± 0.7	8	3.5	± 0.6	4	6.1	± 0.4	8	5.2	± 0.5	8	4.9	± 0.8	4
N-AB	2.3	± 0.4	8	2.8	± 0.8	4	4.7	± 0.3	8	4.9	± 0.5	8	2.8	± 1.2	4

Values (in mm) are given as mean ± SEM; *n* indicates the number of defects.
AB, alveolar bone; IB/ICBM, insoluble collagenous bone matrix-based induced bone; IB/Matrigel®, Matrigel®-based induced bone; N, apical border of the notches on the mesial and distal roots surfaces; N-AB, new alveolar bone; N-C, height of the new cementum; N-F, height of the defect from the notch to the fornix of the furcation; TGF-β, transforming growth factor-β.
There was no significant difference detected by Dunnett's Multiple Comparison Test ($p > 0.05$) for N-F (mesial and distal values), or N-C (the mesial value). Significant differences were noted for the following: N-C (distal) control vs. TGF-β3/muscle implant ($p < 0.05$); N-AB (mesial) control vs. TGF-β3/muscle implant ($p < 0.05$), N-AB (distal) control vs. TGF- β3/muscle implant ($p < 0.01$); N-AB (distal) control vs. IB/Matrigel® ($p < 0.01$).

the amount of alveolar bone regenerated by recombinant human transforming growth factor-β3 on its own was less significant when compared with that of the recombinant human transforming growth factor-β3/muscle and Matrigel®-based heterotopic bone transplant variables (Fig. 2B). This may not be a true reflection of the regenerative capabilities of recombinant human transforming growth factor-β3/Matrigel®, because two of the four furcation defects were implanted in animal 2 and performed well, whereas the remaining two implants were placed in animal 4, a particularly poor responder (Fig. 2C).

The transforming growth factor-β isoforms are site- and tissue-specific (6,22,23), possibly because of the presence or absence of the required responding cells in various anatomical locations, or because of the presence of inhibitory binding proteins (24). This phenomenon was initially noted in studies by Ripamonti *et al*. (25), when transforming growth factor-β1 showed only limited chondro-osteogenesis in calvarial sites but induced large heterotopic ossicles of endochondral bone within the *rectus abdominis* of nonhuman primates (22,23) Subsequent studies, carried out by Ripamonti *et al*. (8), using high doses of recombinant human transforming growth factor-β2 in calvarial defects, demonstrated bone formation that was restricted to the pericranial region of the specimen only, that is, new bone formed only along the muscle-lined periphery of the implant. It is therefore possible that muscle contains the responding cells required for the initiation of osteogenesis within certain bony sites. Studies performed by other researchers have shown that skeletal muscle tissue contains inducible osteoprogenitor cells which, when stimulated by morphogens, are capable of differentiating into osteoblasts (26–30). In the present study, this concept was exploited by transplanting harvested muscle tissue from the *rectus abdominis* of the baboons, combined with recombinant human transforming growth factor-β3, to a periodontal site where superior regeneration of alveolar bone, cementum and periodontal ligament was elicited together with prominent vascularization and the insertion of functionally oriented collagenous fibres (Fig. 3C,D). The formation of new cementum was particularly enhanced in defects implanted with recombinant human transforming growth factor-β3/muscle (Table 1). The quantity of new alveolar bone was significantly greater ($p < 0.05$) in defects implanted with recombinant human transforming growth factor-β3/muscle than in control defects (Fig. 2B).

Experimental methods, using growth factor-induced prefabricated muscular flaps, have been utilized for the manufacture of autogenous bone for the treatment of bony defects (31–34). The graft material is harvested at an optimal time of growth and transplanted to a bony defect site. The time period allowed for growth of autogenous bone is of critical importance. If harvested too early, there may be no evidence of bony transformation, and if harvested too late, resorption may have taken place or the bone may be difficult to excise because of fusion with the surrounding tissue. In the present study, the time period decided upon for heterotopic bone initiation was 40 d. Those ossicles which survived this prolonged period contained an abundance of osteogenic material consisting of trabecular bone covered by osteoid seams, which were populated by contiguous osteoblasts. The fact that all low-dose transforming growth factor-β3 implants in Matrigel® carrier were resorbed may indicate that Matrigel® carrier induces a rapid rate of resorption and that, for a time period exceeding 30 d (or possibly less), higher doses of transforming growth factor-β3 are indicated if a Matrigel® carrier is to be used. Further studies are needed to pinpoint the optimal time period for bone induction by the transforming growth factor-β3 isoform in the *rectus abdominis* of nonhuman primates. In this study, transplanted Matrigel®-based heterotopically induced ossicles yielded the best results for periodontal tissue regeneration (Figs 2B and 3E,F).

Conclusion

This study, with a short-term observation period and only histological analysis as an evaluation parameter, indicated that recombinant human transforming growth factor-β3, delivered by Matrigel® as carrier, induces alveolar bone, periodontal ligament and cementum. The addition of skeletal muscle harvested from the *rectus abdominis* enhanced periodontal tissue regeneration with increased cementum formation, indicating that the harvested muscle tissue retained responding mesenchymal cells capable of transformation into the essential cellular components to engineer periodontal tissue regeneration. The innovative strategy of engineering heterotopic ossicles by the application of recombinant human transforming growth factor-β3 was implemented, which allowed fragmented ossicles to be transplanted to surgically created periodontal defects and resulted in the induction of periodontal tissue regeneration. The rapid induction of mineralized ossicles in the *rectus abdominis* by recombinant human transforming growth factor-β3 is a novel source of induced autogenous bone for transplantation in clinical contexts.

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