J Clin Periodontol 2005; 32: 583–589 doi: 10.1111/j.1600-051X.2005.00729.x

Journal of Clinical Periodontology

Periodontal healing in one-wall intra-bony defects in dogs following implantation of autogenous bone or a coral-derived biomaterial

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Kim C-S, Choi S-H, Cho K-S, Chai J-K, Wikesjö UME, Kim C-K. Periodontal healing in one-wall intra-bony defects in dogs following implantation of autogenous bone or a coral-derived biomaterial. J Clin Periodontol 2005; 32: 583–589. doi: 10.1111/j.1600–051X.2005.00729.x. © Blackwell Munksgaard, 2005.

Abstract

Aim: Autogenous bone grafts and bone biomaterials are being used as part of protocols aiming at reconstruction of periodontal defects. There is a limited biologic information on the effect of such materials on periodontal healing, in particular aberrant healing events that may prevent their general use. The objective of this study was, using histological techniques, to evaluate periodontal healing with focus on root resorption and ankylosis following implantation of autogenous bone and a coral-derived biomaterial into intra-bony defects in dogs.

Methods: One-wall intra-bony periodontal defects were surgically created at the distal aspect of the second and the mesial aspect of the fourth mandibular premolars in either right or left jaw quadrants in four Beagle dogs. Each animal received particulated autogenous bone and the resorbable calcium carbonate biomaterial into discrete one-wall intra-bony defects. The mucoperiosteal flaps were positioned and sutured to their pre-surgery position. The animals were euthanized 8 weeks post-surgery when block sections of the defect sites were collected and prepared for qualitative histological analysis.

Results: There were no significant differences in periodontal healing between sites receiving autograft bone and the coral-derived biomaterial. A well-organized periodontal ligament bridging new bone and cementum regeneration was observed extending coronal to a notch prepared to delineate the apical extent of the defect. Osteoid and bone with enclosed osteocytes were formed onto the surface of both autograft and coral particles. Although small resorption pits were evident in most teeth, importantly none of the biomaterials provoked marked root resorption. Ankylosis was not observed.

Conclusion: Particulated autogenous bone and the coral-derived biomaterial may be implanted into periodontal defects without significant healing aberrations such as root resorption and ankylosis. The histopathological evaluation suggests that the autogenous bone graft has a limited osteogenic potential as demonstrated in this study model.

Key words: ankylosis; autogenous bone; bone grafting; calcium carbonate; dogs; histology; intra-bony defect; root resorption

Accepted for publication 26 October 2004

One objective of periodontal therapy is regeneration of the periodontal attachment including cementum, a functionally oriented periodontal ligament, and alveolar bone, which may be achieved by guided tissue regeneration. Bone grafting is also being used as part of surgical protocols aimed at regeneration of periodontal structures. Autogenous bone grafts, bone derivatives, and bone substitutes have been used for this purpose. Among the biomaterials, autogenous bone has been adopted as the gold standard because of: (1) autograft bone includes cells participating in osteogenesis; (2) a tissue reaction is induced without inducing immunological reactions; (3) there is a minimal inflammatory reaction; (4) there is rapid revascularization around the graft particles; and (5) a potential release of growth and differentiation factors sequestered within the grafts (Marx 1994). In perspective, autograft bone has been considered to yield a high osteogenic potential and has thus been used with the intent to improve outcomes of periodontal regenerative procedures (Dragoo & Sullivan 1973, Hiatt & Schallhorn 1973, Renvert et al. 1985). However, the use of autograft bone presents potential disadvantages such as a need for a wider or second surgery region; restricted donor sites make its use in cases with need for extensive grafting impractical. Moreover and perhaps most importantly, the use of autograft bone has been reported to be attributed to result in tooth debilitating ankylosis and root resorption in human (Schallhorn & Hiatt 1972, Dragoo & Sullivan 1973) and dog intrabony defects (Levin 1975).

Resorbable calcium carbonate is processed from natural coral for use as a bone substitute in the surgical management of skeletal defects. It has a $>45\%$ porosity with pores of about $150\,\mu m$ in diameter and will gradually resorb to be replaced by bone when implanted into bone sites. It has been reported to be biocompatible and osteoconductive. Its potential use as a bone substitute in the management of alveolar and periodontal defects has been reported (Guillemin et al. 1987, Kim et al. 1996, Moon et al. 1996, Yukna et al. 1998, Reynolds et al. 2003, Wikesjö et al. 2003a). However there is limited biologic information on the effect of this and related biomaterials on periodontal healing, in particular aberrant healing events that may prevent their general use. The objective of this study, using histological techniques, was to evaluate periodontal healing with focus on root resorption and ankylosis following implantation of autogenous bone and a coral-derived bone biomaterial into intra-bony defects in dogs.

Material and Methods

Animals

Four 2-year-old Beagle dogs, approximate weight 15 kg, were used. The animals exhibited an intact dentition with a healthy periodontium. Animal selection and management, surgery protocol, and periodontal defect preparation followed a protocol approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea. The animals were fed a soft diet throughout the study in order to reduce the chance of mechanical interference with healing during food intake.

Surgical protocol

The surgical procedure was performed under general anesthesia induced by intravenous injection of atropin (0.04 mg/kg; Kwangmyung Pharmaceutical Ind. Co. Ltd., Seoul, Korea) and intramuscular injection of a combination of xylazine (Rompun, Bayer Korea Co., Seoul, Korea) and ketamin (Ketara, Yuhan Co., Seoul, Korea) followed by inhalation anaesthesia (Gerolan, Choongwae Pharmaceutical Co., Seoul, Korea). Routine dental infiltration anaesthesia was used at the surgical sites.

The mandibular first and third premolars were extracted prior to the experimental surgery and the extraction sites were allowed to heal for 2 months. The remaining dentition received oral prophylaxis in conjunction with the extractions. The experimental surgery involved elevation of buccal and lingual mucoperiosteal flaps to surgically create ''box-type'' one-wall intra-bony defects ($4 \times 4 \times 4$ mm) at the distal aspect of the second and the mesial aspect of the fourth mandibular premolars in either the right or left jaw quadrants (Kim et al. 2004). Following root planing, a reference notch was made with a round bur on the root surface at the base of the defect.

The unilateral mandibular defects each received one of the two experimental protocols: implantation of a particulated autogenous bone graft or a coral-derived resorbable calcium carbonate biomaterial (Biocoral®, Inoteb, Saint Gonnery, France). Treatments were alternated between premolar defect sites in subsequent animals. Contra-lateral jaw quadrants including sham-surgery controls were used for separate experiments to be reported elsewhere.

Autogenous bone was obtained from the retromolar area using 6 mm trephine (TRE 05, 3*i*, Palm Beach Gardens, FL, USA). The bone plugs were particulated using a bone mill. Graft materials were condensed to fill respective defects. Primary, tension free, wound closure was accomplished; the gingival flaps being positioned and sutured completely covering the defect sites and implants. Sutures were removed at approximately 10 days post-surgery.

Post-surgery management

Post-surgery management included intramuscular administration of antibiotics and daily topical application of a 2% chlorhexidine solution (Hexamedin®, Bukwang Pharmaceutical Co., Seoul, Korea). Observations of the experimental sites with regards to gingival health, suture line closure, oedema, and evidence of tissue necrosis or infection were made daily until suture removal, and at least twice weekly thereafter. The animals were euthanized 8 weeks post-surgery using an intravenous injection of concentrated sodium pentobarbital (Entobar®, Hanlim Pharmaceutical Co., Seoul, Korea).

Histological procedures

Block sections including the surgical sites were removed at sacrifice. The sections were rinsed in sterile saline and fixed in 10% buffered formalin for 10 days. After rinsing in sterile water, the sections were decalcified in 5% formic acid for 14 days and embedded in paraffin. Serial sections, $5\,\mu m$ thick, were cut in a mesial–distal direction at intervals of $80\,\mu m$. The four most central sections from each block were stained with haematoxylin/eosin and examined using light microscopy.

Results

Clinical healing was uneventful. All defect sites maintained suture line closure throughout the healing interval without signs of inflammatory reactions.

Autogenous bone grafts

New bone and cementum regeneration, greater than that in the sham-surgery control (Fig. 1), was observed extending coronal to the notch in sites receiving autogenous bone graft (Fig. 2). The newly formed cementum appeared thicker in the notch area and thinner more coronally (Fig. 2). A perpendicular and oblique periodontal fibre orientation was observed (Fig. 2). Above the alveolar crest, cementum with few cellular elements formed a thin strip along the root surface. The collagenous fibres presented a more oblique orientation and paralleled the root surface in this area. The boundary between old and new bone was indistinguishable; osteoblast-like cells exhibiting a dense arrangement at the crest of the newly

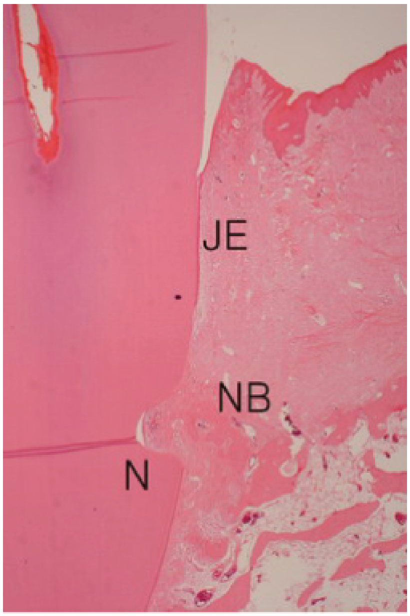

Fig. 1. Photomicrograph of the sham-surgery control section showing junctinal epithelium (JE) down-growth, connective tissue adhesion and limited new bone (NB) formation above the notch (N) (H–E original magnification × 20).

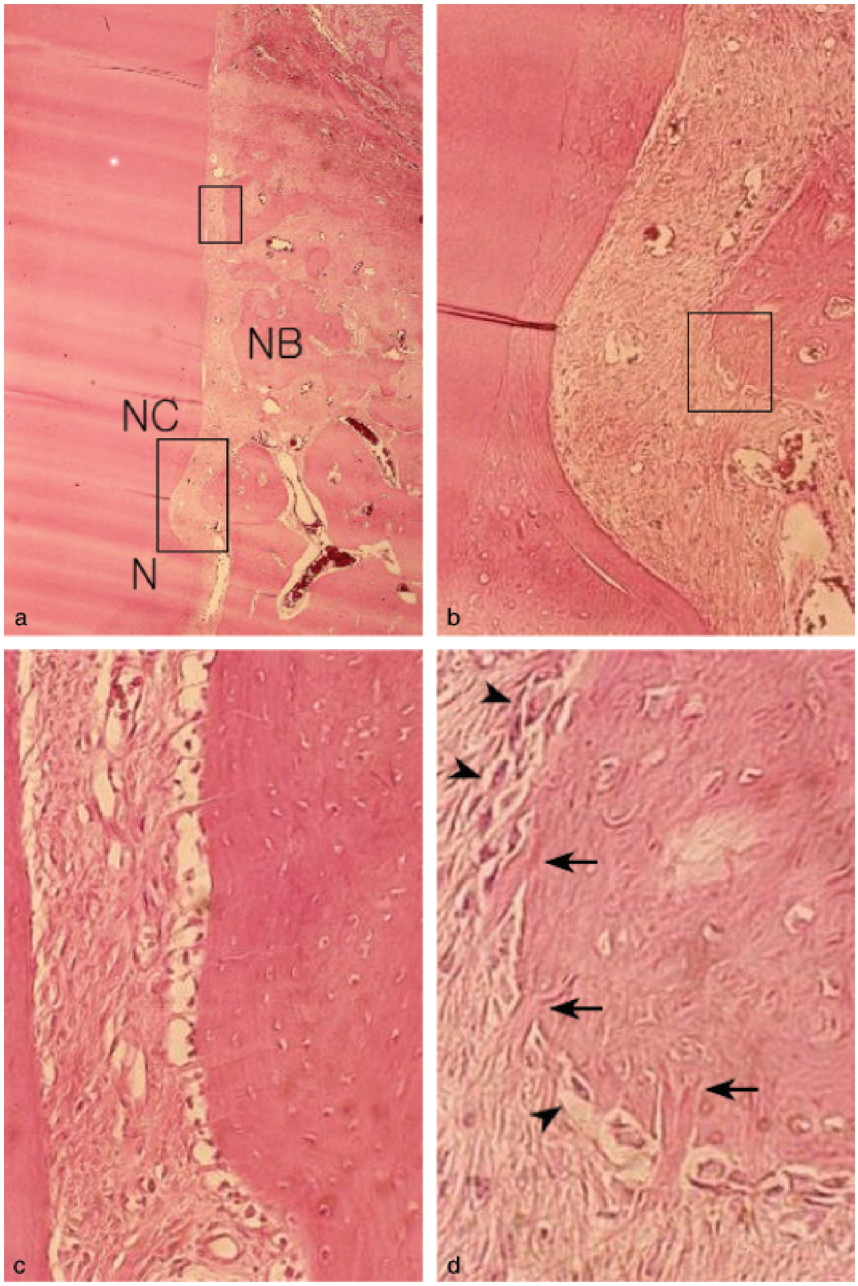

Fig. 2. (A) Photomicrograph of site receiving autologous bone showing new bone (NB) and cementum (NC) formation in the notch area (N) (H–E, original magnification × 20). The higher magnifications show the oblique or perpendicular collagen fibre arrangement. (B) A thick layer of new cementum with an oblique or perpendicular collagen fibre arrangement was observed in the notch area (H–E, original magnification × 100). (C) A well-organized periodontal ligament exhibiting a thinner new cementum layer was observed more coronally (H–E, original magnification × 200). (D) Newly formed bone trabeculae were lined with osteoblast-like cells (arrowhead) and exhibited perpendicularly oriented fibres (arrows) (H–E, original magnification × 400).

formed bone, and facing the periodontal ligament space (Fig. 2) suggesting continued bone apposition at the 8-week observation. Collagenous fibres were found inserted perpendicularly to the newly formed bone (Fig. 2).

The autograft bone particles could be found throughout the defect area. The autograft particles exhibited a wide variety in size and shape. The bone particles rarely exhibited signs of vitality; osteocytes were rarely observed within lacunae of the particles that also exhibited several crack-lines (Fig. 3). Osteoclast-like cells could be observed adjacent to the autogenous bone particles in some specimens suggesting an active resorption process (Fig. 3). The autograft bone particles were embedded in newly formed bone, osteoid, and connective tissue. Autogenous bone particles located at the apical and central aspect of the defects were generally incorporated in newly formed bone. In the central aspect, particles embedded in connective tissue were also observed. However, in the coronal area a majority of the autograft bone particles were embedded in connective tissue. Sometimes the most superficially located particles appeared partially surrounded by epithelium. Evidence of osteogenic activity, such as osteoblast-like cell lining, osteoid and bone apposition along the particles was rarely observed.

Although relatively marked root resorption could be observed in one specimen (Fig. 4), no significant root resorption was observed in all other specimens. However, small resorption pits were evident in most teeth (Fig. 5). These limited resorption pits were mainly detected on the root surface coronal to newly formed cementum. There was no evidence that the autogenous bone particles induced inflammatory reactions, root resorption, or ankylosis.

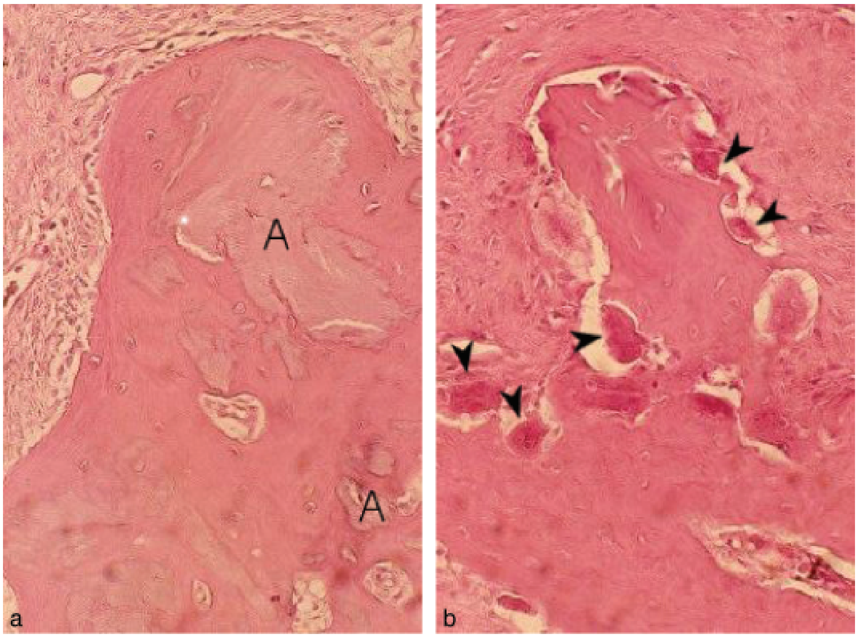

Fig. 3. (A) Several residual autogenous bone particles (A) were observed embedded within the newly formed bone. (B) Osteoclasts (arrowheads) could occasionally be observed around the autogenous bone particles (H–E, original magnification × 200).

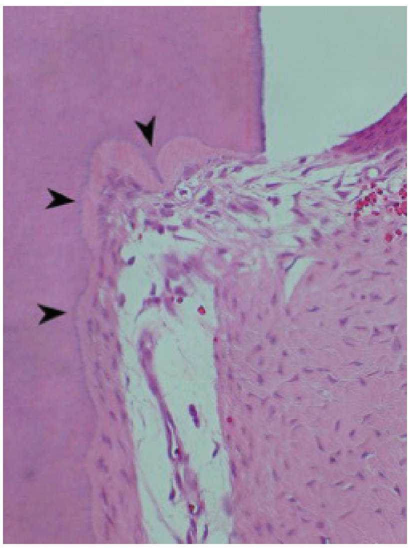

Fig. 4. A relatively marked root resorption (arrowheads) could be observed immediately below the cemento–enamel junction in one specimen (H–E, original magnification × 200).

Coral-derived biomaterial

Observations regarding the magnitude of new bone and cementum formation were similar to that observed for defect sites grafted with autogenous bone (Fig. 6). New bone and cementum formation were observed extending coronal to the notch. New bone formation above the notch area extended to the level of the newly formed cementum. The new cementum, exhibiting perpendicularly oriented collagen fibres, appeared thicker in the notch area and thinner more coronally. A well-organized periodontal ligament was observed (Fig. 6). Overall the periodontal healing associated with coral-derived biomaterial was similar to that of the autogenous bone graft. Several coral particles could be observed in the defect area. The coral particles were embedded within osteoid and newly formed bone, which might originate from the base of defect and the periodontal ligament (Fig. 6). The edge of the particles exhibited irregular features suggesting a resorptive process. Similar to defect sites receiving autogenous bone, sites receiving the coral-derived biomaterial exhibited irregular resorption pits (Fig. 5). Importantly, no marked root resorption or ankylosis was observed.

Discussion

The objective of this study was to evaluate periodontal healing with focus on root resorption and ankylosis following implantation of particulated autogenous bone or a coral-derived biomaterial into one-wall surgically produced intra-bony pockets in dogs. Healing was evaluated using qualitative histologic evaluations following an 8-week healing interval. Although healing resulted in regeneration of bone, cementum, and a fibrous attachment, there were limited signs of root resorption and no evidence of ankylosis suggesting that particulated autogenous bone and the coral-derived biomaterial may be implanted safely into periodontal defects without significant healing aberrations.

As shown in Fig. 1, histologic observations of periodontal regeneration in one-wall intra-bony defects herein corroborate our previous reports (Kim et al. 2002, Park et al. 2003, Kim et al. 2004). Limited periodontal regeneration following gingival flap surgery alone following an 8-week healing interval has been reported for this model system, alveolar bone, and cementum regeneration not exceeding 30–35% of the defect height. Although the 8-week histological observations may not fully explain early healing events, an 8-week healing interval has been considered useful to study periodontal repair/regeneration in dog models (Kim et al. 2002, Choi et al. 2002, Park et al. 2003, Wikesjö et al. 2003a, b, c, Kim et al. 2004). In perspective, Moon et al. (1996) could not discern cementum regeneration until week 6 in a dog intra-bony defect model using light microscopy suggesting that wound maturation must progress over several weeks until cementum formation may be appreciable by light microscopy. Moreover, Choi et al. (2002) observed no additional bone and cementum formation between an 8- and 24-week healing interval for sham-surgery controls in a dog intra-bony defect model suggesting longer observation intervals may not be necessary to capture the osteogenic potential and cementogenesis. Our previous studies have suggested the one-wall intra-bony defect as a relevant reproducible model to evaluate candidate technologies for periodontal regeneration. Although a histometric analysis was not used in the present study, the histopathological observations suggest a significant increase in both bone and cementum regeneration following implantation of the biomaterials compared with our present and previous observations for sham-surgery controls.

Resorbable calcium carbonate has been stated to be biocompatible and osteoconductive material. When implanted in bony tissue. It will gradually resorb and replaced by bone (Guillemin et al. 1987, Ouhayoun et al. 1992). It has

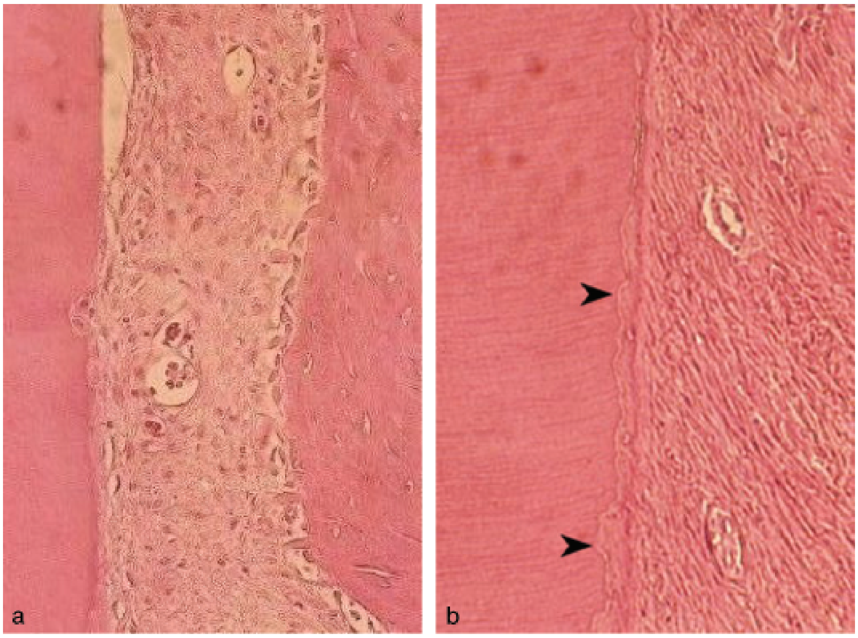

Fig. 5. Small irregular resorption pits were observed in most teeth irrespective of treatment: (A) site receiving autograft bone and (B) site implanted with the coral-derived biomaterial (H–E, original magnification × 200).

been demonstrated to support bone regeneration in a variety of settings including repair of long bone defects (Gao et al. 1997, Guillemin et al. 1989), alveolar augmentation (Piattelli et al. 1997), and periodontal regeneration (Guillemin et al. 1987, Moon et al. 1996, Kim et al. 1996, Yukna et al. 1998, Reynolds et al. 2003, Wikesjö et al. 2003a).

Autogenous bone grafts, implanted into intra-bony periodontal defects, also have been reported to produce favourable clinical results compared with gingival flap surgery alone. Hiatt & Schallhorn (1973) reported an average bone fill of 3.4 mm in a study of 166 sites grafted with intra-oral cancellous bone and marrow. Renvert et al. (1985) also reported predictable results using intra-oral autogenous bone grafts in deep intra-bony defects. In perspective, particulated autogenous bone grafts have been reported to support a pronounced revascularization and may thus potentially enhance osteogenesis in the grafted site (Axhausen et al. 1956, Rivault et al. 1971). In consequence, more favourable periodontal healing would be expected in sites receiving autogenous bone grafts compared with sites implanted with the coral-derived biomaterial. However, no discernable differences in bone and cementum formation were observed among the experimental sites in this study suggesting that the autograft bone exhibited limited effect on osteogenesis and cementogenesis.

The healing response to the implanted graft and biomaterial particles was similar. The histopathological evaluation of sites receiving autograft bone did not provide evidence for an osteogenic, osteoconductive, or osteoinductive potential beyond that observed at sites implanted with the coral-derived biomaterial that does not include cells or factors in support of osteogenesis or osteoinduction. In fact, osteocytes within the lacunae of the graft particles were rare and several crack-lines were evident in the graft particles suggesting poor graft survival leaving the graft particles to merely serve as an osteoconductive scaffold or ''filler'' much like the coral biomaterial without contributing innate biologic activity. In perspective, Ellegaard et al. (1975) reported the fate of vital and devitalized cancellous bone grafts implanted into periodontal furcation defects in non-human primates. They observed similar reactions to vital and non-vital autogenous bone particles. Although a few osteocytes close to the surface of the vital bone particles were observed, osteocyte lacunae were devoid of cells in both vital and non-vital autogenous bone particles after 1 week. Becker et al. (1998) also found non-vital bone particles in the histologic evaluation following implantation of intra-oral autogenous bone into extraction sockets. More recently, Pallesen et al. (2002) evaluated the early stage of bone healing in the defects grafted with autogenous bone of different particle size. They reported that osteocytes within the lacunae of the graft were rare after 4 weeks while lacunae with osteocytes could still be discerned in the 1- and 2-week specimens.

Root resorption appears a common sequel to connective tissue repair following periodontal reconstructive surgery (Wikesjö & Nilvéus 1991). Undermining resorption is not uncommon in the cervical region of large periodontal defects in particular when the connective tissue flaps have been adapted directly onto a denuded instrumented root surface. In contrast, advanced root resorption is rare following protocols including provisions for guided tissue regeneration (Sigurdsson et al. 1994, Wikesjö et al. 2003b, c). Nevertheless, resorption of surface erosion character encompassing a larger part of the exposed root appears inherent to early wound healing following periodontal reconstructive surgery irrespective of specific protocol (Wikesjö & Selvig 1999). Frank et al. (1974) suggested that root resorption and subsequent exposure of dentinal collagen fibres always preceded new cementum formation. Moreover, Schroeder (1992) suggested that formation of a cementum matrix might prevent resorption. The observations in this study generally corroborate these observations and suggestions. Most teeth exhibited limited superficial resorption, appearing more pronounced when the connective tissue immediately interfaced dentin rather than a root surface covered by new cementum.

Ankylosis, that is the obliteration of the periodontal ligament space and progressive replacement of dentin and cementum by bone, was not observed in this study. Ankylosis appears to occur in sites where bone formation takes place without regeneration of a periodontal ligament (Wikesjö et al. 2003b). Ankylosis has been reported as a complication to autogenous bone grafting (Schallhorn & Hiatt 1972, Dragoo & Sullivan 1973). However, ankylosis has usually been observed following use of fresh iliac cancellous bone and marrow suggesting the limited usefulness of this autograft bone in periodontal therapy. When the results in the present study are considered in conjunction with previous reports, it might be concluded that

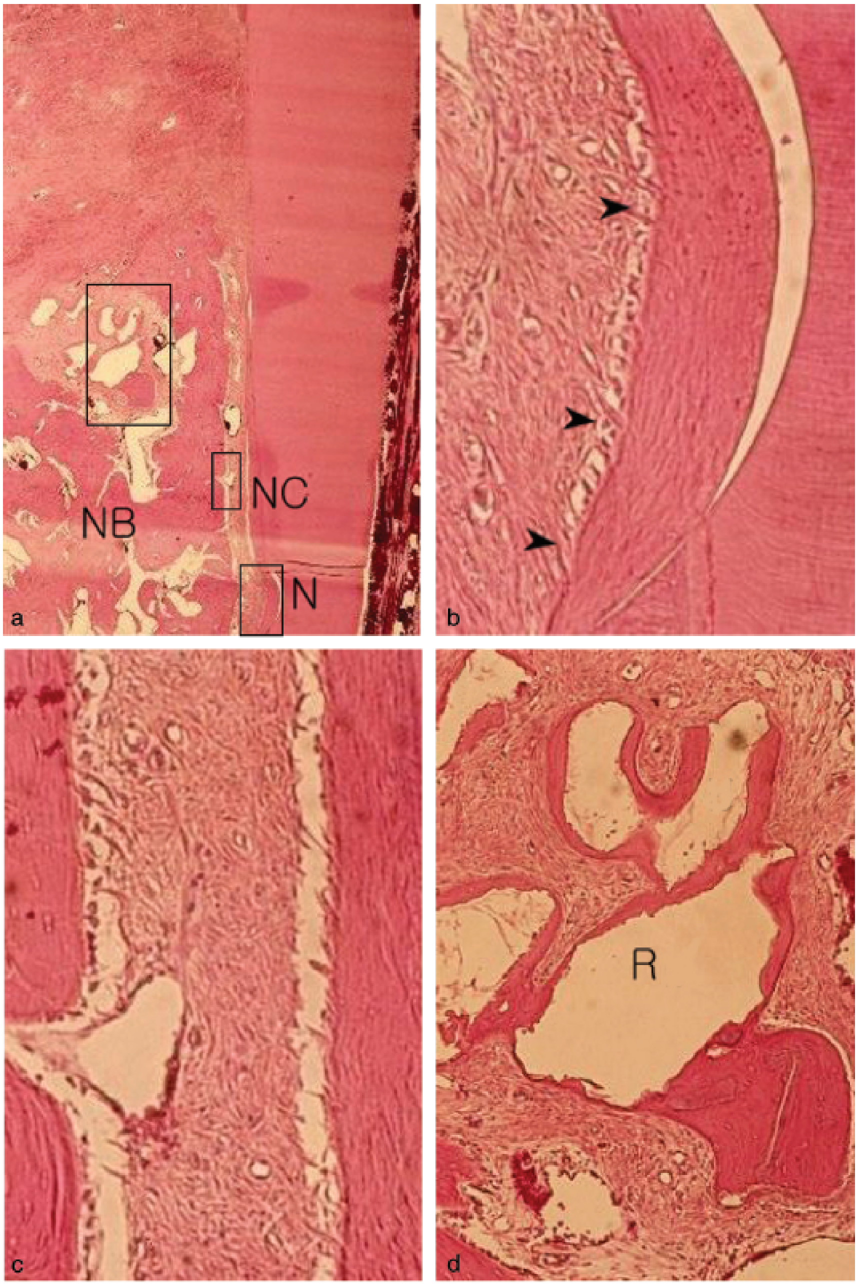

Fig. 6. (A) Photomicrograph of site implanted with the coral-derived biomaterial showing new bone (NB) and cementum (NC) formation in the notch area (N) (H–E, original magnification × 20). The higher magnifications show the oblique or perpendicular collagen fiber arrangement. (B) A thick layer of new cementum with an oblique or perpendicular collagen fibre arrangement (arrowheads) was observed in the notch area (H–E, original magnification × 100). (C) A well-organized periodontal ligament exhibiting a thinner new cementum layer was observed more coronally (H–E, original magnification × 200). (D) Newly formed osteoid tissue and bone with enclosed osteocytes were deposited around coral-derived biomaterial particles (R) (H–E original magnification × 200).

autogenous bone grafts from intra-oral sources do not induce ankylosis.

Conclusion

Particulated autogenous bone and the coral-derived biomaterial may be implanted into periodontal defects of dogs without significant healing aberrations such as root resorption and ankylosis. The histopathological evaluation suggests that the autogenous bone graft has a limited osteogenic potential as demonstrated in this study model.

Acknowledgements

This work was supported by Grant No. R13-2003-13 from the Medical Science and Engineering Research Program of the Korea Science & Engineering Foundation.

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