JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **16** (2005) 753–758

Tyrosine derived polycarbonate membrane is useful for guided bone regeneration in rabbit mandibular defects

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Standardized bilateral through-and-through defects (12 × 6 mm) were created extraorally in the mandibular angle of 18 New Zealand White rabbits. Animals were divided in to three groups ($n = 6$) according to the intended healing time. On the left side, defects were covered with a poly(desaminotyrosyl-tyrosine-ethyl ester carbonate) (PDTE carbonate) membrane wrapped around the inferior border of the mandible and fixed with bioabsorbable sutures. On the right side, the defects were filled with a mesh made of bioactive glass 13–93 and 3 wt% chitosan. The defects were covered with the same membranes. Periosteal flap was sutured over the membrane. Radiographically, bone ingrowth was seen in all specimens at 12 weeks postoperatively. At 24 weeks, completely ossified area remained approximately at the same level as at 12 weeks, but the non-ossified area decreased to almost zero. However, the bioactive glass mesh did not improve the results. Nevertheless, enveloping the defect with PDTE carbonate membrane seemed to play a crucial role in new bone formation. Based on these results, we conclude that tyrosine polycarbonate is a promising new material for guided bone regeneration.

1. Introduction

Bioabsorbable materials suitable for clinical use in oral and maxillofacial surgery have been under extensive research for over three decades [1–4]. One significant task has been the reconstruction of masticatory system when there is not enough bone available, for example, due to trauma or tumor ablation. Bone defects in both, the mandible and maxilla, create a problem in the reconstruction of fully functioning masticatory system and occlusion. Today, defects are mainly filled with autogenous bone, with either free or microvascular bone grafts, which subjects the patient to another operation site, longer operation and more morbidity [5, 6].

Various studies have shown that missing bone can be aided to regenerate by using different kinds of membranes, both bioabsorbable and non-absorbable [7–10]. They inhibit soft tissue invasion to bony defects, which is considered the most important factor contributing to bone formation [10–12]. At present, expanded polytetrafuoroethylene (e-PTFE) is a clinically widely used membrane. Because it is non-absorbable it needs to be removed from the patient in a second operation. The removal procedure subjects the patient to an unnecessary risk of infection and increases the costs of the treatment. Stripping the periosteum, to remove the membrane, might also enhance bone resorption [13]. The time needed for the whole treatment protocol is therefore often prolonged.

By using a bioabsorbable membrane instead of bone grafts, several benefits can be achieved: less morbidity and risk of infection and shorter treatment periods, not to forget the economical aspects, either [6, 14, 15]. Bioabsorbable materials differ from each other in handling properties, malleability, elasticity and absorption rates. However, no material supercedes the other one so far according to the literature [16].

An ideal bioabsorbable material in guided bone regeneration should be able to enhance bone growth in

bone defects, while at the same time establish a firm mechanical barrier against soft tissue invasion into the defect area. The material should also be easy to handle and manufacture and should degrade after tissues have healed. Additionally it should not elicit too strong, clinically significant foreign body reaction [17, 18].

Currently, commercially available bioabsorbable polymers approved for clinical use, are polylactides (PLAs), polyglycolides (PGAs), polydioxanone (PDS) and their copolymers [16]. PLAs and their copolymers are today perhaps the most commonly used and studied bioabsorbable polymers in oro-maxillofacial and orthopedic surgery [3, 19]. By changing the composition of the polymer and the manufacturing procedure, the resorption time, handling properties, and mechanical durability can be adjusted to suit the needs of the patient [20, 21]. In the literature several reports have been published on sterile fluid accumulation, cyst formation and foreign body reaction in association with PLA and PGA homopolymers [22–24]. It is assumed that this may be caused by too fast degradation of impure homopolymeric implants, which exceeds the metabolizing capacity of the surrounding tissues [21].

Tyrosine derived polycarbonates are new bioabsorbable polymers suggested for use in medical applications. Tyrosine-based pseudo-peptide polymers were first introduced in 1987 by Kohn and Langer [25]. They have proven to be biocompatible, biodegradable, non-toxic, and non-immunogenic with good processing properties including solubility, thermal stability, and moldability [26–28]. Various tyrosine polycarbonates derived from the ethyl, butyl, hexyl, or octyl esters of desaminotyrosyl-tyrosine, can be prepared by condensation polymerization [29]. A polymer carrying an ethyl ester pendent chain, PDTE carbonate, has been established as a promising orthopedic implant material, exhibiting bone apposition when in contact with hard tissue [30].

Tyrosine-derived polycarbonates incorporate two *in vivo* hydrolytically labile bonds in each repeat unit, a carbonate bond that connects the monomer units and an ester bond connecting a pendent chain. Degradation rate and products of the polymer are determined by the relative hydrolysis rate of these two labile bonds. Carbonate bond is hydrolyzed at a faster rate than the pendent chain ester bond [31]. They, however, are relatively stable and degrade only very slowly *in vitro*. No mass loss could be detected after three years *in vitro* degradation [32]. Tyrosine-derived polycarbonates were not found to be associated in "acid dumping" or the release of acidic residues found during the degradation of poly(D,L-lactic acid) [32].

The degree of surface hydrophobicity is related to the length of the alkyl ester pendent chain, with the polymer carrying longer alkyl ester pendent chains being more hydrophobic [29]. The least hydrophobic polycarbonate (having a short ethyl ester pendent chain) was a more stimulating substrate for cell growth than the more hydrophobic polymers (carrying longer alkyl ester pendent chains) [29]. Surface hydrophobicity has proven to contribute to reduced swelling during the degradation process compared to poly(alpha-hydroxy acids) [32].

Bioactive glass (BAG) composites as possible fillers in tissue defects have been studied since 1970s [33]. It has been proven that bioactive glass either as bulk or as particles can be used as substrates for bone growth [34]. The bone bonding capability of bioactive glasses depends on the concentrations of ions released interacting with surrounding cells/tissues. Certain bioactive glasses in the system Na_2O-K_2O-MgO-CaO-P_2O_5-SiO_2 have proven to be capable to bone bonding. Bioactive glasses in this system differ from commercially available Bioglass® by their K_2O-MgO portions, however, if the MgO concentration is less than 7.8% their bone bonding abilities are similar to the bioactive glasses developed so far [35].

The aim of the present study was to evaluate the use of tyrosine derivative polymer membrane and bioactive glass mesh in the treatment of artificially created defects in rabbit mandible. Poly(desaminotyrosyl-tyrosine-ethyl ester carbonate) (PDTEcarbonate) was selected to the study to test its suitability to guided bone regeneration (GBR). Filling the defects prior to covering them with membrane to improve the bony growth was considered as an advantageous procedure and thus bioactive glass 13-93 was selected to increase the bioactivity in the covered defect.

2. Materials and methods

This study was approved by The Research Animal Committee of University of Helsinki and the Provincial Administrative Board, according to Finnish law.

2.1. Materials

Batches of PDTE carbonates were supplied by Integra LifeSciences Corporation (New Jersey, USA). These polycarbonates having M_w from 200 000 to 220 000 (weight average molecular weight) were prepared according to previously published procedures [36, 37]. Materials were stored in the form of powder at −18 °C temperature prior to processing in airtight containers. Three days before processing, powder was ground in liquid nitrogen to eliminate larger particles. The homogenized powder was dried in vacuum chamber at 53 °C for 48 h. Solid plates (85 × 85 × 3.3 mm) were compression moulded at 165 °C from the raw material powder. Moulded plates were biaxially oriented in one phase to a draw ratio of 2.2 × 2.2 at 75 °C with a plate-stretching machine Karo IV (Brueckner GmbH, Siegsdorf, Germany).

Bioactive glass 13-93 (BAG) (Vivoxid Ltd., Turku, Finland) was spun to fibers, which were prepared to mesh fixed with 3 wt% chitosan (Chitech®, Medicarb, Sweden). Samples were sized 12 × 6 × (2–4) mm (Fig. 1) and used as filling material in the defects on the right side. BAG composition in weight percentages was Na_2O 6%, K_2O 12%, MgO 5%, CaO 20%, P_2O_5 4% and SiO_2 53%.

Manufacturing of all the specimens was done at the Institute of Biomaterials (Tampere University of Technology, Tampere, Finland). All samples were sterilized with gamma irradiation, minimum dose 2.5

Mrad. (Willy Rütsch AG, Kernen-Rommelshausen, Germany).

2.2. Animals

Eighteen adult female New Zealand White rabbits (HsdPoc strain) weighing 2500–3000 g were used as experimental animals. No preoperative fasting was required. The animals were divided in to three groups ($n = 6$) according to the intended healing time (6, 12 and 24 weeks).

2.3. Surgical procedure

Preoperatively, the animals received trimethoprim-sulfadiazine (Duoprim vet®, Schering-Plough, Brussels, Belgium) 0,3 mg/kg subcutaneously (s.c.) for infection phrophylaxis. Anesthesia was induced with medetomidine (Domitor®, Orion Pharma, Turku, Finland) 300 μg/kg and ketamine (Ketaminol vet®, Intervet International, Boxmer, The Netherlands) 25 mg/kg (s.c.).

Mandible was shaved on both sides and skin was rinsed and scrubbed with chlorhexidine digluconate (Klorhexol® 5 mg/ml, Leiras, Turku, Finland). A skin incision was made along the inferior border of the rabbit's mandible. The periosteal flap was lifted and standardized through-and-through defects (12 × 6 mm, 72 mm^2) were created with oscillating saw bilaterally in the mandibular angle (Fig. 2). On the left side the membrane (Fig. 3) (sized 20 × 25 mm) was mounted and wrapped around inferior border of the mandibular angle and fixed superior to the defect with absorbable sutures through holes drilled in the mandible. Periosteal flap was sutured over the implanted membrane and the incision was closed in layers with absorbable sutures (Vicryl® 3–0, Ethicon, Somerville New Jersey, USA).

On the right side , the defect was filled with Bioactive glass (BAG) mesh prior to fixation of the membrane (Fig. 4). In all defects blood clot formation was ensured. For postoperative pain control the animals received 0.02–0.05 mg/kg (s.c.) buprenorphinum (Temgesic®, Schering-Plough, Brussels, Belgium) immediately after the operation and every 12 h for the next two days. For euthanasia, pentobarbital (Mebunat®, Orion Pharma, Turku, Finland) 30 mg/kg was used intravenously (i.v.).

2.4. Radiographical analysis

Radiographs were taken of all animals 3, 6, 12 and 24 weeks postoperatively. The samples from 12 and 24 weeks were also radiographed with a "standard pearl Ø 5 mm" after dissection, using Heliodent DS® (Siemens Co, Munich, Germany) unit at 60 kV, 7 mA and 0,005 s on Digora® digital imaging plate (Soredex Co, Helsinki, Finland). The imaging plate was read by Digora FMX® (Soredex Co, Helsinki, Finland) laser scanner. On the digital radiographs a specialist in oral radiology[2,5] marked areas of complete or partial ossification using Adobe Photoshop®7 (Adobe System Inc, San Jose, USA) software. The marked areas were then calculated with Matlab®(MathWorks Inc, Natic, USA) and divided into two groups: radiographically completely non-ossified area and completely non-ossified area together with partly ossified area (where bone mineralization is not yet radiologically complete).

2.5. Statistical evaluation

All 12 and 24 week specimens were included in statistical analysis. Differences between non-ossified areas on the left and right side were evaluated using paired samples T-test.

3. Results

3.1. 3 weeks

At three weeks all the wounds had healed properly and clinically no signs of infection were seen. All animals were eating normally. Plain radiographs were taken. However, no reliable conclusions regarding the ossification could be made based on these radiographs.

3.2. 6 weeks

Clinically no signs of infection were seen. After dissection a small clear fluid filled cyst was seen in the left mandible of one rabbit (membrane only). This, however, did not seem to have any significant effect on the ossification process evaluated from the plain radiographs. No reliable conclusions about the size of ossificated defect area could be made from the plain radiographs.

3.3. 12 weeks

Clinical inspection revealed no signs of infection. Radiographical analysis of the specimens ($n = 6$) revealed that on the right side (membrane + BAG) 13.6% (range 0.0-26.9%) of the defect area remained non-ossified and 44.6% (range 12.3–84.7) was fully ossified. On the left side (membrane only) 4.5% (range 0.0–16.6%) was non-ossified and 58.7% (range 30.7–100%) was fully ossified. The mean difference between non-ossified areas calculated was 6.6 mm^2 (standard deviation 4.0), which was statistically significant ($p < 0.05$).

3.4. 24 weeks

No signs of infection were clinically evident in any animal. Radiographic analysis of the specimens ($n = 6$) revealed on the right side (membrane + BAG) (Fig. 5.) 10.7% (range 0.0–31.4%) of the defect area was non-ossified and 47.1% (range 27.7–100%) was fully ossified. On the left side (membrane only) (Fig. 6.) 0.8% (range 0.0–5.1%) was non-ossified and 58.5% (range 44.3–100%) was fully ossified. The mean difference of non-ossified areas calculated was 7.1 mm^2 (standard deviation 4.1), which was statistically insignificant ($p > 0.05$). Summary of the radiographical analysis is presented in Fig. 7.

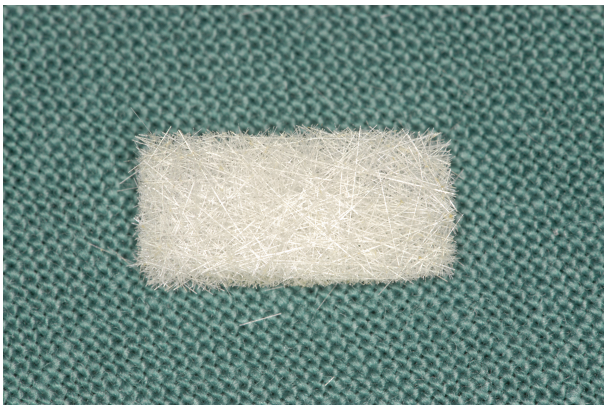

Figure 1 Bioactive glass 13-93 mesh with 3 wt% chitosan.

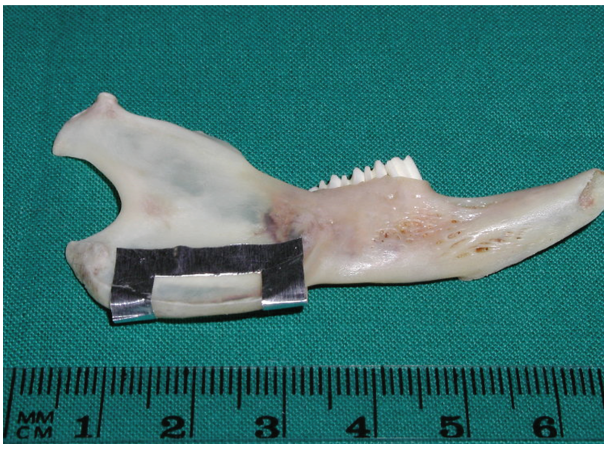

Figure 2 Rabbit mandible and the template used to create standardized defects.

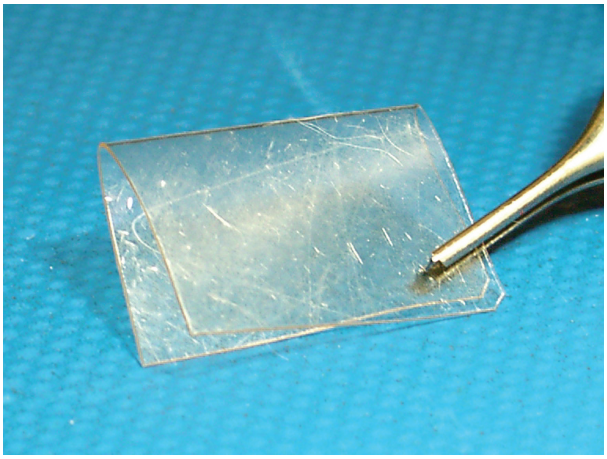

Figure 3 Folded PDTE carbonate membrane.

4. Discussion

In the current study, poly(desaminotyrosyl–tyrosine - ethyl ester carbonate) (PDTEcarbonate) was selected due to previously experimented mechanical properties and processability (unpublished data). The membrane showed excellent bending and handling properties at the operation. Edges of the membrane could be rounded with scissors and needle perforated it easily. Blood clot formation and correct positioning of the membrane could be ensured because the membrane was transparent.

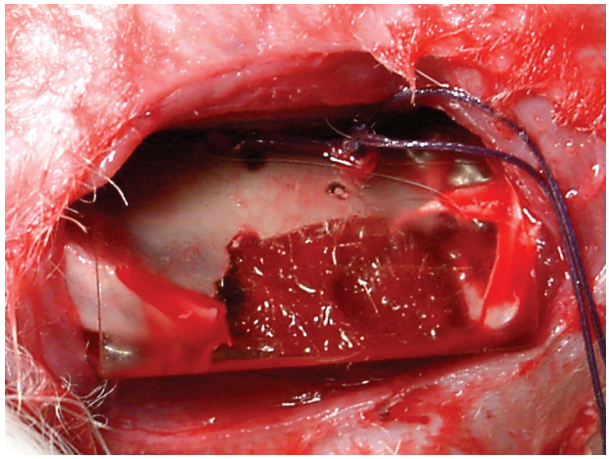

Figure 4 Implanted PDTE carbonate membrane and bioactive glass 13-93 mesh with 3 wt% chitosan fixed with absorbable suture. Correct placement and bioactive glass is easily seen through the transparent membrane.

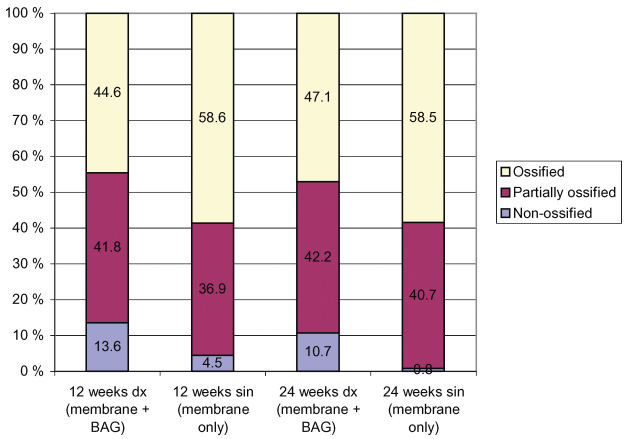

Figure 7 Summary of the radiographical analysis after 12 and 24 weeks of healing.

Most of the bioactive glass formulations are not processable and thus need to be used either as larger blocks or as crushed particles [34]. From certain glass recipies, including the bioactive glass 13-93, it is possible to manufacture for example fibres [38]. The mesh manufactured for the current study tended to disintegrate when in contact with blood. Thus it was more difficult to handle and place, compared to the membranes.

Clinically all animals went through an uneventful healing process except one, which due to wound dehiscence, needed two additional sutures in the mandible on the first postoperative day. All animals started eating normally within 24 h after the operation. This suggests that no excessive functional damage was caused by the operation.

Plain radiographs revealed little of the actual ossification due to the difficulties in positioning rabbit's mandible at the right angle. This was why another technique was chosen to further study the animals. One animal died due to sedation complication at three weeks in radiographic examination.

Postmortem digital radiographical analysis of the non-ossified area suggested, surprisingly, that no additional benefit is gained at 12 weeks when using bioactive glass with PDTE carbonate membrane. On the

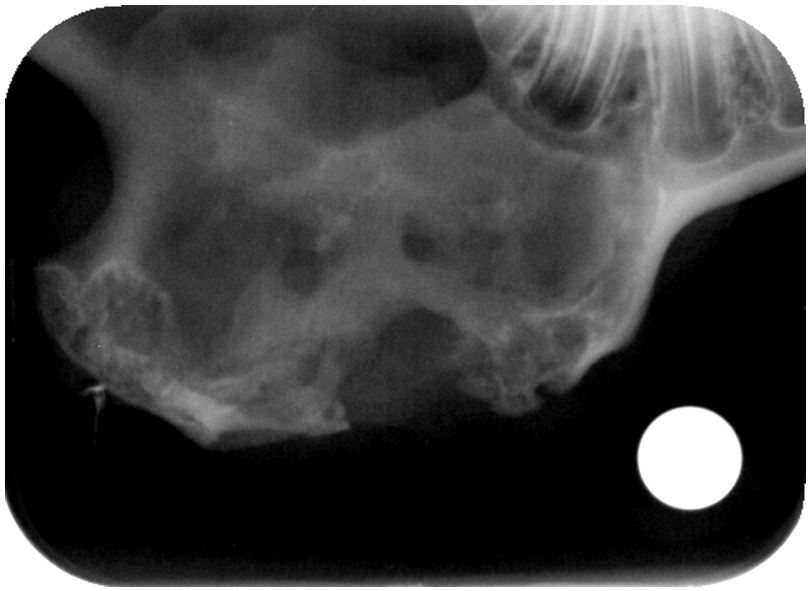

Figure 5 Digital radiograph from the right side (membrane + BAG) at 24 weeks. Mineralization of the defect is not yet complete.

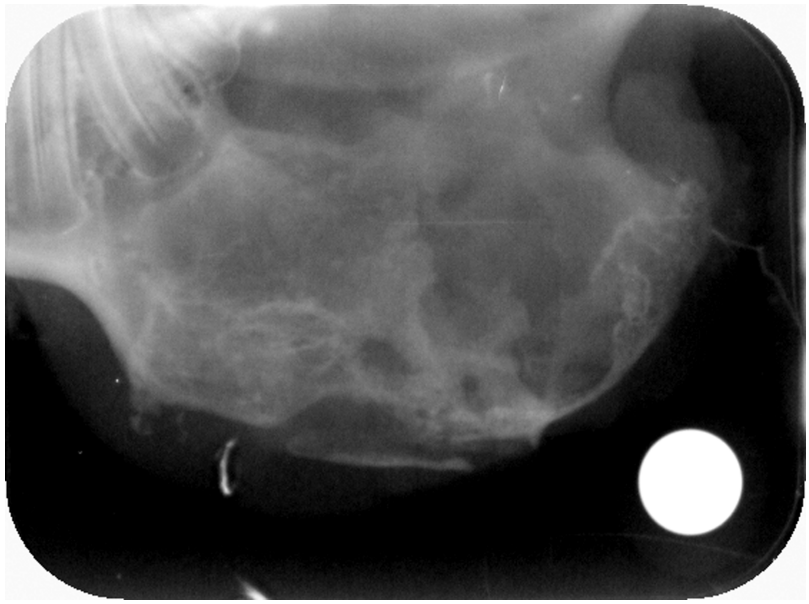

Figure 6 Digital radiograph from the left side (membrane only) at 24 weeks. The defect is almost completely ossified and it seems as if bone is growing along the membrane.

contrary, it seems that BAG slowed down mineralization when it was used. Partially mineralized or non-ossified area remained almost at the same level whether BAG was used or not. According to the statistical evaluation the difference between the non-ossified areas was statistically significant ($p < 0.05$) at 12 weeks.

At 24 weeks completely ossified area remained approximately at the same level as at 12 weeks. However, the non-ossified area on the left side (membrane only) diminished to almost zero (on the average 0.84%). On the right side (membrane + BAG), non-ossified areas remained approximately at the same level as at 12 weeks. The difference between non-ossified areas on the right and left sides was statistically insignificant ($p > 0.05$).

Ossification of defect areas was calculated from digital radiographs taken perpendicular to the buccal side of the mandibular angle. The tube was applied as close to the mandible as possible. This should give an optimal result especially when the dissected areas were radiographed. The final results were calculated from radiographs of dissected mandibles. The fact that marking of the partly and non-ossified areas was done manually on the screen can, however, cause some inaccuracy.

As shown above, the results from this study indicate that the ossification of the defect area proceeded well in both groups, but BAG seemed to slow it down. The reason(s) for this unexpected phenomenon remain unsolved in this. One reason for this is that thin bioactive glass fibres may resorb too fast to obtain an optimal healing response in bone. This can be confirmed by further studies comparing differently sized bioactive glass particles and fibres. It may also be that BAG resorption products while trapped in the defect under the slowly absorbable covering membrane, may exceed the capacity of the tissues to effectively clear metabolites

and thus slow down the mineralization. Third theory is that otherwise beneficial and bone growth enhancing ion release from BAG [39] may influence the osteoblasts' production of regulating factors as well as covering the defect may concentrate the regulating factors turning the effect to a negative one. Confirming of these assumptions need to carried out in further experiments where more set-ups (for example, comparison of an empty defect to BAG filled and membrane covered BAG defects) need to be cross-checked.

Studies of tyrosine derivatives for medical purposes have been in progress since 1987 [25]. However, to our knowledge, this is the first time a PDTE carbonate membrane was used to cover segmental bone defects. Our study suggests that the use of this material could benefit the ossification in bone defects without additional augmentation materials. Further studies are naturally needed to confirm its behavior in larger animals. Also, long-term behavior in healed tissues needs to be studied because tyrosine derivatives degrade very slowly [32].

Acknowledgments

Support received from the Finnish Dental Society Apollonia and the European Commission (European Union R&D Project QLRT-2000-00487) are greatly appreciated. The authors thank Mr. Juha-Matti Perkkiö for his assistance with Matlab program and Mr. Pasi Ohtonen, MSc, for statistical analysis.

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Received 6 April
and accepted 17 December 2004