Implant Placement Enhanced by Bioactive Glass Particles of Narrow Size Range

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Poor bone quality and quantity are often related to implant failure. Synthetic bone grafts may be used to enhance the formation of new bone in bone defects. The purpose of this animal study was to determine the efficacy of bioactive glass particles of narrow size range (300 to 335 µm, Biogran) in the treatment of bone defects prior to implant placement. On both sides of the mandible of six beagle dogs, areas of partial edentulousness were created by the removal of the intra-alveolar septa to obtain large defects, instantly filled on one side with bioactive glass particles. The other side was left empty as a control. After a healing period of 4 months, three oral implants each were placed in the glass-treated area and in the control zone. In three dogs, the implants were left subgingival for 3 months after which histologic sections were made. In the remaining three dogs, the implants were functionally loaded with a fixed partial prosthesis for 7 weeks before sacrifice. Qualitative and quantitative analysis of both groups revealed statistically significantly more bone tissue and higher remodeling activity at the interface and at a distance of implants placed in glass-treated areas, compared to implants placed in untreated regions. Implant placement in bioactive glass-filled defects was not jeopardized, on the contrary.
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Key words: beagle dog, bioactive glass, bone remodeling, fixed partial prosthesis, fluorescence microscopy, histomorphometry, occlusal loading, oral implants

A prerequisite for successful placement of oral implants is the preparation of a congruent implant bed resulting in tight adaptation of the bony bed to the implant surface.[1,2] Bone defects around oral implants are often seen when implants are placed in areas with inadequate alveolar bone, ie, dehiscence defects, fenestration defects, residual intraosseous defects, in extraction sockets, or around failing implants.[3,4] Bone regeneration in these defects by means of bone grafts or substitutes may improve the long-term prognosis of the implant.

Guided tissue regeneration and bone fillers of various sizes and origins have been used to promote bone formation in osseous deformities, either before or in conjunction with endosseous implant placement.[5–8] Bioactive glass is a ceramic that can be used as a particulate material. Previous animal experiments revealed a superior response to bioactive glass particles of narrow size range (300 to 355 µm) (Biogran, Orthovita, Malvern, PA) compared to hydroxyapatite (HA) granules (Calcitite, Calcitek, San Diego, CA, and Interpore-200, Interpore International, Irvine, CA).[9] More osteoconductive bone growth starting from the wall of the defects was seen around the bioactive glass particles than around the HA particles. In addition, trabecular bone growth was observed in the center of the defect. These bone trabeculae were associated with bioactive glass particles, which exhibited an osteophilic nature, while mostly fibrous tissue separated the bone tissue from the hydroxyapatite particles. It was clearly demonstrated that the bioactive glass particles of narrow size range showed an internal erosion via small cracks. In these protective pouches new bone tissue that was not connected to any external bone tissue was observed. Bioactive glass particles of narrow size

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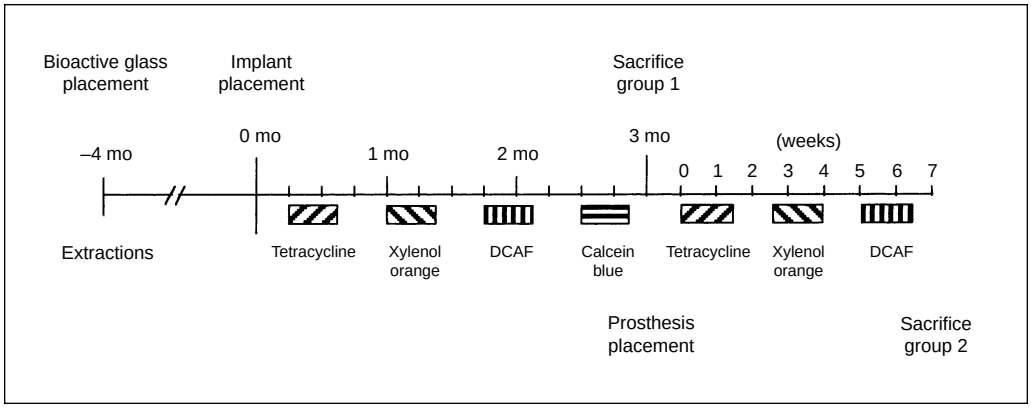


Fig 1 Timeline of the animal experiment, indicating the times of tooth extraction and bioactive glass placement at selected sites (–4 months), implant placement (0 months), subgingival healing period of 3 months with four fluorescent labeling intervals (tetracycline, xylenol orange, DCAF, and calcein blue), sacrifice of group 1 (3 months), prosthesis placement in group 2 (3 months), functional loading period of 7 weeks with three fluorescent labeling intervals (tetracycline, xylenol orange, DCAF), and sacrifice of group 2 (3 months and 7 weeks).

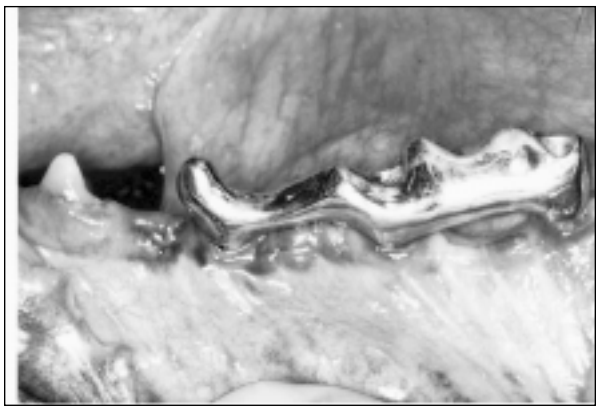


Fig 2 Fixed partial prosthesis supported by three implants in the mandible of one of the beagle dogs in group 2.

range produced differentiation of osteoprogenitor cells into osteoblasts. The islands of newly formed bone functioned as nuclei for enhanced repair.

The purpose of this animal study was to determine the efficacy of bioactive glass particles of narrow size range (300 to 355 µm, Biogran) in the treatment of bone defects prior to implant placement. Bone formation was evaluated quantitatively and qualitatively after a subgingival healing period of 3 months and an additional functional loading period of 7 weeks.

Materials and Methods

Surgical Procedure. Partially edentulous areas were created on both sides of the mandibles of six beagle dogs by extraction of all premolars and the first molar (Fig 1). The intra-alveolar septa were removed to obtain large defects. On one side, these defects were immediately filled with bioactive glass particles (Biogran), and on the other side they were left empty as a control, which is the procedure in most clinical situations. After a healing period of 4 months, three IMZ implants (Friatec AG, Mannheim, Germany), 10 mm in length and 3.3 mm in diameter, were placed both in the glass-treated (test) areas and in the untreated (control) zones. The implants were placed with the use of slowly rotating inner-cooled burs of progressive diameter under coverage of a long-lasting penicillin (Penadur LA, SmithKline Beecham Pharma, Genval, Belgium).[10] All surgical treatment was performed under premedication consisting of an intramuscular neuroleptic analgesic (Thalamonal, Janssen Cilag, Berchem, Belgium), followed by intravenous anesthesia with a pentobarbiturate (Nembutal, Ceva under license from Abbott Laboratories, Brussels, Belgium).

After 3 months of subgingival healing, three animals were sacrificed with an overdose of pentobarbiturate (group 1). The remaining three animals received a fixed partial prosthesis, and the implants were functionally loaded for 7 weeks before sacrifice (group 2). These prostheses, cast in silver-palladium alloy (Pallorag 33, Cendres and Métaux, Biel, Switzerland), had an average length of 38.02 mm (SD = 1.86 mm) with an average mesial extension of 9.5 mm (SD = 3.19 mm) and were placed on rigid titanium connectors without intramobile elements (Fig 2). Corrections were made to distribute the occlusal loads equally over the prostheses and the natural teeth.

Histologic Processing. Undecalcified serial sections were made labiolingual and mesiodistal to each implant as described earlier.[11,12] These sections were ground and polished to a thickness of 50 to 60 µm. Bone formation and remodeling around the implants were evaluated histologically on a qualitative and quantitative basis. Some sections were stained with a combination of Stevenel's blue and Von Gieson's picro-fuchsin in preparation for qualitative light microscopic analysis.

Histometric Assessments. The intravenous sequential administration of different fluorochrome labels during the experiment allowed quantification of the bone formation and remodeling around the implants (Fig 1). Four labels were injected in group 1 and three in group 2 three times each in the course of 1.5 weeks. An interval of 1.5 weeks and 1 week, respectively, was provided between the injection of two consecutive labels to prevent overlap of the fluorescent bands.[13,14] The first label, oxytetracycline hydrochloride (bulk) powder (25 mg/kg body weight) dissolved in a 0.9% sodium chloride solution of 10 mL in addition to vitamin C, had a yellow color.[12] The second label, xylenol orange (UCB 3104, Vel, Leuven, Belgium) prepared as a 3% solution of 90 mg/kg body weight in distilled water, was red.[15] The third label, DCAF (4',5'-bis[N,N-di(carboxymethyl) aminoethyl] fluorescein) (Fluka Chemie AG, Switzerland) (20 mg/kg body weight) dissolved in 0.5 N potassium hydroxide in distilled water with a concentration of 100 mg DCAF per 7.5 mL, was green.[16] The last label consisted of calcein blue (Fluka Chemie AG) (20 mg/kg body weight) prepared as a 3% solution in 2% sodium bicarbonate.[14]

Histomorphometry was performed manually using a highly sensitive, color video camera (JVC TK-1085E, Heta, Japan) and a high resolution video monitor (JVC TM-1500PS) to which a 10-µm precision scale was attached. For each 10 µm, the kind of tissue (fibrous tissue, nonremodeled and remodeled bone tissue fluorescing in various colors) was determined at the mesial, distal, buccal, and lingual interface and at a distance of 3 mm from the mesial and distal interface. For each region, the total amounts of the different tissues were converted to percentages and processed by dividing the coating of the implants into a coronal, middle, and apical third. The marginal bone level, defined as the first marginal bone contact relative to the titanium plasma-spray coating (upper border is 0 value), was histometrically measured at four sites around the implants. The intra- and interreproducibility of these measurements were confirmed in an earlier study, in which a similar histomorphometric technique was used.[17] The intensity of the remodeling at the interface and at a distance over time was derived from the respective proportional amounts of remodeled bone tissue at the interface and at a distance of each separate label.

Statistical Analysis. Each implant was considered the unit for measurement, and therefore a mean value was calculated for four interfacial surfaces (mesial, distal, vestibular, and lingual) and for the two distant registrations (mesial at a distance and distal at a distance) for both the test and the control groups. The data were analyzed using a linear mixed model, taking into account the correlation between measurements from the same animal or implant. The implant was taken as a random factor. All analyses were performed with SAS, Proc Mixed, version 6.12. The level of significance was determined as $P < .05$.

Results

Surgical and labeling treatments were uneventful. The bioactive glass particles formed a cohesive mass when wetted with blood, which allowed very easy manipulation and packing into the extraction sockets. Oral implant placement in the glass-treated area was possible because these granules transformed fully and their hardness was similar to that of the surrounding bone tissue. The vascularization of the glass-treated implant bed was more pronounced than on the control side. The primary stability of all implants was excellent, and was histologically confirmed by intimate contact of all implants with the surrounding bone tissue.

Histometric Results After the 3-Month Subgingival Healing Period. The use of bioactive glass prior to implant placement resulted in a statistically significant ($P = .0340$) higher mean marginal bone level (+0.6 mm; SE = 0.29): –0.5 mm (SD = 0.71) for the test implants and –1.1 mm (SD = 1.38) for the control sites under the titanium plasma-spray coating border of the neck of the implant.

Also, statistically significantly ($P = .0001$) more interfacial bone tissue was measured around implants placed in the test sites than those in the control sites. Implants placed in glass-treated sites showed 51.3% interfacial bone tissue, or 52.1% (SE = 3.56) more than at the control sites. At a distance of 3 mm from the implant surface, 134.7% (SE = 4.31) more bone tissue was found in the test sites than in the control sites. This difference was statistically significant ($P = .0001$) (Fig 3 and Tables 1 and 2). The statistically significant differences between the test and the control sites at the interface and at a distance were found in the coronal, middle, and apical one-third parts of the implants.

Approximately 90% of the interfacial bone tissue and more than 30% of the distant bone tissue remod-

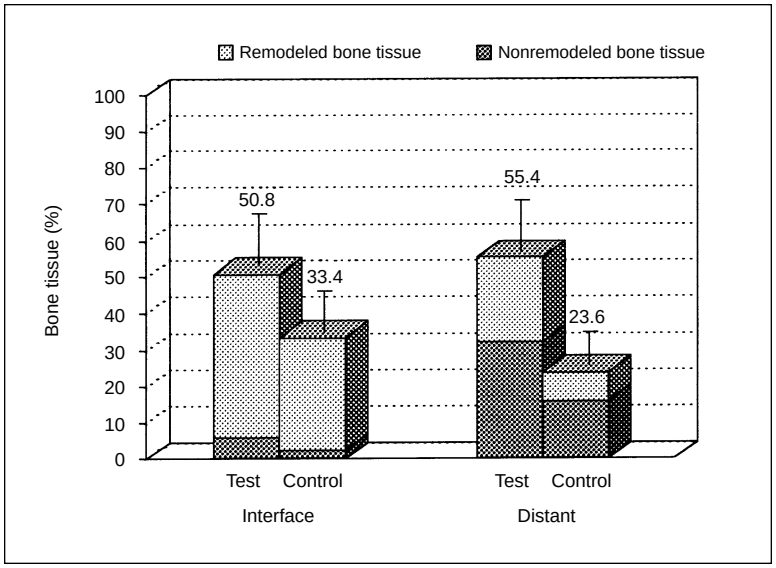


Fig 3 Mean amounts of nonremodeled and remodeled bone tissue at the interface (mean of the mesial, distal, buccal, and lingual interfacial values) and at a distance of 3 mm from the interface (mean of the mesial and distal distant values) of implants placed in bioactive glass-treated (test) sites and in untreated (control) sites after 3 months of subgingival healing. Values and variations of the sum of nonremodeled and remodeled bone tissue are indicated.

eled at the test and control sites. The test sites showed approximately 50% and 200% more remodeled bone tissue than the control sites at the interface and at a distance, respectively (Fig 3). Over time, statistically significantly more bone tissue was in remodeling at 2.5 weeks, 5.5 weeks, 8.5 weeks, and 11.5 weeks both at the interface and at a distance in the test sites than was at the control sites (Fig 4 and Table 2). Most intense remodeling processes were activated within the first 2 weeks to 1 month after implant placement, after which remodeling strongly decreased (Fig 5).

Histometric Results After an Additional 7-Week Functional Loading Period. Additional functional loading of the implants did not result in a statistically significant (P = .9404) difference in the marginal bone level between the test and control sites. The first mean marginal bone contact was found –0.7 mm (SD = 0.69) under the titanium plasma-spray coating border for the implants in the test sites, and –1.1 mm (SD = 0.75) for the implants in the control sites.

However, the additional occlusal loading did result in a mean of 36.0% (SE = 3.56) more interfacial bone contact in the test regions (57.1%, SD = 29.15) than in the control regions (42.0%, SD = 22.13). At a distance of 3 mm from the implant surface, a mean of 77.5% more bone tissue was found in the test sites (61.6%, SD = 27.95) than in the control sites (34.7%, SD = 26.23) (Fig 6 and Table 3). These differences were statistically significant (P = .0001) (Table 4). As in group 1, in group 2 the statistically significant differences between the test and control sites at the interface and at a distance were found in the coronal, middle, and apical one-third parts of the implants.

Approximately one half of the interfacial bone tissue and only one tenth of the distant bone tissue in the test and control zones remodeled during the loaded period (Fig 6). Over time, the remodeling activity was most intense during the first 2 weeks of loading, and then it decreased rapidly. The interfacial and distant remodeling activity proceeded parallel for the implants in the test and the control regions (Fig 7). Except at the interface at 1.5 weeks, the test sites did not remodel statistically significantly more than the control sites (Table 4).

Light Microscopic Evaluation. After 3 months of subgingival healing, bone growth in the control areas started from the surrounding cortical and trabecular bone structures toward the implant surface. This resulted in increased interfacial bone contact at the coronal part of the implant (Fig 8a). The interfacial bone contact in the middle and apical parts of the implant was established by preexisting bone trabeculae contacting the implant surface, with limited osteoconductive bone growth. The trabecular bone remained rather thin. Additional functional loading increased this osteoconductive bone growth (Fig 8b), although the surrounding bone trabeculae remained rather thin.

After 3 months of healing, advanced bone growth was observed in the test regions (Fig 9a). Strong osteoconductive bone growth was seen around the glass particles, which enlarged the bone contact area with the implant and substantially thickened the cortical bone structure. The upper parts of the spongiosa turned into a dense, almost cortical bone-like structure, by which many particles were entirely engulfed. The glass granules are used as a scaffold for osteoconductive bone growth. Functional loading further

Table 1 Mean (and SE) Amounts (%) of Nonremodeled (NR) and Remodeled (R) Bone Tissue at Various Sites Around Subgingivally Healed Implants

		Mesial interface		Distal interface		Buccal interface		Lingual interface		Mesial at a distance		Distal at a distance	
		Test side	Control side	Test side	Control side	Test side	Control side	Test side	Control side	Test side	Control side	Test side	Control side
Animal 1, implants subgingivally healed for 3 months													
Implant 1	NR	2.7 (4.62)	2.7 (3.06)	0.0 (0.00)	1.7 (2.08)	5.3 (4.73)	1.7 (2.89)	4.7 (7.23)	1.7 (2.89)	21.3 (10.97)	4.7 (1.53)	50.3 (12.58)	20.7 (7.51)
	R	47.3 (19.35)	41.7 (15.95)	40.0 (38.43)	35.0 (30.64)	49.7 (43.66)	21.0 (15.00)	38.0 (9.17)	70.3 (1.15)	7.7 (0.58)	7.3 (3.51)	15.0 (3.00)	7.7 (2.52)
Implant 2	NR	10.7 (1.53)	3.3 (3.06)	4.7 (6.43)	0.7 (1.15)	2.0 (2.65)	5.7 (9.81)	5.7 (6.03)	7.7 (7.09)	31.7 (23.12)	6.3 (8.39)	17.0 (20.81)	16.7 (25.48)
	R	65.0 (19.16)	50.0 (20.66)	54.7 (37.29)	29.0 (24.52)	29.0 (33.72)	29.7 (15.31)	49.3 (24.50)	45.7(16.29)	24.0 (22.87)	10.3 (12.74)	14.7 (9.45)	9.7 (9.81)
Implant 3	NR	1.7 (2.89)	0.3 (0.58)	7.3 (7.51)	1.3 (2.31)	7.0 (3.61)	0.0 (0.00)	2.7 (3.06)	4.0 (6.93)	17.0 (20.81)	11.7 (10.41)	52.3 (30.35)	16.0 (27.71)
	R	48.3 (33.38)	5.0 (8.66)	51.3 (17.01)	7.0 (12.12)	33.7 (14.74)	2.0 (2.65)	30.0 (11.14)	19.7 (11.85)	14.7 (9.45)	4.7 (6.43)	11.0 (9.54)	0.7 (1.15)
Animal 2, implants subgingivally healed for 3 months													
Implant 1	NR	1.0 (1.73)	0.3 (0.58)	2.7 (4.62)	0.3 (0.58)	5.3 (9.24)	1.0 (1.73)	2.7 (4.62)	2.0 (3.46)	21.3 (25.93)	11.0 (10.54)	42.3 (36.67)	22.0 (12.77)
	R	77.3 (9.29)	30.0 (30.00)	40.0 (35.55)	29.3 (15.31)	41.7 (31.07)	34.3 (47.08)	53.3 (47.06)	50.0 (12.12)	50.0 (19.52)	8.7 (10.69)	10.7 (9.45)	7.7 (3.51)
Implant 2	NR	7.0 (12.12)	5.3 (2.08)	9.3 (8.62)	3.0 (5.20)	4.0 (5.29)	0.7 (1.15)	3.0 (5.20)	1.7 (1.53)	28.3 (35.39)	22.3 (14.43)	33.0 (7.00)	17.7 (15.50)
	R	49.0 (14.73)	47.7 (30.62)	34.7 (37.54)	23.7 (22.03)	47.3 (32.33)	33.0 (28.93)	49.7 (30.02)	40.3 (21.22)	11.3 (4.16)	10.0 (4.36)	45.0 (14.11)	6.0 (7.21)
Implant 3	NR	22.0 (6.24)	1.3 (0.58)	10.7 (11.02)	0.0 (0.00)	0.3 (0.58)	5.7 (8.96)	3.0 (5.20)	4.0 (6.93)	30.0 (33.41)	25.0 (31.22)	40.3 (35.73)	13.3 (11.93)
	R	32.7 (25.32)	42.3 (4.93)	41.3 (37.69)	28.3 (26.69)	38.7 (27.02)	27.3 (27.02)	61.0 (27.00)	40.3 (9.02)	38.0 (18.36)	6.7 (7.64)	18.3 (16.50)	5.0 (4.58)
Animal 3, implants subgingivally healed for 3 months													
Implant 1	NR	17.3 (11.68)	4.3 (7.51)	4.0 (6.93)	5.0 (8.66)	4.0 (4.00)	0.0 (0.00)	10.7 (18.48)	2.7 (3.06)	21.3 (13.65)	21.3 (17.04)	22.7 (16.44)	14.7 (16.17)
	R	28.0 (20.66)	16.0 (21.93)	54.0 (40.85)	25.3 (20.03)	31.7 (27.47)	27.7 (15.14)	55.7 (33.84)	52.3 (25.40)	16.3 (13.01)	7.3 (4.93)	52.7 (17.21)	4.3 (5.13)
Implant 2	NR	7.0 (12.12)	5.3 (7.57)	3.0 (3.00)	0.7 (1.15)	4.3 (5.13)	0.3 (0.58)	6.3 (4.93)	0.7 (1.15)	29.7 (21.39)	18.7 (2.52)	36.0 (19.97)	3.0 (5.20)
	R	41.7 (39.07)	35.7 (15.31)	49.3 (40.62)	13.0 (11.27)	24.0 (26.06)	7.3 (11.85)	31.3 (17.67)	34.0 (9.54)	21.3 (8.14)	16.7 (5.13)	32.0 (33.15)	11.0 (9.17)
Implant 3	NR	12.3 (10.79)	1.3 (2.31)	3.7 (4.04)	4.3 (4.51)	3.3 (5.77)	3.0 (3.61)	10.3 (11.68)	4.3 (5.86)	46.3 (22.81)	20.7 (22.81)	37.7 (11.06)	20.0 (23.58)
	R	45.3 (16.50)	23.0 (19.92)	56.0 (13.00)	21.3 (20.13)	41.0 (37.59)	21.7 (21.50)	61.3 (5.51)	44.0 (23.81)	21.7 (13.01)	8.0 (10.39)	13.7 (12.66)	11.0 (7.00)

Table 2 Probability of the Difference in Amounts of Nonremodeled and Remodeled Bone Tissue at the Interface and at a Distance Between Treated and Control Areas in Group 1

	P values	
	Interface	Distant
Nonremodeled and remodeled bone tissue	.0001	.0001
Nonremodeled bone tissue	.0863	.0001
Remodeled bone tissue	.0001	.0001
Remodeled bone after healing:		
2.5 weeks	.046	.0001
5.5 weeks	.0002	.0001
8.5 weeks	.0001	.0001
11.5 weeks	.0007	.0026
Marginal bone height	.034	—

stimulated this tendency (Fig 9b). Toward the implant surface, the number and size of these glass particles decreased. No direct contact of glass granules with the implant surface was observed. Near the implant surface, almost all of the granules had a reduced diameter and widened entrance to the internal lumen of the granules (Fig 10). Some of the glass granules disintegrated completely. At a greater distance from the interface, the granules showed their characteristic internal excavation, in which bone formation independently from the surrounding bone tissue could be observed (Fig 11). This indicates that bone growth also started from the internal protective pouches, which acted as nucleation sites for enhanced bone repair.[11] Only glass granules that were embedded entirely in fibrous tissue occasionally demonstrated phagocytosing cells, next to the more frequent active and resting osteoblasts (Fig 12).

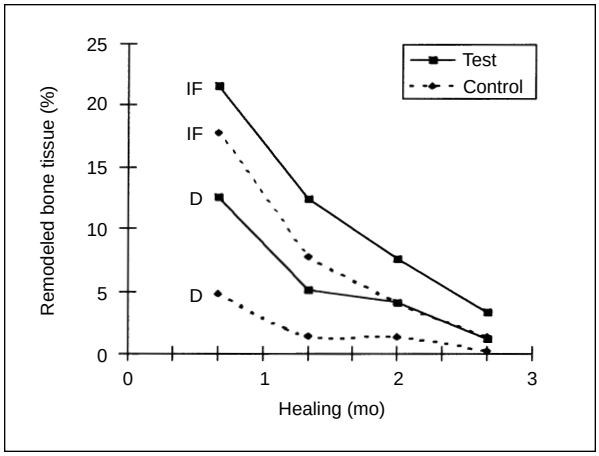


Fig 4 Amount of remodeled bone tissue at the interface (IF) and at a distance of 3 mm (D) for implants placed in bioactive glass-treated (test) areas and in untreated (control) areas during the 3-month subgingival healing period.

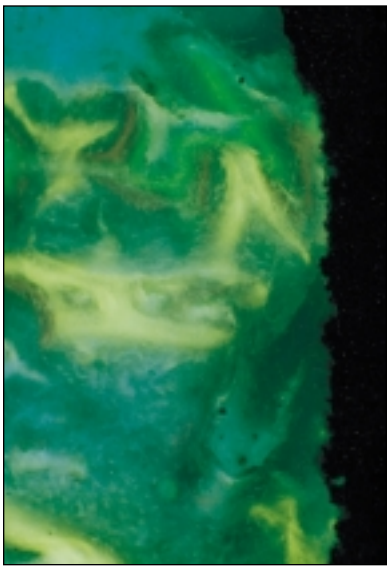


Fig 5 Representative intense remodeling in the cortical bone at the marginal border of an implant placed in a glass-treated site after the 3-month subgingival healing period. The four fluorescent bands are clearly visible: yellow, red, green, and blue (fluorescence microscopy; original magnification × 100).

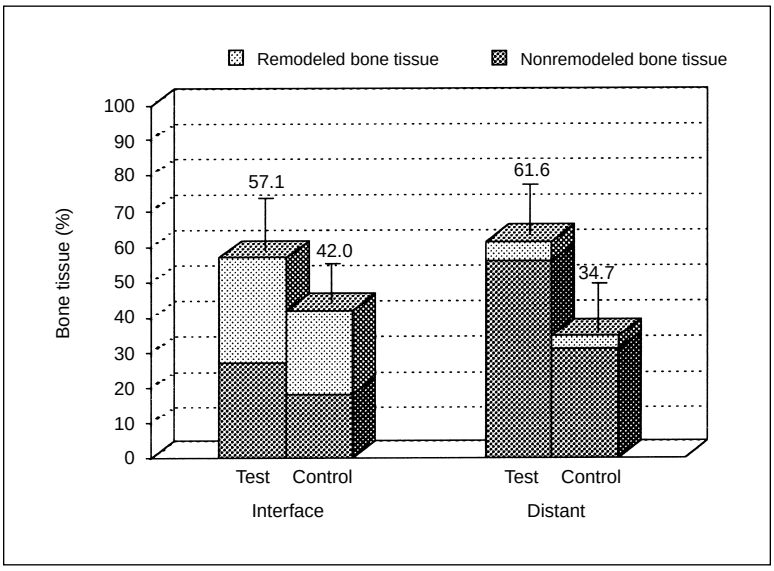


Fig 6 Mean amounts of nonremodeled and remodeled bone tissue at the interface (mean of the mesial, distal, buccal, and lingual interfacial values) and at a distance of 3 mm from the interface (mean of the mesial and distal distant values) of implants placed in bioactive glass-treated (test) sites and in untreated (control) sites after 3 months of subgingival healing and 7 weeks of loading. Values and variations of the sum of nonremodeled and remodeled bone tissue are indicated.

Table 3 Mean (and SE) Amounts (%) of Nonremodeled (NR) and Remodeled (R) Bone Tissue at Various Sites Around Subgingivally Healed and Functionally Loaded Implants

		Mesial interface		Distal interface		Buccal interface		Lingual interface		Mesial at a distance		Distal at a distance	
		Test side	Control side	Test side	Control side	Test side	Control side	Test side	Control side	Test side	Control side	Test side	Control side
Animal 4, implants subgingivally healed for 3 months and functionally loaded for 7 weeks													
Implant 1	NR	12.3 (10.69)	14.0 (15.62)	55.7 (26.10)	17.0 (11.14)	4.0 (6.93)	13.3 (15.95)	33.7 (24.66)	11.3 (4.16)	50.3 (8.08)	24.0 (9.85)	77.3 (18.45)	28.7 (19.66)
	R	58.3 (21.55)	25.0 (13.11)	33.3 (20.31)	22.3 (12.74)	17.7 (15.50)	33.7 (5.86)	33.7 (10.26)	28.3 (10.79)	10.3 (8.62)	6.0 (2.65)	13.3 (10.07)	1.7 (2.08)
Implant 2	NR	8.3 (5.86)	1.3 (2.31)	1.7 (2.08)	12.7 (10.60)	2.0 (3.46)	10.0 (12.12)	16.7 (20.11)	30.0 (28.48)	47.7 (32.02)	20.3 (24.83)	79.0 (15.72)	23.7 (6.11)
	R	31.7 (21.22)	11.7 (10.41)	25.3 (20.84)	16.0 (5.57)	4.3 (7.51)	21.7 (12.86)	35.3 (9.29)	19.0 (6.56)	3.7 (1.53)	2.3 (3.21)	4.0 (3.61)	3.7 (4.04)
Implant 3	NR	34.7 (24.85)	8.0 (8.54)	24.0 (21.00)	8.3 (8.50)	18.7 (16.65)	16.7 (8.08)	34.3 (18.04)	29.0 (29.46)	84.7 (13.87)	74.0 (14.11)	76.3 (25.42)	37.3 (32.39)
	R	37.0 (21.63)	49.7 (22.37)	21.7 (10.50)	14.0 (5.00)	36.0 (30.20)	25.3 (12.34)	43.3 (13.65)	30.3 (26.03)	6.3 (6.03)	3.3 (0.58)	4.7 (1.53)	4.3 (4.04)
Animal 5, implants subgingivally healed for 3 months and functionally loaded for 7 weeks													
Implant 1	NR	46.3 (19.09)	27.0 (22.52)	17.0 (16.52)	20.3 (13.32)	28.7 (38.18)	20.0 (29.60)	18.0 (11.79)	32.7 (8.14)	46.7 (45.37)	41.3 (39.53)	49.0 (25.94)	17.7 (28.88)
	R	44.3 (12.42)	25.0 (9.17)	42.0 (28.93)	17.3 (7.09)	10.0 (10.58)	15.3 (17.24)	25.0 (18.33)	21.7 (4.73)	3.3 (2.89)	2.7 (2.31)	6.7 (5.51)	2.7 (3.79)
Implant 2	NR	33.0 (28.62)	5.7 (8.96)	19.3 (18.15)	16.3 (17.62)	18.0 (16.09)	11.7 (13.87)	23.0 (19.31)	27.3 (18.18)	35.0 (34.51)	24.3 (20.26)	38.0 (6.08)	29.7 (28.29)
	R	23.3 (23.50)	26.7 (18.01)	34.7 (25.79)	19.3 (17.21)	39.0 (23.00)	20.7 (15.70)	54.0 (17.00)	34.3 (6.35)	3.7 (4.04)	2.0 (1.73)	6.3 (7.57)	3.0 (4.36)
Implant 3	NR	34.3 (19.35)	12.3 (18.01)	35.3 (28.68)	41.3 (17.62)	39.0 (20.07)	16.7 (15.28)	32.0 (19.52)	20.0 (23.52)	31.3 (7.02)	16.7 (15.28)	57.0 (25.71)	48.3 (36.61)
	R	26.0 (10.00)	18.3 (15.95)	41.0 (12.12)	23.3 (10.97)	26.3 (15.18)	32.3 (26.54)	20.7 (14.05)	16.3 (10.79)	5.3 (5.86)	8.3 (7.64)	10.7 (5.51)	5.7 (4.04)
Animal 6, implants subgingivally healed for 3 months and functionally loaded for 7 weeks													
Implant 1	NR	14.7 (23.69)	17.3 (10.21)	33.7 (20.13)	7.0 (8.19)	25.3 (30.37)	23.0 (14.53)	25.7 (23.07)	26.3 (29.19)	25.0 (25.51)	11.3 (14.74)	39.0 (27.22)	21.0 (7.21)
	R	22.3 (10.50)	30.7 (24.44)	34.3 (9.29)	15.0 (6.00)	8.0 (9.85)	18.3 (9.87)	27.0 (4.36)	29.3 (20.21)	1.7 (2.08)	1.7 (1.53)	3.3 (2.52)	4.3 (4.16)
Implant 2	NR	23.3 (8.33)	20.3 (5.51)	62.3 (19.86)	14.7 (15.18)	21.3 (19.04)	32.7 (24.01)	37.7 (26.50)	34.3 (26.27)	57.0 (27.87)	14.0 (16.46)	74.0 (22.07)	35.3 (27.54)
	R	42.3 (9.61)	22.0 (2.00)	20.7 (15.95)	20.0 (11.79)	15.3 (17.21)	11.0 (6.24)	28.3 (17.10)	14.0 (4.00)	3.0 (4.36)	4.0 (3.61)	3.3 (2.08)	2.7 (1.15)
Implant 3	NR	33.3 (28.57)	3.3 (1.15)	33.3 (28.57)	25.7 (17.67)	32.0 (29.46)	24.7 (14.05)	46.7 (25.79)	12.0 (14.42)	74.0 (22.07)	31.0 (20.07)	66.7 (25.17)	60.7 (24.34)
	R	34.0 (28.93)	30.3 (20.79)	34.0 (28.93)	25.3 (12.90)	22.0 (13.45)	36.0 (7.55)	24.0 (4.58)	29.7 (13.58)	3.3 (2.08)	5.3 (1.53)	8.0 (5.29)	1.3 (2.31)

Table 4 Probability of the Difference in Amounts of Nonremodeled and Remodeled Bone Tissue at the Interface and at a Distance Between Treated and Untreated Control Areas in Group 2

	P values	
	Interface	Distant
Nonremodeled and remodeled bone tissue	.0001	.0001
Nonremodeled bone tissue	.0001	.0001
Remodeled bone tissue	.0278	.2211
Remodeled bone after loading for:		
1.5 weeks	.0332	.1796
4.0 weeks	.3236	.7122
6.5 weeks	.2460	.7623
Marginal bone height	.9404	—

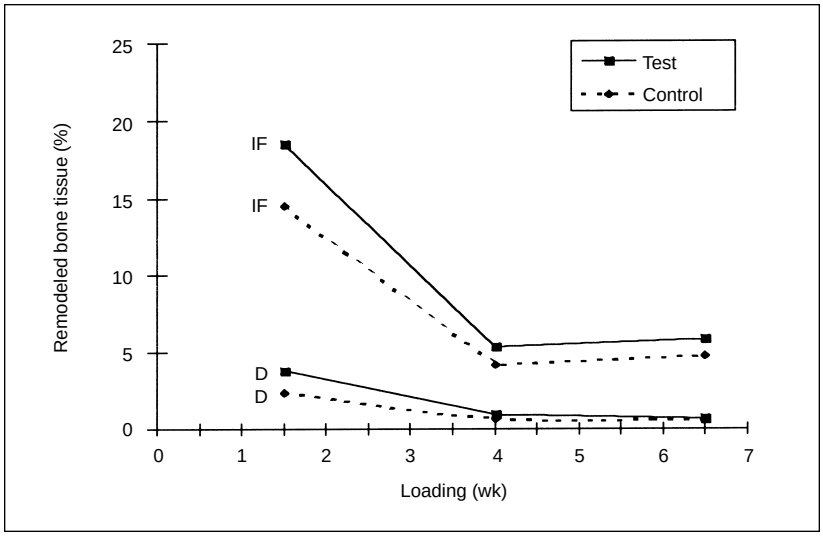
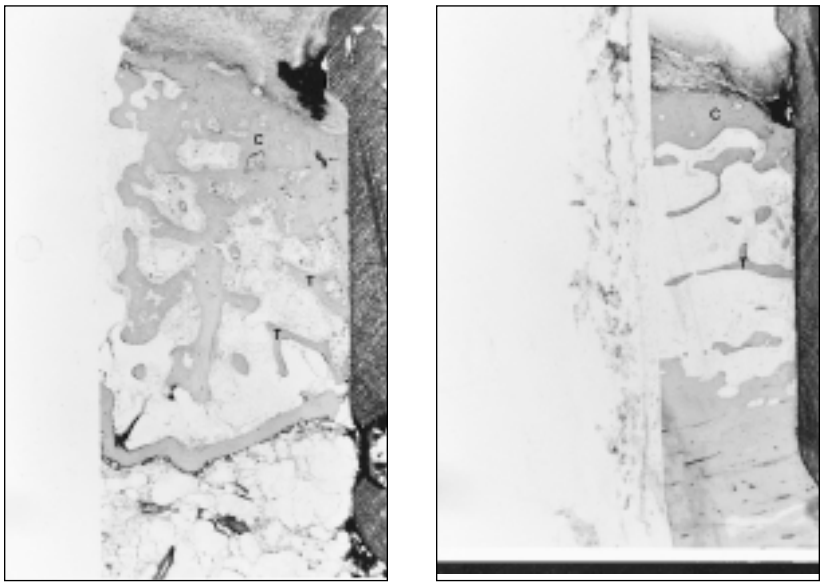
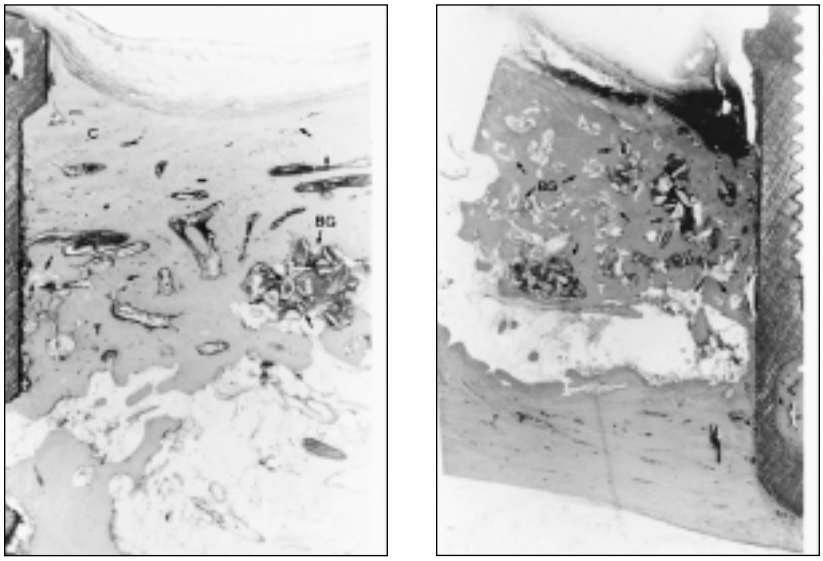


Fig 7 Amount of remodeled bone tissue at the interface (IF) and at a distance of 3 mm (D) from implants placed in bioactive glass-treated (test) areas and in untreated (control) areas during the 7-week functional loading period.



Figs 8a and 8b Bone formation at the distal side of an implant placed in an untreated (control) area after the subgingival healing period of 3 months *(left)* and after the additional functional loading period of 7 weeks *(right)*. Bone growth starts from the surrounding cortical (C) and trabecular (T) bone tissue and proliferates to and along the implant surface. The surrounding cortical and trabecular bone remains rather thin (combined Stevenel's blue and Van Gieson's picro-fuchsin stain; original magnification × 8).



Figs 9a and 9b Considerable bone formation at the distal and mesial side of an implant placed in a glass-treated (test) site after the subgingival healing period of 3 months *(left)* and after the additional functional loading period of 7 weeks *(right)*. Strong osteoconductive bone growth is observed starting from the surrounding cortical (C) and trabecular (T) bone tissue and proliferating towards the implant surface, using the bioactive glass particles (BG, *arrow*) as a scaffold for osteoconduction. The number and size of the glass granules decreases towards the implant surface (combined Stevenel's blue and Van Gieson's picro-fuchsin stain; original magnification × 8).

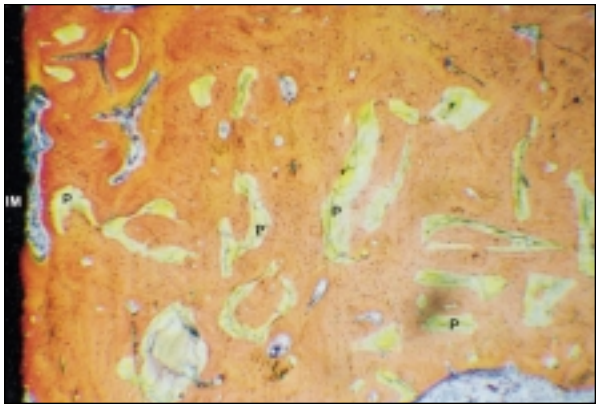


Fig 10 Near the implant surface (mesial side after 3 months of subgingival healing), partially reduced glass particles (P) are entirely encapsulated in the surrounding bone tissue. Because of the intense remodeling at the interface, no direct contact of glass particles with the implant surface (IM) was observed (combined Stevenel's blue and Van Gieson's picro-fuchsin stain; original magnification × 40).

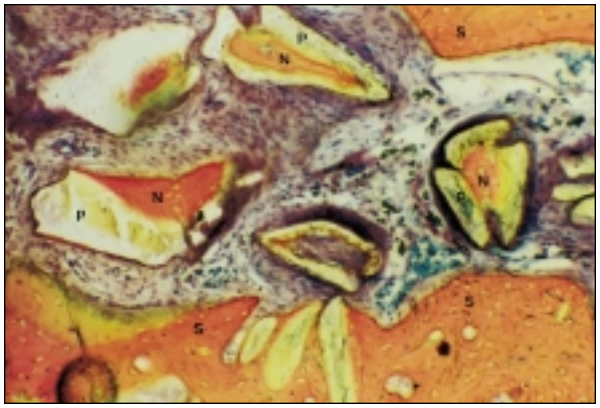


Fig 11 Typical excavation of the bioactive glass particles of narrow size range (P). New bone tissue is formed (N) in the internal eroded protective pouches, independently from the surrounding bone tissue (S). These islands of newly formed bone tissue act as nuclei for enhanced repair (combined Stevenel's blue and Van Gieson's picro-fuchsin stain; original magnification × 100).

Discussion

Filling extraction sockets with bioactive glass granules of narrow size range did not impede the delayed placement of oral implants. After 4 months of healing, a similar, even increased density of the bony implantation bed ensured primary stability of the implants. This was confirmed by Furusawa et al,[18] who measured the microhardness of reacted Biogran granules in human biopsies 7 months after implantation. In contrast to unreacted granules, which have a hardness of approximately 3,000 to 6,000 N/mm^2, transformed glass granules have hardness values of 1,000 to 1,300 N/mm^2. New bone tissue that was formed in the internal lumen showed hardness values of 200 to 390 N/mm^2, which is almost equal to the hardness of preexisting bone tissue (256 to 405 N/mm^2). Hence, delayed implant placement in Biogran-augmented subantral sinal cavities posed no problems.

The success of implant placement in Biogran-grafted sites can also be explained by the transformation of the glass particles into excavated granules covered by a carbonated calcium-phosphorous (Ca-P) layer.[19] This biologically, in vivo–formed calcium phosphate is equivalent to the mineral phase in bone tissue and therefore submitted to the remodeling processes of the surrounding bone tissue.[20] This study shows evidence of complete replacement of glass granules by bone tissue in regions with high remodeling activity. No granules in direct contact with the implant surface were found, where the histometric results showed that, during healing of the implant, approximately 90% of this interfacial bone tissue remodeled. Near the implant surface, most of the granules underwent resorption, which reduced their diameter and widened the entrance to the internal lumen. At a distance, where limited remodeling activity took place, the granules are larger and less reduced. Animal experiments showed that these glass granules in sites with low remodeling activity, such as the edentulous mandible, can persist for up to 24 months after implantation. This property, in combination with abundant blood vessels, provides the necessary osteoprogenitor cells for osseointegration of the implant.

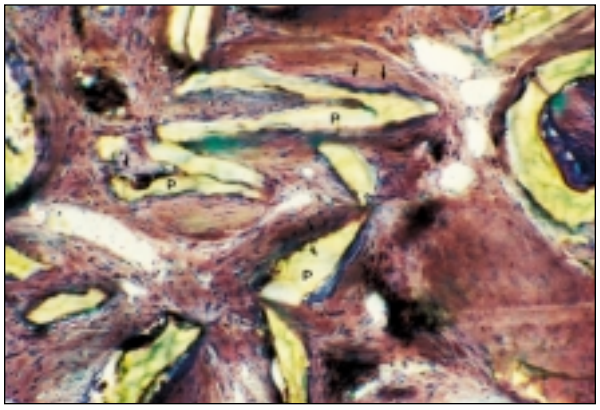


Fig 12 Phagocytosing cells *(arrow)* at the outer surface of the bioactive glass particles (P) are occasionally seen when the particles are entirely encapsulated in dense fibrous tissue at some distance from the interface. Most of the glass particles show the internal erosion, but without internal bone formation (combined Stevenel's blue and Van Gieson's picro-fuchsin stain; original magnification × 100).

The delayed placement of implants in untreated tooth sockets, 4 months after tooth extraction, led to a mean interfacial bone contact of 33.4% after 3 months of subgingival healing. When the implants were subsequently functionally loaded with a fixed partial prosthesis for 7 weeks, the total interfacial bone contact area increased to 42%. The use of bioactive glass particles of narrow size in fresh extraction sockets prior to delayed implant placement promotes interfacial bone contact after healing and subsequent loading by 52.1% and 36.0%, respectively. At a greater distance from the interface, the differences were even larger. The higher-end results are in part related to a greater new-bone formation rate during the experimental periods, which occurred at all sites. In all regions, from the coronal to the apical parts of the implants, statistically significantly more bone tissue was formed at the test sites than at the control sites. On the other hand, it is also reasonable to assume that at the time of implant placement, more bone tissue was present in the glass-treated than in the untreated areas.

Earlier studies in the beagle dog indicated that these glass granules of narrow size range are able to stimulate new bone formation after 3 to 4 months.[11] If the amount of distant nonremodeled bone tissue offers any indication of the amount of bone tissue at the time of implant placement, it can be hypothesized that the glass-treated mandibular sites contained approximately twice as much bone tissue as the control sites at the time of implant placement (minimal 32.1% versus 15.9%). This, of course, increases the primary stability of the implants. Greater amounts of bone tissue and more new bone formation also had a positive effect on the marginal bone level, which appeared to be better stabilized in the test sites after the 3-month healing period. Functional loading did not interfere with these results, although the loading period was too short to detect significant marginal bone changes.

The beneficial effect of bioactive glass on bone formation is a result of the osteoconductive and osteostimulatory properties of specifically sized glass particles. The narrow granule size range of 300 to 355 μm leads to an interfacial ion exchange throughout the particles, followed by a specific cellular response.[11] Like other bioactive glass particles of similar composition but different size range, not only do they form a Ca-P–rich layer on their outer surface, which is responsible for the extensive osteoconductive properties, but the particles themselves are eroded internally by phagocytosing cells entering via small cracks. After resorption of the silicon-rich centers of the particles, the internal surface of the Ca-P–rich layer is exposed to interstitial fluids. This protected harbor allows easy adherence of osteoprogenitor cells. New bone is formed inside the particles, unconnected to any external bone tissue, and acts as nuclei for improved bone growth. As a consequence, many particles are entirely embedded by bone tissue, especially close to the implant surface. Because of the intense remodeling near the interface, some of the granules here are completely replaced by a dense bone structure. In bone areas of lower density, bone tissue jumps from one particle to another, using the granules as a scaffold for osteoconduction.

Conclusion

The use of bioactive glass granules in extraction sockets prior to delayed implant placement statistically significantly increases the interfacial and distant amount of bone tissue compared to implants placed in untreated control areas. After the subgingival healing period of 3 months, 52.1% more interfacial bone tissue and 134.7% more distant bone tissue was found around the implants in the test sites. After an additional 7-week functional loading period with a fixed partial prosthesis, the Biogran-grafted sites still had 36.0% more interfacial and 77.5% more distant bone tissue than the untreated sites. Grafting of extraction sockets with bioactive glass granules of narrow size range results in increased primary stability of the implant at the time of implant placement and faster and higher level of secondary osseointegration during the subgingival healing period and initial functional loading period. These beneficial effects are the direct result of the osteoconductive and osteostimulative properties of these bioactive glass granules of narrow size range.

Acknowledgments

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