

Investigation of the bone tissue response to glass-ionomer microimplants in the canine maxillary alveolar ridge

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Abstract: This paper reports the results of experimental use of glass-ionomer microimplants in the augmentation of the maxillary alveolar ridge in dogs. The study included ten adult mongrel dogs 5 years of age, weighing between 50 and 70 pounds (25-30 kg), divided into 2 groups of 5 dogs each. In both groups, the maxillary 4th premolar and 1st molar were removed after the elevation of a buccal mucoperiosteal flap. The alveolar bone adjacent to the extracted teeth was also removed. In the experimental group (5 dogs), Ionogran[®] a glass-ionomer microimplants (GIMIs) (Ionogran[®] particle size of 0.5 - 1.0 mm, IONOS Medizinische Produkte GmbH & Co. KG, D-8031 Seefeld, Germany) were used for augmentation and were inserted in the created defects. The extraction sockets and bone defects were augmented with an average amount of 2 g of GIMIs. In the control group, the bone defects were left unfilled as a control for bone healing. Histological examination showed that the glass-ionomer microimplants were extremely osteoconductive and inert materials. Stimulation of growth of new bone tissue in contact with the glass-ionomer microimplants was evident. No inflammatory cells were detected on or adjacent to the GIMIs. In the control group, incomplete bone healing with fibrous scar tissue and inflammatory cells was noted. These results indicate that glass-ionomer microimplants represent highly osteoconductive and biocompatible materials for use in bone surgery. (J Oral Sci. 45, 207-212, 2003)

Key words: glass-ionomer microimplant; augmentation; canine; alveolar ridge, maxilla.

Introduction

Preservation and augmentation of the alveolar ridge is still a challenge for surgeons and an important part of the experimental investigation of alloplasts. It is accepted that bone replacement material should be synthetic, sterile, non-toxic, immunologically acceptable and available in sufficient amounts and shapes (1). Additionally, the material should mechanically prevent the fibrous ingrowth or interposition of muscle tissue into the bone defect. The material should also stimulate cell differentiation in the bone, to produce new bone cells or act as a scaffold for new bone formation (1). In the last two decades, hydroxyapatite (HA) has been used extensively for augmentation of the alveolar ridge, despite reports of complications (2-4).

Glass-ionomeric cement (GIC) was introduced into dentistry by Wilson and Kent in 1972 (5), for its unique characteristic of adhering to the hydroxyapatite of enamel and dentine under moist conditions. Glass-ionomer materials (GIMs) have rarely been used in orofacial surgery, and form their own group of hybrid materials, neither organic or inorganic. However, the newer alloplasts, glass-ionomer microimplants (GIMIs), are beginning to be more commonly used in reconstructive bone surgery.

GIMIs (Ionogran[®]) are porous granulate microimplants formed by the process of neutralization of an alkaline ion leachable powder (calcium-aluminium fluorosilicat) and polycarboxylic acid (copolymer of acrylic and maleic acid). Ionogran[®] is a permanent bone scaffold replacement material for spongy bone.

Several studies on the applicability of GIC and GIMIs

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in different anatomical regions have been conducted (6-9), also including comparisons of the characteristics of GIC and hydroxyapatite (10).

Pathological conditions frequently and easily lead to the loss of the spongy maxillary alveolar ridge. Nevertheless, almost no research has been reported on the use of GIMIs in this anatomical region. For these reasons, an experimental study in the maxilla was planned and conducted.

This experimental study aimed to:

1. determine histopathologically the biocompatibility and biofunctionality of GIMIs in contact with the bone tissue;
2. establish clinically the existence of inflammatory signs and rejection in the region of implanted GIMIs.

Material and Methods

Experimental animal model

Ten adult mongrel dogs 5 years of age, weighing between 25 and 30 kg were used. Care and feeding of the animals met all criteria of the Institute for Experimental Medicine in Niš. The dogs were divided into 2 groups of 5 dogs each.

Surgical procedure

Dogs in the experimental group were premedicated intravenously with 0.02 - 0.06 ml 1% Kombistres[®] (acepromazin, VANA, Austria) under sterile conditions. Intravenous general anesthesia was obtained with Ketalar[®] 10 mg/kg, and the maxillary 4th premolar and 1st molar were removed after elevation of a buccal mucoperiosteal flap. The alveolar bone adjacent to the extracted teeth was also removed. Ionogran[®] glass-ionomer microimplants (particle size 0.5-1.0 mm, IONOS, Medizinische Produkte GmbH & Co. KG, D-8031 Seefeld /Germany) were used for augmentation and insertion into the created bone defects. An average amount of 2 g of GIMIs was used to augment the extraction sockets and bone defects. In the control group, the bone defects were left unfilled as a control for bone healing. The mucoperiosteal flap was mobilized and directly sutured using 4-0 Vicryl[®] (Ethicon[®], Edinburgh, Scotland) in a simple interrupted fashion in both groups. All dogs were given 200,000 U of penicillin G, intramuscularly, every 6 hours daily for 5 postoperative days. Dogs were fed with soft food for 5 days postoperatively, and thereafter they were maintained on normal food until the time of death.

Harvest procedure

Four months postoperatively the dogs were killed with ten times the previous dose of Ketalar[®]. The dogs' heads were immediately perfused via the carotid arterial system

until clear outflow was obtained from the jugular veins. Continuous perfusions were maintained with 2 L of 10% buffered formalin solution. The augmented maxilla was excised using surgical burs, and stored in 10% buffered formalin. In all dogs, the augmented maxillary alveolar ridge was checked for any signs indicating rejection of the GIMIs (inflammation, fistula, mobility of the ridge etc.).

Specimen preparation

The histological investigation was conducted at the Institute of Pathological Anatomy in Niš, and the Institute of Pathology of the Dental School in Belgrade. Alveolar ridges augmented with GIMIs and alveolar ridges without GIMIs were sectioned from the midportion with the Reichart Ultracut-E (C.Reichart AG, Vienna, Austria). The bone specimens with GIMIs were decalcified using an equal mixture of 50% formic acid, and 20% sodium citrate for a period of 6-8 weeks. Specimens were then postfixed in a solution of 10% acetic acid and 10% formalin. Histological sections were prepared at 5 µm and stained with hematoxylin and eosin. Alveolar bone tissue and the GIMIs were analyzed under an Olympus BX-50 light microscope (Tokyo, Japan), at 2 to 40 times magnification. Criteria for cellular events were: 1. type of cellular reaction noting the presence of fibrous scar tissue and encapsulation; 2. presence or absence of ossification on/around or in direct contact with GIMIs (11).

Results

Healing was uneventful in all dogs. In the experimental group, all implants demonstrated good consolidation and a favorable ridge form.

Clinical examinations of the oral cavity and operative region during the period of investigation (4 months) showed no fistulous openings or inflammatory signs in the region of the implanted material. In addition, there was no evidence of migration of the implants which would indicate explantation. Palpation of the filled defect showed the same firmness as the surrounding bone.

Analyses of the histological preparations of bone defects with inserted GIMIs showed similar histological characteristics in all preparations (Table 1). Implantation of GIMIs in the maxillary bone defect stimulated new bone formation. The formation of new bone tissue positioned between microimplants and "old bone" was clearly noticeable in the histological preparations (Fig. 1). Stimulation of osteogenesis occurred in the area immediately surrounding the glass-ionomer microimplants. New bone formation occurred in immediate contact with the glass-ionomer implants whereas the old bone was still noticeable. New bone formation with numerous cellular

elements i.e. hypercellular osteoblasts was observed. Reinforced ossification of young bone tissue developed in the area surrounding the microimplants. There were no inflammatory cells or rejection of the implants. Foreign body giant cells were not present (Fig. 2). In the control group, connective scar tissue was observed (Fig. 3), and

inflammatory cells were present in most preparations (Fig. 4).

Table 1 Data of histological features

Animal	Group	Bone formation	Fibrous scar and inflammatory cells
1	E	+++	/
2	E	+++	/
3	E	++	/
4	E	+++	/
5	E	+++	/
6	C	*	/
7	C	*	***
8	C	*	**
9	C	**	***
10	C	*	*

E: experimental group: +++; full bone formation and apposition onto the GIMI, ++; partial bone formation and apposition onto the GIMI, +; poor bone formation and apposition onto the GIMI.

C: control group: *; presence of fibrous scar with minimal evidence of inflammatory cells, **; presence of fibrous scar and inflammatory cells less than 1/2 of the magnifying field; ***; presence of fibrous scar and inflammatory cells more than 1/2 of the magnifying field.

Note: In the control group the following symbols in the bone formation column represent:

*; poor; **; mild; ***; severe.

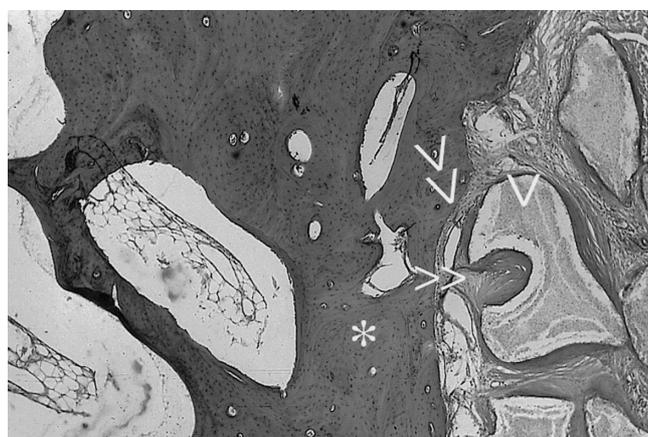


Fig. 1 There is still evidence of creation of “young bone”(>>>) in direct contact with the GIMI - (>) and adjacent bone - (*) 4 months after implantation (original magnification, HE, $\times 20$).



Fig. 2 Complete osseointegration (>>) with adjacent bone 4 months after implantation of GIMIs - (>). It is almost impossible to distinguish the GIMIs from the adjacent bone (*)- (original magnification, HE, $\times 20$).

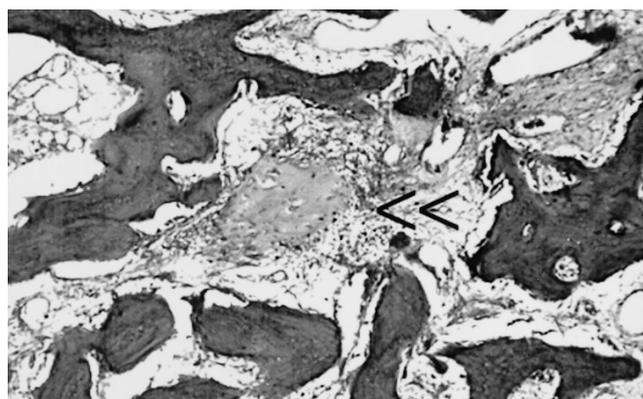


Fig. 3 Incomplete bone regeneration with presence of fibrous scar - (>>) 4 months after implantation (original magnification, HE, $\times 20$).

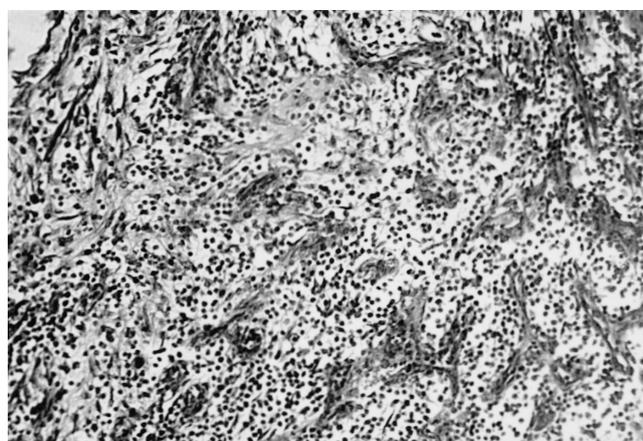


Fig. 4 Presence of inflammatory cells in the control group 4 months after implantation (original magnification, HE, $\times 20$).

Discussion

In this study, all histological analyses of maxillary alveolar ridges augmented with GIMIs provided an identical picture - characterized by the absence of any "foreign body" type reaction. Although absolute biocompatibility is a utopian notion, such a finding is interesting for it concurs with the excellent biocompatibility of GIMIs observed by other authors (12-20).

Ossification of implants depends on the biochemical congruence of the implanted material and natural bone, as well as on dynamic chemical processes on the implant surface (21), which probably influence the final osteoconductive potential of implants. GIMIs with an active surface of $70 \pm 10 \text{ m}^2/\text{g}$, is considerably more favorable than hydroxyapatite with an active surface of only $10 \text{ m}^2/\text{g}$, for providing the accommodation of cells in relation to implant (21).

In the course of *neutralization*, GIMIs create a matrix which represents a three-dimensional net in which polycarboxylic acid chains are cross-linked to ions of calcium and aluminium. In this net, cations (sodium, potassium, magnesium, calcium, etc.), as well as anions (fluorides, chlorides, phosphates, etc.) strike the balance of ions in GIMI and those in adjacent tissue, without compromising the stability of matrix (21). It is observed that ions of aluminium and silica, as well as of fluoride, calcium, and phosphate, pass the bone / GIMIs interface freely (17). It is likely that fluoride, a constituent of GIMIs, plays a pivotal role in the stimulation of osteogenesis; since favorable effects of fluoride on stimulation of osteoides have already been recorded in patients with osteoporosis (22). Slow and long-lasting release of fluoride ions from GIMIs provides the stimulus for osteoblastic activity that improves the binding of an implant to the bone. In addition, fluoride strongly influences the mytogenic processes of cartilage and bone. Presumably, these processes of stimulation develop due to the increasing effect of growth factor and its synergistic effect with calcitonine (21,22).

In bone reparation and regeneration, the pore size and shape of the implant play a significant role. GIMIs have a specific, connected system of micro pores (1 - 10 mikm) and macro pores (100 - 400 mikm), which enables the formation of osteoid and facilitates the ingrowth of bone minerals (23). This system of mutually bonded pores makes up 60% of the total volume of material (21,24,25). Unlike HA, which is characterized by even and consistent porosity, GIMIs have a three-dimensional intercanalicular system of numerous and diverse micro and macro pores, enabling a free flow of the intercellular fluid. In this way, electrolytes are "free" to circulate as in their natural

environment - *bone* (21). GIMIs, as a permanent osteoconductive material, allow the formation and organization of osteons and Haversian canals as in normal bone. The GIMIs granule size of 0.5 - 1 mm enables both the free circulation of interstitial fluid and the functional adaptation of implants (21). Pores of GIMIs have rounded edges to avoid mechanical irritation. As already pointed out, probably the size of pores is of importance in enabling bone tissue ingrowth. According to literature (6,21), the stimulatory effect of GIMIs on osteoblast and osteoblast-like cells is extremely favorable. It is considered that the bone cells in contact with granules of GIMIs recognize and accept the surface of implants as a "bone" and migrate from the adjacent bone tissue to the GIMIs (6). A surprising finding is that these polygonal cells are actually attached to the surface of the GIMIs by stimulation of collagen fibers which anchor onto the implant (6,21), creating an undisturbed continuous coating (17). The adhesive proteins fibronectin and tenascin, present on the surface of GIMIs, are responsible for such positive cellular events. Tenascin is associated with mesenchymal condensation, which is an important process preceding osteogenesis (26). Fibronectin is strongly associated with successful endochondral ossification (27).

Similar positive reactions of bone tissue to GIMIs were observed in our investigations. All microtome sections revealed strong osteoblastic reactions and formation of new bone between the GIMIs and old bone. The hypercellularity of osteoblasts observed in this investigation was identical to the findings of other authors(21). In addition, no significant difference was observed between the experimental models in which the defect was filled with spongy bone and those in which the defect was filled with GIMIs (21). In both cases, bone marrow cells, hematopoietic cells, star shaped cells, and cells of fatty tissue were observed, so that the major histological impression of normal bone development in a well organized and differentiated tissue was confirmed in both this and related investigations (21).

Comparative analysis of the quality of cellular elements observed during bone healing stimulated by GIMIs and HA showed considerable difference (21). In samples with GIMIs, highly specialized and well-organized tissue was formed, containing bone marrow and hematopoietic tissue cells. HA, on the other hand, stimulated the formation of highly active fibrous tissue with no bone marrow or hematopoietic tissue cells. Osteoclasts, absent in the group with GIMIs, were found in the group stimulated by HA, followed by lymphocytes and numerous monocytes, suggesting HA irritation. The ability of GIMIs to absorb and release proteins (28) is extremely important, bearing

in mind the possibility of inserting high molecular weight binding proteins into the GIC matrix of GIMIs. This particularly refers to GIMIs and GIC as potential carriers of certain drugs and growth factors (28). The absence of inflammatory cells or leukocyte infiltrate in the preparations presented in this study is consistent with the results of other authors (6,21).

In the control samples, the formation of scar tissue and the presence of leukocyte infiltrate were observed. The absence of bone tissue formation was expected, since other authors have confirmed that bone healing requires both bone lamellas (29).

The results obtained indicate the biocompatibility and biofunctionality of glass-ionomer microimplants in relation to the tissues examined in this study. It can be concluded that GIMIs i.e. glass-ionomer materials represent a new application of alloplastic materials in bone tissue surgery, and that there is a possibility of their use in human jaw surgery. However, in spite of outstanding results and easy handling, GIMIs should only be used in certain clinical cases where long-term postoperative follow-up is possible.

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