HISTOPATHOLOGIC EFFECTS OF GLASS IONOMER BONE CEMENTS APPLICATION TO MAXILLOFACIAL AREA: AN EXPERIMENTAL STUDY IN RABBITS

Ediz Yorgancilar¹, Ugur Fırat², Ramazan Gun¹, Salih Bakır¹, Suleyman Dasdag³, Zeki Akkus⁴, Osman Gokalp⁵, Ismail Topcu¹
¹Dicle University, Medical School, Department of Otorhinolaryngology, Head and Neck Surgery, Diyarbakir, Turkey
²Dicle University, Medical School, Department of Pathology, Diyarbakir, Turkey
³Dicle University, Medical School, Department of Biophysics, Diyarbakir, Turkey
⁴Dicle University, Medical School, Department of Biostatistics, Diyarbakir, Turkey
⁵Dicle University, Medical School, Department of Pharmacology, Diyarbakir, Turkey
Correspondence to: Ediz Yorgancilar
E-mail: edzyrg@hotmail.com

ABSTRACT
Reconstruction of the maxillofacial bone defects and fractures poses a challenge to the surgeons. Various alternatives and materials have been described for these defects and fractures. Glass ionomer bone cements (GICs) have been used extensively in dentistry but recently they have also been utilized in otolaryngology. We hypothesized that GIC can be an alternative material for maxillofacial reconstruction. However, their biocompatibility is of primary importance because this material will be in direct contact with the tissue for a prolonged time and might affect it. Therefore the aim of this study was to investigate the tissue responses to GIC in the maxillofacial area in rabbits. The study was carried out on 16 New Zealand White rabbits, which were divided into study (n: 8) and control (n: 8) groups. Experimental defects and fractures were created in the nasal bone, maxilla and zygoma in both the study and the control group. The experimental fractures and defects were reconstructed by GIC in the study group. However, the rabbits in the control group were left to natural healing process. The inflammatory reaction and fibrosis in the rabbits of both the study and the control group were compared by using descriptive histopathological analysis 180 days after application. The tissue reactions were graded. GIC showed a slight inflammatory and fibrous reaction in the rabbit of the study group. Nevertheless, statistical difference between the groups was not observed in terms of inflammatory reaction and fibrosis (P > 0.05). The results of this study indicated that GIC is a well tolerated material in maxillofacial reconstruction.

Keywords: bone cements, glass ionomer, maxillofacial, reconstruction

Introduction
The glass ionomer cements (GIC) were developed by Wilson and Kent (18) primarily for dental purposes. Favorable properties of GIC are as follows: it binds firmly to bare bone surfaces, sets with minutes, leaving time for manipulation, and forms a hard, bone-like substance that is water-resistant after setting (3, 10). These favorable properties have generated interest in other fields of medicine, too. Since the popularity of bone cement in otolaryngology it has begun to be used in the middle ear surgery (2, 11).

Maxillofacial reconstruction still remains a considerable surgical problem demanding search for new and better materials. Glass ionomer bone cement may be an alternative material in this area for reconstruction of selected fractures or defects. It has been shown in many experimental and clinical studies that glass ionomer bone cements have a high affinity and good adhesiveness to bone (7, 9, 14). The only question that remains is how the surrounding tissue reacts to GIC in this area. There are only a few reports on the tissue reactions to GIC in the maxillofacial area. Due to lack of research such as on the biocompatibility of GIC the aim of the present study was to histologically investigate the bone and soft tissue response to GIC applied to nasal bone, maxilla and zygoma defects.

Materials and Methods
Sixteen female New Zealand White rabbits, weighing 3-4 kg (6 months old), were used in this study. The animals were housed under standard conditions (21 ± 2 °C) in the Animal Health and Research Center of Dicle University (DUSAM). The study protocol was approved by the Animal Research Committee (DUHADEK) of Dicle University, Turkey. The animals were housed one per cage, fed ad libitum with water and standard laboratory animal diet and carrots, under the care of trained wardens.

Experimental protocol
The animals were randomly divided into two groups, 8 rabbits each. All the rabbits in the study and control groups were healthy. Abnormalities were not observed during the examination. Surgery and experiments were performed in the Health Research Center of Dicle University (DUSAM).

Anesthesia
For the surgical procedure, the rabbits were anesthetized by intramuscular injections of ketamin hydrochloride 40 mg/kg...
were then embedded in paraffin. Sections (5 µm thick) were cut with a microtome, stained with Hematoxylin-Eosin (H&E) and Masson-Tricrom stains. The state of the surrounding tissue, the occurrence and location of fibrous tissue, as well as various types of inflammatory cells were examined under a light microscope (Nikon, Eclipse, 80i, Japan) by the same pathologist blind to study groups. Fibrosis and inflammatory reaction around the bone cement were graded and scored in 10 different randomly chosen fields at a magnification of ×40. Inflammatory reactions were graded and scored as: minimal changes or no reaction = 0; focal, slight to moderate reaction = 1; severe reaction = 2. Fibrosis around the bone cement was graded and scored by evaluation of Masson-Tricrom stain samples as: no fibrosis = 0; sparse, moderate fibrotic bands = 1; massive, diffuse fibrotic bands = 2. An average value for each group was obtained from the sum of all scores which rated in 10 randomly selected separate areas.

**Statistical analysis**

The results for the study and the control group were compared statistically by SPSS software (ver. 15.0; SPSS, Chicago, IL, USA). Student’s t test for two independent groups was used for the comparisons of inflammation and fibrosis.

**Results and Discussion**

All the animals survived without any complications until the end of the study. The postoperative healing was uneventful; clinically healthy skin in the nasal dorsum and both zygomatic areas and, healthy mucosa in the mouth, without signs of infection, had already covered the defects in all animals after 180 days.

Macroskopically, no change was observed in the size and shape of the material. However, an excessive fibrous tissue on the nasal area was observed in two GIC treated rabbits.

The histopathological changes in the surgical fields in both groups were compared. GIC applied and non-applied groups showed similar histologic findings. In the study group, the bone cavity was almost completely occupied by GIC. A direct bone-material contact was still observed in the bone region. The cement was covered with thin fibrous tissue with a slight inflammatory response (Fig. 1 and Fig. 2). Macrophages were sparsely scattered outside the fibrous tissue. Some of the material was lost and replaced predominantly by fibrous connective tissue that had grown into the defect, which is indicative of the dissolution of the GIC over time. No severe response, such as degeneration and necrosis, was observed in either soft or hard rabbit tissue.

In the control group, defect and osteotomy areas were covered with thin fibrous tissue. Only minimal inflammation was observed (Fig. 3). The defects were healed spontaneously but with a remaining concavity.

In this study, the late tissue responses of GIC showed slight inflammation and development of well formed fibrous capsule was observed. The result of the present study demonstrated that GIC is well tolerated by the tissue in the maxillofacial area.

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In this animal study, no statistical difference was observed between the Gic applied and non-applied groups in terms of a histopathological reaction (Table 1).

**Fig. 1.** Control group. Mild fibrosis and inflammation in section of the nasal bone periosteum: healing tissue and fibrosis (right-left arrows); healing area on the nasal bone defects (arrowheads); Hematoxylin-Eosin, 40× magnification.

**Fig. 2.** Experimental group. Mild inflammation and moderate fibrosis around the bone cement: healing tissue and fibrosis (arrow); glass ionomer bone cement (star); Masson-Tricrom, 100× magnification.

**Fig. 3.** Experimental group. Mild inflammation and fibrosis around the bone cement in zygoma: healing tissue and fibrosis (arrows); glass ionomer bone cement (star); Masson-Tricrom, 100× magnification.

**TABLE 1:**

<table>
<thead>
<tr>
<th>Tissue Reaction</th>
<th>Control Group (n=8) (x±SD)</th>
<th>Study Group (n=8) (x±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxilla</td>
<td>1.0±0.3</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>Zygoma</td>
<td>0.9±0.4</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>Nasal bone</td>
<td>1.2±0.4</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td><strong>Fibrosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxilla</td>
<td>1.2±0.4</td>
<td>1.4±0.3</td>
</tr>
<tr>
<td>Zygoma</td>
<td>1.1±0.2</td>
<td>1.2±0.4</td>
</tr>
<tr>
<td>Nasal bone</td>
<td>1.2±0.3</td>
<td>1.5±0.4</td>
</tr>
</tbody>
</table>

The results are expressed as mean±SD.

The treatment of bony defects in the cranial and maxillofacial regions is a common clinical problem. Various alternatives have been described for either skeletal fixation after maxillofacial fracture or bony defects in this region. Among these alternatives, wire fixation, bicortical miniplates, and compressive and noncompressive miniplate systems are reliable options for rigid fixation (8, 13, 15). An ideal alloplastic material for maxillofacial reconstruction should be biocompatible, available in sufficient quantities, strong enough, and easy to shape to fit the defect and regional anatomy, easily fixable, not prone to migration, and bioresorbable with minimal foreign body reaction (1, 15). Miniplate systems are expensive, and except for biodegradable miniplates, re-operation is needed for removal (8). This might be considered a disadvantage. Moreover, miniplates and screws have a complication rate with various rates in different studies. Due to these disadvantages, GIC could be an alternative material for treating patients with maxillofacial fractures which have thin and weak bone fragments. Using GIC for this condition may reduce the need for miniplates. We also suggest that the use of mini- or microplates in combination with bone cement could also improve the stabilization and enhance the healing of maxillofacial fractures.

GICs are substances produced by an acid-base reaction (2). This means that formulated powder is mixed with a liquid to generate a mixture that hardens through a reaction.
Classification of cements can be performed according to their chemical contents as follows: phosphate cement (ZnO powder and phosphoric acid liquid), carboxylate cement (ZnO powder and polyacrylic acid liquid), silicate cement (glass powder and phosphoric acid liquid), and glass ionomer cement (glass powder and polyacrylic liquid). GIC is slowly biodegradable, bioactive and biocompatible. GICs have been reported to have antibacterial properties owing to their fluoride content and low pH (16). Based on these properties GIC bone cements for wider surgical applications have been developed, including applications in otology for reconstruction of the ossicular chain and cementation of implants (2, 11).

Because the bone of the anterior maxilla is thin and weak, it is frequently difficult to reduce and adequately stabilize without the use of alloplastic materials. Bone cement has been shown to give sufficient stabilization of isolated, single fractures by fixation with just one microplate along the external oblique ridge. Thus, it may be possible to maintain the stability of the fracture site using a less rigid fixation system than has been previously thought necessary (4). In our study we observed adequate stabilization of fractured fragments in the zygomatic area.

There are only a few studies about maxillofacial area usage of GIC. Tamimi et al. (17) demonstrated a minimally invasive vertical bone augmentation procedure with brushite based cements. They presented that this procedure was an attractive alternative to current surgical procedures in terms of increased simplicity, reduced trauma, and lower cost of surgery. Gosain et al. (6) presented a safety and efficacy report for bioactive glass for craniomaxillofacial reconstruction and supported the clinical applications of bioactive glasses in this area. Peltola et al. (12) have a report on the use of bioactive glass particles to obliterate the frontal sinus in 30 patients over a 10-year period. Although there was some decrease in radiologic density of the obliteration material over time, they reported the material to be well tolerated with no loss of volume. Smeets et al. (15) stated that, for clinical application of reconstruction plates, the dentine bone bonding agent has the advantage of providing an alternative fixing technique for the plates if fixing with screws is impossible. They showed achievable bond strength in a tension test with this material. Copcu et al. (5) reported long term results in reconstruction of maxillofacial segmental bone defects with bioactive glass in six cases and they performed bioactive glass reconstruction of the median mandibular cyst with excellent results. We obtained similar findings in maxilla and zygomatic area in our study. But on the nasal bone we observed excessive fibrous tissue around the GIC. We believe that it is a problem for the patients with thin nasal skin as there may be bad cosmetic results. Therefore, it should be used very carefully in thin skin areas. In other words, maximum attention should be paid in such cases.

The risk of implant exposure and local infection is a potentially serious disadvantage of all alloplastic materials. However, there were no serious complications observed with GIC in this study.

Conclusions
In the present study, the tissue biocompatibility of the tested glass ionomer bone cement in the maxillofacial area was investigated. Overall, the severity of the inflammatory response was minimal. Consequently, we conclude that glass ionomer bone cement may be a good alternative material for bone defects in the maxillofacial area. However, it must be used very carefully in thin skin areas. Because of the small sample size and the short observation period, more performance is necessary to evaluate glass ionomer bone cement and the tissue response.

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REFERENCES