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Original Research

Hydroxyapatite agarose composite gels as a biochemical material for the repair of alveolar bone defects due to cleft lip and palate☆☆

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ABSTRACT

Purpose: Hydroxyapatite agarose composite gel (HAp gel) is a radiolucent and absorbable bone–graft material that differs from hydroxyapatite ceramic; it is more rapidly absorbed and replaced by new bone than existing materials. This study examined the usefulness of HAp gel in the repair of alveolar bone defects due to cleft lip and palate.
Materials and methods: HAp gel was prepared by an alternate soaking technique. HAp gel was mixed with an equal volume of autologous bone and applied to bone defects due to cleft lip and palate (mixture group). As a reference group, 10 cases were treated with autologous bone graft alone (bone group). To determine the presence of osteogenesis, bone density on X-ray film was measured at the transplanted site and surrounding normal bone, and the ratio was calculated. The marginal bone level was assessed by intraoral radiography.
Results: In the mixture group, the bone density of the transplanted site increased on radiography 1 month postoperatively and was maintained thereafter. A similar increase in bone density was observed in the bone group. The marginal bone level in the alveolar cleft was similar between groups. There were no complications such as infection and prolonged inflammation of the transplanted bone in association with the use of HAp gel.
Conclusions: The mixture of HAp gel and autologous bone is as effective as autologous bone in the treatment of bone defects due to cleft lip and palate. HAp gel can reduce the weight of autologous bone required.

1. Introduction

Although autologous bone is considered the best bone–graft material for the reconstruction of jaw bone defects, it has many disadvantages, despite its popularity. Limited supply of bone at the donor site may require additional surgery to harvest sufficient bone, resulting in further trauma to the patient and postoperative complications. In order to overcome these disadvantages due to taking large amounts of bone, several artificial bone graft biomaterials mainly composed of hydroxyapatite (HAp) ceramic and/or beta-tricalcium phosphate (β-TCP) have been developed. However, most of these materials are either slowly absorbed or not absorbable.

The formation of organic–inorganic composites is common in biological systems; for example, HAp and collagen make up bone and teeth. We previously report the use of agarose as an organic component because of its biodegradability in the body and developed a biomaterial with organic–inorganic composites, HAp composite agarose gel (HAp gel), by using an alternate soaking method [1–3]. The method is based on the wet process for HAp preparation, in which organic composites are alternately soaked in $CaCl_2$ and Na_2HPO_4 aqueous solutions [4]; as a result, nanometer-sized HAp particles are formed on and in the organic polymer matrix. Unlike commercially available HAp ceramics, HAp gel is radiolucent at the applied site. Furthermore, it is degraded and resorbed in the body in accordance with new bone formation [4].

We previously demonstrated that HAp gel is a useful bone–graft material in an in vitro cell system and animal models of bone defect

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[4–8]. Its ability was enhanced in the presence of mesenchymal stromal cells [8]. If the amount of bone required is reduced, the damage to the patient would be reduced. Taking this into consideration, we decided to use HAp gel in combination with autologous bone to ensure bone formation and reduce the amount of bone used.

Therefore, this clinical study investigated whether a 1:1 mixture of HAp gel and bone is as effective as autologous bone alone as a bone graft material for the treatment of bone defects due to cleft lip and palate.

2. Materials and methods

Biological safety criteria in accordance with ISO-10993 were enforced: the ISO-10993 guidelines entail a series of standards for evaluating the biocompatibility of a medical device before a clinical study. This clinical study was accepted as translational research by the Ethics Committee of Osaka University Dental Hospital, and has been ongoing since July 2009. The research complies with the World Medical Association Declaration of Helsinki on medical research protocols and ethics. The results of our clinical study are reported herein.

2.1. Generation of HAp gel

Tabata et al. described the procedure for generating HAp gel [4]. In brief, a boiled aqueous solution containing 3% (w/v) agarose (NuSieve; Cambrex Bio Science Rockland, Rockland, ME, USA) was poured into molds created between 2 glass slides at 0.5-mm intervals and then cooled. The resultant 0.5-mm thick agarose gels were punched out into discs 4-mm in diameter. These gel discs were alternately soaked in aqueous solutions of $CaCl_2$ (pH 7.4, 200 mmol/L) and Na_2HPO_4 (120 mmol/L) at 4 °C, followed by washing in ultrapure water after each immersion. Soaking in each ionic solution and subsequent washing was defined as 1 cycle; each disc was subjected to 12 cycles. HAp gel discs were prepared by the alternate immersion technique described above, homogenized, transferred to a 1-mL syringe, and injected in approximately 0.5-mL aliquots, which were sealed and sterilized by 25-kGy gamma-ray irradiation (Koga Isotope, Shiga, Japan).

2.2. Candidate patients

All candidate patients had alveolar cleft due to cleft lip and palate. Patients were divided into 2 groups. Ten cases of alveolar cleft were treated with a mixture of HAp gel and autologous bone (mixture group); a 1:1 (w/w) mixture of HAp gel and bone taken from chin region of the mandible (chin bone) was used to fill the defect. Meanwhile, as a reference group, 10 cases of alveolar cleft were treated with autologous bone alone (bone group); the iliac bone was used in 9 cases, and the jawbone from the chin was used in 1 case.

2.3. Surgical procedure

The preoperative state of an alveolar cleft and suturing of the nasal mucosa are shown in Fig. 1A; the base of the bone graft is illustrated in Fig. 1B. HAp gel was dispensed by a syringe, although pouring would have also been feasible. The cancellous bone was extracted from the chin region of the mandible (Fig. 1C). Gel was transferred to a dish and mixed with autologous bone (Fig. 1D). The mixture of HAp gel and autologous bone was injected into the bone defect, and the wound was tightly sutured (Fig. 1E and F). In all cases, the operation was performed by 2 maxillofacial surgeons who had sufficient experience with the standard surgical approach.

The presence or absence of postoperative infection and prolonged inflammation were also documented clinically.

2.4. Evaluation

Outcomes were evaluated using dental and occlusal X-ray films obtained preoperatively and 1, 3, and 6 months postoperatively. To determine osteogenesis radiographically, bone density on X-ray films was measured by ImageJ (NIH, Bethesda, MD, USA) at the cleft (Fig. 2B, white closed line) and surrounding normal bone [9,10]. The background value of each film was subtracted from these results. The values at 2 points of the surrounding normal bone were averaged (Fig. 2B, white closed dotted line). The density ratio was calculated as follows: density ratio = (density of transplantation area – density of film background)/(average density of surrounding normal bone – density of film background),

The marginal bone level relative to the teeth adjacent to the alveolar cleft was assessed by intraoral radiography at 6 months postoperatively as described by Enemark et al. [11]. On the basis of the level from the apex (0%) to the enamel–cement junction (100%) of neighboring teeth, bone formation was scored from 1 to 4 as follows: 1, 75–100%; 2, 50–75%; 3, 25–50%; 4, 0–25%.

2.5. Statistical analyses

Data were analyzed by using Student's *t*-test. The level of significance was set at $P < 0.05$.

3. Results

The background characteristics of the patients were examined. In the mixture group, the mean age was 11 years, ranging from 9 to 17 years. There were 4 males and 6 females. Regarding diagnosis, there were 6 cases of cleft lip and alveolar only (CLA) and 4 cases of unilateral cleft lip and palate (CLAP) (Table 1). In the bone group, the average age was 10.7 years, ranging from 8 to 13 years. There were 6 males and 4 females. There were 5 cases each of CLA and unilateral CLAP, respectively (Table 2). There was no significant difference between groups with respect to age distribution ($P < 0.05$).

Patients were followed postoperatively, and clinical findings were recorded. No patients in either group had postoperative infection, or prolonged inflammation (Tables 1 and 2).

In all cases of the mixture group, the alveolar cleft was filled with new bone, which had a structure similar to that of surrounding bone (Figs. 2 and 3, arrows). All cases in the bone group exhibited similar results (Fig. 4).

The density on X-ray films was compared between the transplanted sites and surrounding normal bone immediately postoperatively, showing a bone density ratio of approximately 0.4. The density ratio reached approximately 0.8 after 1 month and was maintained until 6 months postoperatively (Fig. 5A). Similar results were obtained in the bone group (Fig. 5B).

We also evaluated the marginal bone level relative to the teeth adjacent to the alveolar cleft at 6 months postoperatively. In the mixture group, the images of case 2 were difficult to evaluate, because the permanent teeth obstructed assessment. Meanwhile, the remaining 9 cases had a score of 1, indicating that most of the alveolar bone defect had been filled with new bone. In the bone group, case 9 had a score of 2, while the other cases had a score of 1 (Table 2).