Adipose-derived stem cells in alveolar cleft management: looking for a better scaffold

Aus Fettgewebe gewonnene Stammzellen im Alveolarspalt-Management: Suche nach einem besseren Gerüst

Abstract

Background: Although various sources of bone graft material have been suggested in the literature for alveolar cleft reconstruction including autogenous, allogenic, xenogenic, and alloplastic grafts, autogenous bone graft from either the iliac crest or the tibial plateau remains the gold standard against which other sources are evaluated. However, the procedure is invasive and associated with a potential risk of early complications such as bleeding, pain, infection, fracture or late complications such as chronic pain, scarring, paresthesia, and gait abnormalities. Moreover, its failure rate is about 15%.

In an effort to improve outcome and decrease donor site morbidity, we tried to find a better scaffold for adipose-derived stem cells (ACSs) as an alternative to autologous bone graft for alveolar cleft reconstruction.

Aim of the study: To study the efficacy of demineralized bone matrix (DBM) versus autologous bone graft as a scaffold for adipose-derived stem cells (ASCs) and to compare both techniques to the standard autologous iliac crest bone graft (ICBG).

Methods: 54 patients underwent alveolar cleft reconstruction at the age of mixed dentition over a 3-year period. Their mean age was 11.4 years and their mean postoperative follow-up was 12.4 months. Of these, 18 constituted the ICBG group (standard group), 20 constituted the ACSs with ICBG scaffold (ASCs/ICBG) group, whereas the remaining 16 patients made up ACSs with DBM (ASCs/DBM) group. Results were assessed by rating the radiographs obtained 6 months postoperatively according to Bergland scale.

Results: Alveolar cleft repairs using cancellous bone only (ICBG group) were 72.2 percent successful, alveolar cleft repairs using cancellous bone enhanced with ASCs (ASCs/ICBG group) were 90 percent successful, and alveolar cleft repairs using DBM enhanced with ASCs (ASCs/DBM group) were 62.5 percent successful. However, there was no statistical difference between the groups. A significantly shorter operative time (average time saving was 103 minutes/case) and higher infection rate (18.8 percent) were observed in ASCs/DBM group as compared to the other two groups.

Conclusion: Although not being significantly better than DBM, ICBG appears to be a good scaffold for ASCs that improves results of alveolar cleft reconstruction.

Keywords: alveolar cleft reconstruction, stem cells, demineralized bone matrix
Zusammenfassung

Hintergrund: Obwohl in der Literatur verschiedene Quellen für Knochentransplantatmaterial für die Alveolarspaltenrekonstruktion einschließlich autogener, allogener, xenogener und alloplastischer Transplantate vorgeschlagen werden, bleibt autogenes Knochentransplantat aus dem Beckenkamm oder dem Tibiaplateau der Goldstandard, anhand dessen andere Quellen bewertet werden. Das Verfahren ist jedoch invasiv und mit einem möglichen Risiko für frühe Komplikationen wie Blutungen, Schmerzen, Infektionen und Frakturen oder späte Komplikationen wie chronische Schmerzen, Narbenbildung, Parästhesien und Ganganomalien verbunden. Darüber hinaus beträgt die Misserfolgsrate etwa 15%. In dem Bestreben, das Outcome zu verbessern und die Morbidität auf Seiten der Spender zu verringern, haben wir versucht, ein besseres Gerüst für aus Fettgewebe gewonnene Stammzellen als Alternative zu autologem Knochentransplantat für die Alveolarspaltenrekonstruktion zu finden.


Ergebnisse: Alveolarspaltenreparaturen nur mit Spongiosa (Beckenkammknochentransplantat) waren in 72,2 Prozent erfolgreich. Unter Verwendung von Spongiosa, die mit aus Fettgewebe gewonnenen Stammzellen verbessert wurden, waren die Alveolarspaltenreparaturen zu 90 Prozent und bei Verwendung von demineralisierter Knochenmatrix, die mit aus Fettgewebe gewonnenen Stammzellen verbessert wurde, zu 62,5 Prozent erfolgreich. Allerdings gab es keinen statistisch signifikanten Unterschied zwischen den Gruppen. Es wurde eine deutlich kürzere Operationszeit (die durchschnittliche Zeitsparnis betrug 103 Minuten/Fall) und eine höhere Infektionsrate von 18,8 Prozent wurden in der Gruppe mit aus Fettgewebe gewonnenen Stammzellen mit entmineralisierter Knochenmatrix im Vergleich zu den anderen zwei Gruppen beobachtet.

Schlussfolgerung: Obwohl Beckenkammknochentransplantat nicht signifikant besser als demineralisierte Knochenmatrix ist, scheint das Beckenkammknochentransplantat ein gutes Gerüst für aus Fettgewebe gewonnene Stammzellen zu sein, wodurch die Ergebnisse der Alveolarspaltenrekonstruktion verbessert werden.

Schlüsselwörter: Alveolarspaltenrekonstruktion, Stammzellen, demineralisierte Knochenmatrix
Introduction

Repairing the alveolar cleft is an important step to create a stable and continuous maxillary dental arch, facilitate closure of the oronasal fistula, improve support of teeth adjacent to the cleft site, permit further orthodontic and orthognathic interventions, and to provide support to the alar base of the nose. Although various sources of bone graft material have been suggested in the literature for alveolar cleft reconstruction including autogenous, allogenic, xenogenic, and alloplastic grafts, autologous bone grafts either from the iliac crest or the tibial plateau remain the gold standard against which other graft materials are evaluated [1], [2], [3]. However, the procedure is invasive and associated with a potential risk of early complications such as bleeding, infection, fracture, and/or late complications such as chronic pain, scarring, paresthesia, and gait abnormalities [4], [5]. Moreover, its failure rate is about 15% [6], [7].

For effective osteogenesis to take place, presence of both osteoinductive factors and osteoconductive scaffolds are needed. Cancellous bone grafts, either from iliac crest or tibial plateau display both osteoinductive and osteoconductive properties, which explain their efficacy in a wide variety of procedures.

Demineralized bone matrix (DBM) is an established group of allograft bone substitutes that has been used extensively in the orthopedic surgery as an osteoconductive scaffold but it has no osteoinductive potentiality. ACSs act not only through direct bone formation in the gap of alveolar cleft, but also due to their paracrine effects: production of extracellular matrix, releasing cytokines and promotion of angiogenesis. ACSs in combination with a proper scaffold have a great potential that has already been proven in animal studies and on humans [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19]. Our aim is to study the efficacy of demineralized bone matrix versus autologous bone graft as a scaffold for adipose derived stem cells (ASCs) and to compare both techniques to the standard autologous ICBG for alveolar cleft reconstruction.

Patients and methods

After getting the approval from the Ethical Committee of Faculty of Medicine, Mansoura University (Study No. CD60), fifty-four patients with unilateral alveolar cleft were collected randomly from the outpatient clinics of the Plastic Surgery Centre at Mansoura University and El Mataria Teaching Hospital over a 3-year period from March of 2012 to February of 2015. All patients with unilateral or bilateral alveolar cleft were included, and patients operated before and needed revision (due to previous failure or inadequate bone formation) were also included. Old patients (>12 years) with neglected alveolar cleft with no aesthetic or functional concerns (e.g., no alar depression, no need for orthognathic surgery or dental implants), patients with small alveolar notch only, and patient with mental disorders were excluded. Their mean age was 11.4 years and their mean postoperative follow-up was 12.4 months. For each patient, age, sex, medical and surgical history was recorded. All patients were requested to have preoperative and 6 months postoperative periapical, occlusal, and panoramic radiograph.

All patients and/or their parents were offered the three modalities of treatment. In addition to standard ICBG, they are offered the possibility of an alternative procedure using ACSs on an off-label basis, along with autogenous ICBG or DBM as a scaffold, and the choice of a specific modality was according to each patient’s (or his/her parents’) preference. Patients were then divided into three groups according to the material(s) used for grafting. Of the 54 patients, 18 constituted the ICBG group (standard group), 20 constituted the ACSs with ICBG scaffold (ASCs/ICBG) group, whereas the remaining 16 patients made up ACSs with DBM (ASCs/DBM) group.

Operative technique

The standard Gingivoperiosteoplasty technique was performed as described by Skoog [20] to prepare the appropriate pocket for introducing our graft (Figure 1). Lidocaine 1% with epinephrine 1:200,000 was infiltrated around the cleft margins, then intraoperative reassessment of the cleft was done as regard its extent, position of the teeth on the margins of the cleft, and presence of nasoalveolar fistula. An incision was made around the labial component of the fistula, first within the loose muscosa, then within the alveolar processes. The incision was continued along the margin of the alveolar cleft vertically toward the crest of the alveolus on each side, positioned equidistant between the labial and palatal surfaces. Once on the alveolar crest, the incisions were carried within the gingival sulci of the teeth on their labial aspect. Within the lesser segment, the incision was typically extended to the second premolar. Next, the mucoperiosteum was dissected from the alveolar processes on the labial aspect using periosteal elevator. This dissection extended to the nasal floor, exposing the lateral aspect of the anterior nasal spine and the lower pyriform rim. Through the labial approach, the mucoperiosteum was elevated off the bony walls of the cleft from the alveolar crest to the nasal floor. The oronasal fistula tract was then dissected and closed by sutures. At this time, before placement of the bone graft, adequate labial soft tissue mobility that will provide a tension-free closure over the bone graft was confirmed. If greater mobility was needed, horizontal scoring of the periosteum at the base of the lesser segment flap was done. Extending the back cut and directing it anteriorly gained further mobility if needed. After introducing the graft material(s), the lesser...
Harvesting cancellous bone from the iliac crest

The cancellous bone is harvested from the iliac crest using the standard technique, taking into consideration not to place the scar on the bone prominence (1 cm posterior and lateral to anterior superior iliac spine (ASIS), Figure 2).

3 cc of the tumescent solution was injected at the incision site; incision was then taken by 15 blade through the skin and continued by diathermy down to the periosteum. Incision was then taken through the periosteum to expose the bone, and an osteotome was used to make a trap door fenestration (two vertical cuts and one from the medial aspect of the crest to connect the vertical cuts), then an anteriorly based cortical bone flap was elevated to expose the cancellous bone. A curette was then used to extract as much cancellous bone as needed. The cortical roof was then reduced back in place to cover the donor site and reduce the postoperative bleeding. If there were any defects or fragmentation of the cortex of the roof, bone wax was used to seal the cancellous bone cavity. Vicryl 3/0 (Ethicon, Inc., Somerville, NJ) sutures were used for the re-attachment of the muscles and for closure of the subcutaneous layer, and Monocryl 4/0 (Ethicon, Inc., Somerville, NJ) sutures were used in a subQ pattern to close the skin. 1 cc of Xylocaine 1% + 1 cc of Marcaine 0.25% was injected to relieve postoperative pain and soft dressing was applied.

Preparation of the demineralized bone matrix

Using sterile technique on a side table with gloves, the demineralized bone matrix powder (Wright Medical Technology, Inc., Arlington, TN) was emptied into the mixing bowl (5 cc).

The mixing solution was then emptied into the bowl gradually and mixed with spatula and the material kneaded against the sidewall of the bowl until the desired consistency was achieved (approximately 30–60 seconds).

After achieving a putty-like consistency, the material can be handled digitally. Material maintains handling characteristics up to 10 minutes after mixing, during that period it was implanted in the already prepared cleft area.

Preparation of adipose-derived stem cells from the fat

Fat grafts were harvested at the end of the procedure. Donor site was the lower abdomen in all cases. In ACSs/ICBG group, the aspiration cannula was introduced through the same incision of bone graft harvesting to avoid additional scarring. In ACSs/DBM group, the aspiration cannula was introduced through a small 0.5 cm
harvesting cancellous bone from iliac crest. A: incision is marked 1–2 cm posterior and laterally to ASIS, B: an anteriorly based cortical bone flap was elevated, C: A curette was used to extract cancellous bone, D: harvested cancellous bone graft stab incision in the umbilicus at 6 o’clock. Liposuction was performed as described by SR Coleman [21]. A long a traumatic 3 mm Mercedes cannula (luer lock type) was used. An average amount of 60 cc was usually harvested (range 50–80 cc). The raw aspirate was then collected for stem cell preparation.

Fat was extensively washed with sterile phosphate buffered saline (PBS) to remove the blood cells, saline, and local anesthetics. Then ACSs were separated from adipose tissue by cell digestion using 0.075% collagenase type I solution (Collagenase NB4 Standard, SERVA Electrophoresis, Heidelberg, Germany) at 37°C for 30 min to one hour.

An equal volume of Dulbecco’s Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (Gibco, Carlsbad, CA, USA) was then added to inactivate collagenase. The digest was centrifuged at 1,500 rpm for 5 min. The supernatant was removed, and the cell pellet, termed the stromal vascular fraction (SVF), was left.

The SVF, containing ASCs, was resuspended in 10% FBS then recentrifuged and filtered through a 100-μm nylon filter. The cell pellet was re-suspended in a 10 ml complete culture medium formed of DMEM, 13% FBS and 1.5% penicillin streptomycin mixture (Lonza, Verviers, Belgium). The cell suspension was cultured in culture flask 25 cm² (Easy Flask, Nunc, Roskilde, Denmark) and incubated in CO₂ incubator (Nuaire, NU 4950E, Autoflow Water Jacketed CO₂ incubator, USA) at 37 °C and 5% CO₂. The ASCs were prepared in a final concentration of 3x10⁶/ml and its identity was confirmed by flow cytometry (positive for CD49, CD71, CD73, CD90, CD105, and negative for CD31, CD34, CD45), and then supplied in a tube for injection.

Postoperative follow-up

All patients instructed to have clear fluids only for the first 48 hours after surgery, then soft diet for 4 weeks. Antibiotics prophylaxis was given before surgery, and for 3 days after surgery. Mouthwash was used regularly after meals for one week. All patients were stable and discharged from the hospital in the second postoperative day.

Follow-up visit was scheduled in the outpatient clinic one week after surgery when checking of the oral wound and dressing of the graft donor site (in ICBG and ASCs/ICBG groups) were done.

Prepared ASCs was injected in the pocket of the bone graft at that time using a 27 gauge needle (for ASCs/ICBG...
Radiographic outcomes

In this study, the success of autogenous bone grafts was assessed through evaluating the radiographs taken at 6 months postoperatively using the indicators of surgical success described by Bergland (Figure 3) [22]. Bone grafts of types I and II, according to the Bergland scale, were considered successful bone grafts, whereas the other types were considered unsuccessful.

Statistical analysis

GraphPad Prism version 5 for Mac (GraphPad Software, Inc., La Jolla, Calif.) was used to carry out statistical tests. The Fisher exact test was used to analyze the data comparing the success rate, the complications rate and the operative time between the ASCs/ICBG group, the ASCs/DBM group and the standard ICBG group. P-value was regarded as significant if less than 0.05.

Results

All groups were similar as regard number of patients, age, sex, follow-up period, and time between alveolar reconstruction and postoperative radiographs (Table 1). Alveolar cleft repairs using cancellous bone only (ICBG group) were 72.2 percent successful, alveolar cleft repairs using cancellous bone with ASCs (ASCs/ICBG group) were 90 percent successful, and alveolar cleft repairs using DBM with ASCs (ASCs/DBM group) were 62.5 percent successful. However, there were no statistical difference between the groups (P-value >0.05) (Table 2, Figure 4, Figure 5).

Figure 3: Bergland scale: The amount of bone produced in the cleft site was evaluated based on the height of the intraalveolar septum (bone bridge).

Heights are measured from the apical extent of the cleft site (a line between the tips of the roots of the adjacent teeth) to the cementoenamel junction coronally. Type 1 is defined as normal height (more than 75% of the normal bone height). Type 2 is defined as less than normal height (50–75% of normal bone height). Type 3 has less than 50% of normal bone height. Type 4 has no bone bridge in the cleft site.

Table 1: Characteristics of the groups by type of repair

<table>
<thead>
<tr>
<th></th>
<th>ICBG group</th>
<th>ASCs/ICBG group</th>
<th>ASCs/DBM group</th>
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<tbody>
<tr>
<td>N, %</td>
<td>18, 77.8%</td>
<td>20, 80.0%</td>
<td>16, 75.0%</td>
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<tr>
<td>Age, yrs.</td>
<td></td>
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<tr>
<td>&gt;7.5–12</td>
<td>14, 77.8%</td>
<td>16, 80.0%</td>
<td>12, 75.0%</td>
</tr>
<tr>
<td>&gt;12–18</td>
<td>2, 11.1%</td>
<td>2, 10.0%</td>
<td>2, 12.5%</td>
</tr>
<tr>
<td>&gt;18</td>
<td>2, 11.1%</td>
<td>2, 10.0%</td>
<td>2, 12.5%</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>12, 66.7%</td>
<td>6, 60.0%</td>
<td>4, 50.0%</td>
</tr>
<tr>
<td>Females</td>
<td>6, 33.3%</td>
<td>4, 40.0%</td>
<td>4, 50.0%</td>
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<tr>
<td>Follow-up, mo.</td>
<td>10.9±5.0</td>
<td>11.7±6.5</td>
<td>11.5±5.0</td>
</tr>
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<table>
<thead>
<tr>
<th></th>
<th>ICBG (Standard group)</th>
<th>ASCs/ICBG</th>
<th>ASCs/DBM</th>
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</thead>
<tbody>
<tr>
<td>Success</td>
<td>13 (72.2%)</td>
<td>18 (90%)</td>
<td>10 (62.5%)</td>
</tr>
<tr>
<td>Failure</td>
<td>5 (27.8%)</td>
<td>2 (10%)</td>
<td>6 (37.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>18 (100%)</td>
<td>20 (100%)</td>
<td>16 (100%)</td>
</tr>
<tr>
<td>Statistical significance compared to standard group</td>
<td>P-value=0.222 (Not significant)</td>
<td>P-value=0.716 (Not significant)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Comparison between groups according to success of bone grafts

and ASCs/DBM groups). By that time, the mucosa was already healed and the pocket is sealed.
Operative time in ASCs/DBM group was significantly shorter than in the other two groups (P-value <0.001), the average time saving was 103 minutes/case (Table 3, Figure 6).

A significantly higher cleft site infection rate was observed in the ASCs/DBM group (18.8 percent) as compared to ICBG group (standard group). The oronasal fistula was completely closed in all patients except two, one in each of ICBG and ASCS/DBM groups.

Table 3: Mean operative time

<table>
<thead>
<tr>
<th></th>
<th>ICBG</th>
<th>ASCs/ICBG</th>
<th>ASCs/DBM</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Operative time</td>
<td>195±35</td>
<td>188±40</td>
<td>110±20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
mesenchymal stem cells (MSCs) from bone marrow exceed the frequency of other multipotent cells such as adipose tissue contains nearly 5,000 ASCs. This greatly. Lipoaspirate provides an easily accessible source of stem cells. Adipose tissue is that it represents an abundant, reliable, noninvasive, and accessible source of stem cells. In this study we chose adipose tissue as a source for the research. Despite the superiority of ICBG, and despite being the “gold standard bone graft” for reconstruction of alveolar cleft and the standardized graft to which different types of bone grafts are compared [23], two major disadvantages remain. First, its associated donor-site morbidities including delayed ambulation, pain, hematoma, discomfort, nerve injury, prolonged hospitalization, and limited volume that can be harvested. Moreover, its failure rate is about 15% [24]. So, in an effort to reduce the donor-site morbidity, many other sources of bone graft material have been suggested in the literature for alveolar cleft reconstruction including allogenic, xenogenic, and alloplastic grafts. The outcomes achieved with different graft materials have been extensively studied and compared in the literature, and each type has its advantages and disadvantages. But the ideal bone graft material remains controversial till now [25]. In the present study, fifty-four patients were chosen randomly and divided into three groups according to the graft material(s) used: ICBG group (the control group), in which we used cancellous bone only; ASCs/ICBG group, in which ACSs with ICBG scaffold was used; and ASCs/DBM group, in which we used ASCs with DBM scaffold. To the best of our knowledge, this is the first prospective study that used the ICBG and/or DBM scaffold with adipose-derived stem cells for alveolar cleft reconstruction till the time of launching our study, and this is the unique part of our research. In this study we chose adipose tissue as a source for the stem cells similar to Gimble et al. [26], Zuk et al. [27], and Yoshimura et al. [28]. Others, like Behnia et al. [16], Hibi et al. [14], and Pradel et al. [15] used mesenchymal stem cells from the bone marrow. The advantage of adipose tissue is that it represents an abundant, reliable, noninvasive, and accessible source of stem cells. Lipoaspirate provides an easily accessible source of ASCs from abdominal adipose tissue in pediatric population and confirmed their proliferation and differentiation properties with an ability of selective osteogenic [31]. In preclinical studies ACSs were found compatible with different scaffolds including collagen, titanium dioxide, and tricalcium phosphate [32], [33], [34]. ACSs were also successfully used in combination with fibrin glue to cover a large calvarial traumatic defect in a 7-year-old patient [35], but no studies used ACSs with a DBM scaffold. Hibi et al. published the first clinical use of stem cells for bone tissue engineering in a 9-year-old patient with 10x13 mm alveolar cleft. Mesenchymal stem cells (MSCs) were obtained from bone marrow, cultured for 4 weeks, and then differentiated into osteogenic lineage. He used a titanium-mesh plate over which MSC, platelets rich plasma (PRP), and calcium chloride solution with thrombin were applied using a syringe. Nine months later, 79.1% of the bone was regenerated, and the canine and the lateral incisor erupted forcing out the mesh plate [14]. Pradel et al. reported spontaneous tooth eruption and complete defect closure after filling the alveolar cleft defect with MSCs harvested from the maxilla in a bovine collagen matrix scaffold in a 10-year-old male [15]. Behnia et al. utilized mesenchymal stem cells from the bone marrow carried on a scaffold that combined demineralized bone and calcium sulfate for alveolar cleft reconstruction. The results suggested that the amount of bone formation was inadequate and indicated that the conventional bone substitute was a favorable scaffold for mesenchymal stem cells for alveolar bone regeneration [16]. In the present study, we used demineralized bone matrix (DBM) as an osteoconductive scaffold for alveolar cleft reconstruction in ASCs/DBM group patients similar to Cameron et al. [36], Behnia et al. [16], Sivak et al. [37], Macisaac et al. [38], and Louis et al. [39]. And we used the cancellous bone harvested from the iliac crest as a scaffold for ACSs for ACs/ICBG group patients similar to Kom et al. [40], Behnia et al. [16], and Yuanzheng et al. [41], 2015 who enhanced the autologous iliac bone with MSCs from the iliac crest. Others, like Benliday et al. [42] used bovine hydroxypetite, Pradel et al. [15] used bovine collagen matrix, De Ruiter et al. used tri-calcium phosphate [43], Jiao et al. used cryopreserved dentin matrix as a scaffold [44]. DBM has the advantages of being osteoconductive. It does cause local foreign-body immunogenic reaction as the antigenic surface structure of the bone is destroyed during demineralization, its degradation does not produce any products that affect new bone formation, and being prepared by acid extraction of allografts, it retains collagen and other proteins as well as bone morphogenetic
proteins (BMPs) which have osteoinductive capability. Although, transmission of diseases has not yet been reported with DBM but is theoretically possible [45].

Many factors have been used in the literatures to enhance either cancellous bone or DBM to improve bone formation. The most frequently used are PRP and BMPs [46], [47].

Backly et al. demonstrated that PRP enhances late stage bone regeneration with osteoinductive effects that last for 8 weeks [48]. Dutra et al. demonstrated that PRP improves bone formation in artificially induced alveolar defects when combined with bioactive glass foams [49]. But, Luaces et al. found no differences between control and study groups who received PRP and autologous bone for alveolar cleft reconstruction [50].

Cameron et al. compared the results of using DBM enhanced with BMPs with the conventional cancellous bone from the iliac crest and they found better results in the group that used DBM with BMPs with success rate of 97.2% compared to 84.2% for the group used cancellous bone from the iliac crest, with significant decrease in the operative time [36]. Canan et al. [51] and Fallucco et al. [52] used recombinant human BMP in treating patients with alveolar clefts and the results were comparable to autologous ICBG. Others, as Neovius et al. had to terminate the study because of severe gingival swelling in patients receiving effective doses of BMP-2 [53].

In this study, a statistically non-significant difference was detected in the success rates between the three groups. However, we found a significant higher infection rate and significant shorter operative time in ASCs/DBM group as compared to the other control group, the average time saving was 103 minutes/case. This is similar to Cameron et al. who found significant shorter operative time when DBM was used [38]. The limitation of our study was the small number of the patient in each group that might be the reason for non-significant results.

Notes

Competing interests

The authors declare that they have no competing interests.

References


