Regenerative Medicine For Alveolar Cleft Treatment

Salem A. Alkaabi

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Salem Ahmed Alkaabi

The studies presented in this thesis were conducted at the Department of Oral and Maxillofacial Surgery, Amsterdam University Medical Center, VU University Medical Center, Academic Center for Dentistry Amsterdam (ACTA), and the Faculty of Dentistry, University of Hasanuddin, Makassar, Indonesia.

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	dr. N. Bravenboer

Dedicated to my masters and my patients

Contents

Chapter 1

General Introduction

Overview

Cleft lip and palate (CLP) is a congenital anomaly caused by abnormal facial development during gestation. Orofacial clefts may occur in isolation or in combination with a syndrome with an incidence of approximately 1:700 live births worldwide. Accordingly, it is considered the most common congenital craniofacial malformation.[1] An alveolus bony defect, called an Alveolar cleft (AC), occurs in up to 75% of CLP patients.

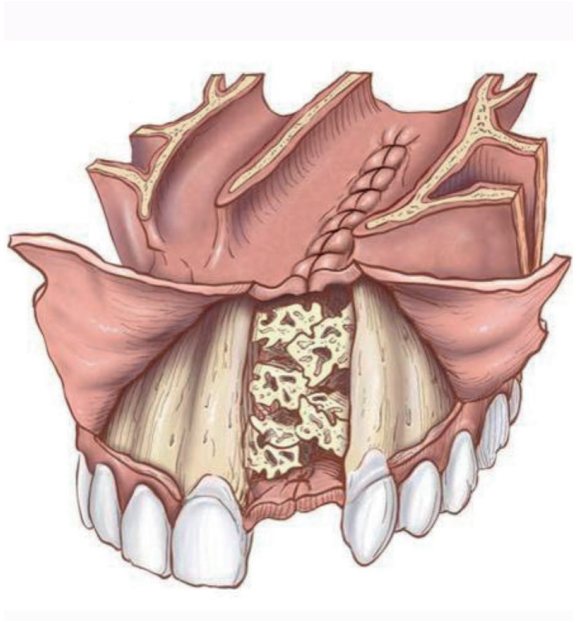

Figure 1: Representation of the alveolar cleft defect shape and the bone graft.

History

In the early 20th century, significant advancements were made in reconstructive surgery for maxillary alveolar defects and cleft closures. Von Eiselsberg and Lexer pioneered the use of autologous bone grafts from pedicled soft tissue and the little finger's bone in 1901 and 1908, respectively. Later in 1914, Drachter published the first report on closing a cleft by utilizing tibial bone and periosteum.[2] However, the popularity of alveolar bone reconstruction remained limited until after the First World War. It was the publication of Axhausen's influential cleft surgery book in 1952 that

sparked widespread interest and adoption of the technique. In his book, Axhausen emphasized the significance of inducing bony healing between the premaxilla and lateral fragments to preserve well-formed incisors, identifying it as the crucial challenge in repairing complete clefts.[3] Axhausen's contributions played a pivotal role in prioritizing functionality and aesthetics for patients with cleft lip and/or palate. Therefore, he deserves recognition as a pioneer in this field.[4]

Growth Aspects

Frontonasal and maxillary prominences develop the palate between 4 and 12 weeks of gestation. During the 4 to 7 weeks of gestation, the primary palate originates from the median palatine process, which is developed from the frontonasal prominence. The primary palate, the lip, alveolus and the hard palate lying anterior to the incisive foramen share the same origin.

Between 7 and 12 weeks, the secondary palate develops from the maxillary prominences recognized as two palatine shelves, which course in specific directions to form the secondary palate. A developmental disturbance of the frontonasal prominences leads to an alveolar cleft between the lateral incisor and the canine.[5]

Classification

Clinically, clefts exhibit significant variations in terms of etiology, size, and pathology, which play a crucial role in communication and treatment. Orofacial clefts have been classified based on their size and location, offering a framework for categorization.[6] These classifications encompass a range of cleft types, including simple notching or submucosal clefts, incomplete or complete clefts, unilateral or bilateral involvement, and combinations of lip, palate, and alveolus. Various classification systems have been employed, such as those proposed by Veau and Borel (1931), Fogh-Andersen (1942), Kernahan and Stark (1958), Kernahan (1971), and Kriens (1989) (McBride et al., 2016).

Although multiple classifications have been used, as mentioned earlier, none of them have gained universal acceptance. However, I can describe a few classifications that are widely used.

Veau proposed a simple classification based on the degree of deformity, utilizing a numerical scale from 1 to 4. According to this classification: 1) a cleft of the soft palate only, 2) a cleft of both the soft and hard palate, 3) complete unilateral cleft lip and

palate (UCLP), and 4) complete bilateral cleft lip and palate (CLP) are distinguished (Veau, 1931)[7].

In 1942, Fogh-Andersen introduced a morphological classification of different types of cleft lip and palate (CLP) based on embryology and genetics. According to this classification: 1) cleft lip and alveolus (CLA) (involving the primary palate), 2) unilateral and bilateral cleft lip and palate (CLP), and 3) isolated cleft palate (CP) extending up to the incisive foramen are identified (Fogh-Andersen, 1942)[8].

Kriens' cleft classification is widely regarded as one of the most descriptive and useful classifications. It utilizes the letters "LAHSHAL" to represent the anatomical areas involved, namely the Lip, Alveolus, Hard Palate, and Soft Palate. The uppercase letters indicate complete clefts, while lowercase letters indicate incomplete clefts. One notable advantage of this classification is that it allows for recording the involvement of each anatomical area on either the right or left side of the cleft, with the exception of the soft palate. To simplify the classification, the Royal College of Surgeons removed the second "H" from the original palindrome (Figure 2)[9].

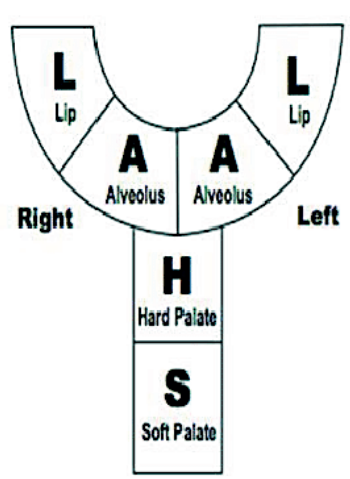

Figure 2: LAHSHAL is a palindrome representing the anatomic structures, proceeding from the patient's right side toward left side.

Treatment Objective

The management of cleft lip or palate reconstructions, from birth to completion, involves multiple treatments and assessments conducted by a multidisciplinary team of experts. Typically, surgical intervention is the primary approach for cleft repair. However, it is crucial to involve other specialized fields, including speech therapy, otorhinolaryngology, maxillofacial surgery, psychology, orthodontics, and dental care[10].

The surgical repair of alveolar clefts aims to achieve both esthetic and functional outcomes. Esthetically, the goal is to enhance the appearance of the alveolus and pyriform area, particularly when the patient smiles, by creating a well-formed curved arch. Functionally, the objectives include closing any vestibular or oronasal fistulas, providing sufficient bone grafting to facilitate the eruption of permanent teeth (such as the central incisor of the maxilla, lateral incisor, and canine), establishing a foundation for the nasal pyriform skeleton architecture required for symmetrical reconstruction of the nasolabial muscles, creating a functional floor for the nasal airway, and replacing missing bone for potential dental implant use[11].

Current Management of Clefts

Cleft lip and palate, including alveolar clefts, require multiple surgeries to address the anatomical deformities. According to current Western guidelines, primary surgical lip repair is recommended around three months of age, while primary soft tissue reconstruction of the palate is typically performed at around 12 months of age[12]. In the past, primary alveolar cleft grafting was often combined with these procedures. However, it is now discouraged due to the potential risk of growth disturbances in the middle third of the facial skeleton[13-14]. The secondary alveolar cleft reconstruction procedure is typically conducted during the mixed dentition phase, usually between 9-12 years of age, specifically just before the eruption of the permanent canine[15].

The reconstruction of an alveolar cleft typically involves the use of marrow-cancellous bone obtained from the iliac crest or other autologous grafts, which is considered the gold standard for alveolar cleft grafting[16]. The iliac crest graft is known for its high regeneration potential and success rate, making it a reliable choice for alveolar cleft reconstruction (Kazemi et al., 2002)[17]. Alternatively, other materials such as allografts,

xenografts, and synthetic grafts have also been utilized for alveolar graft reconstruction[18]. Each option has its own advantages and disadvantages. Autologous grafts require an additional surgical site for harvesting, which is a notable drawback. On the other hand, other bone substitutes primarily possess osteoconductive and/or osteoinductive properties but lack the osteogenic properties found in autologous bone[19].

Regenerative Medicine

In the past three decades, regenerative medicine has gained significant popularity and emerged as an innovative field of biotechnology that integrates cells, molecular biology, and tissue engineering to regenerate and replace tissues[20]. Tissue engineering is defined as a process that modifies the structure and architecture of viable and non-viable tissues to enhance the effectiveness of the construct within biological environments[21]. In the case of managing bone loss, tissue engineering techniques can be employed to promote bone formation through the use of scaffolds, growth factors, stem cells, or a combination of these approaches[22].

Bone regeneration is a complex process involving the generation of new bone tissue along with associated changes in blood supply and inflammation. This process relies on the recruitment and activation of various cells, both locally present and recruited from other locations. Numerous studies have highlighted the significance of various factors, such as bone morphogenetic proteins (BMPs), transforming growth factors (TGFs), and insulin-like growth factors (IGFs), in the process of bone development. Particularly, the role of BMP-2 in promoting the differentiation of osteogenic precursor cells into osteoblasts has been well-established[23].

PolyP as a Novel Regenerative Medicine Compound

This thesis aimed to assess a novel bioactive material as a potential alternative to autologous bone for the reconstruction of alveolar cleft defects. The material under investigation is Ca-polyP microparticles (MPs), which are composed of polyphosphate complexed with calcium. When the high-energy phosphate bonds within the compound are cleaved, it generates energy. Preclinical studies have demonstrated that Ca-polyP-MPs possess osteoinductive and angiogenic properties, making them a promising class of bone substitute materials with unique characteristics (Figure 2)[24].

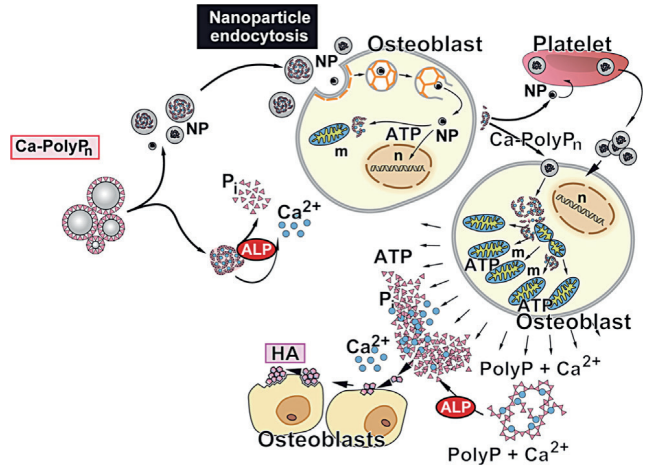

Figure 3. A synopsis of the (potential) biological role of polyP, packed as ''Ca-polyP$_2$'' nanoparticles, during bone mineral formation. The nanoparticles are taken up by clathrin-mediated endocytosis by bone and/or bone-associated cells, resulting in an increase in the intracellular level of ATP, the number of mitochondria (m), and the cell nucleus (n). It is likely that polyP is primarily stored in platelets and synthesized in osteoblasts or osteoblast-like cells. Both ATP and polyP are released by the cells and hydrolyzed by ALP to the building blocks of HA, P_i, and Ca^{2+}. (Muller et at, 2015).

A preclinical study investigated the effects of polyphosphate on wound healing in both diabetic and normal mice. The findings from these studies demonstrated that the presence of polyphosphate microparticles significantly reduced the healing period by accelerating the re-epithelization of wounds[25].

In another preclinical study, conducted in a rat critical size calvarial defect model, the use of amorphous polyphosphate microspheres led to increased bone mineral healing and mineralization after implantation, particularly within 8-12 weeks. These results were compared to the effects of β-tri-calcium phosphate[26].

Outline of the Thesis

In addition to reviewing the currently tested regenerative graft materials for maxillary sinus augmentation and alveolar cleft augmentation, the purpose of this thesis is to assess the safety and viability of this novel regenerative graft material, Ca-Polyphosphate, in a human alveolar cleft defect model. As demonstrated in Chapter 2, we first conducted a systematic literature review and meta-analysis to evaluate the available regenerative graft materials for maxillary sinus augmentation in randomized

clinical trials, as this is the most prevalent clinical model for bone augmentation in the craniofacial region.

In Chapters 3 and 4, systematic reviews and meta-analyses were conducted on stem cell-based tissue engineering of cleft defects in animal models (Chapter 3) and on regenerative alveolar graft materials in clinical trials (Chapter 4) to provide a comprehensive overview of what has been accomplished in alveolar cleft repair utilizing regenerative medicine approaches. Chapter 5 presents a study protocol for a pilot randomized controlled trial involving Polyphosphate (polyP) for alveolar cleft repair. In Chapter 6, we conducted a clinical trial safety and feasibility study on the use of Polyphosphate (PolyP) in repairing alveolar clefts.

In Chapter 7, a prospective study conducted in Indonesia investigated the impact of patient-related variables on intraoperative blood loss during double opposing Z-plasty, Furlow palatoplasty, and buccal fat pad coverage. A prospective clinical trial protocol utilizing micro-fragmented fat (MFAT) and BCP has been proposed for alveolar cleft repair (Chapter 8) in light of the observed benefit of using adipose tissue for bone regeneration. Chapter 9 discusses the outcomes of the topics covered in this thesis and suggestions for future research. Finally, a summary of this thesis in English is provided.

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Chapter 2

Regenerative Graft Materials for Maxillary Sinus Elevation in Randomized Clinical Trials: A Meta-Analysis

S. A. Alkaabi; G. A. Alsabri; D. S. Natsir Kalla; S. A. Alavi; T. Forouzanfar; R. Nurrahma; M. N. Helder. Regenerative graft materials for maxillary sinus elevation in randomized clinical trials: A meta-analysis. Advances in Oral and Maxillofacial Surgery, ISSN: 2667-1476, Vol: 8, Page: 100350. DOI10.1016/j.adoms.2022.100350.

Regenerative graft materials for maxillary sinus elevation in randomized clinical trials: a meta-analysis.

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Short title: Sinus augmentation trials, a meta-analysis.

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Abstract

Background:

Maxillary sinus floor augmentation (MSFA) is a procedure to restore vertical bone defects in the posterior maxilla. Randomized clinical trials (RCT) are considered a golden standard for investigating the efficacy of treatments. Therefore, we aimed to conduct a systemic review and meta-analyses of RCTs using regenerative materials for MSFA and to evaluate the risk of bias (RoB) which can still affect trial validity.

Methods:

Cochrane Oral Health Group's Trials Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE and Google Scholar were searched until December 2020. After outcome assessments and meta-analyses, the articles underwent quality assessment methods (according to the Jadad scale and the Delphi list) to evaluate the RoB.

Results:

Thirty-two studies were included. The meta-analyses found no significant difference between regenerative materials and non-regenerative grafts in new bone formation, augmented bone height, soft tissue area, total bone volume and bone density. However, they displayed a significant difference in terms of residual bone graft. None mentioned quality assessment methods in their trial. Eighteen out of 32 failed to describe the way of randomization, 23 studies did not declare a double blinded approach, and 30 studies failed to clarify their blinding procedure. Moreover, allocation concealment (28 studies), intention to treat (32 studies), and patient awareness (29 studies) were not described or mentioned properly in the trials.

Conclusion:

Meta-analysis showed no significant preference in using regenerative over non-regenerative grafts except when using bone substitutes. The high RoB observed in RCTs implies that quality improvement of CTs is necessary.

Keywords:

Maxillary sinus floor augmentation, Maxillary sinus floor elevation, Bone substitutes, Risk of bias, Quality assessment.

Introduction

Edentulism, especially in the posterior maxillary region as a result of resorption of the alveolar ridge resorption due to pneumatization, often leads to complexities in prosthetic restoration [1,2]. Lack of stimuli by occlusal forces is the primary reason for this alveolar bone resorption, which may reach 40 - 60 % of the original bone volume due to osteoclastic activity. In severe posterior maxillary resorption cases, maxillary sinus floor elevation is the most used augmentation technique, so-called maxillary sinus floor augmentation [3,4]. Autologous bone graft is still considered the golden standard for bone augmentation since it contains osteogenic, osteoconductive, and osteoinductive properties. Nevertheless, it also comes with drawbacks such as an additional surgical intervention, donor site morbidity, need for hospitalization and costs [5].

Therefore, numerous alternative graft materials such as allografts, xenografts, bone substitutes, alloplastic materials and growth factors are used in maxillary sinus floor augmentation (MSFA) [6,8,9]. Those grafts are characterized by osteoconduction or osteoinduction. The main advantages of such substitutes are easy sterilization, no further surgical intervention, and the amount of preference available [7]. Researchers are putting much effort into clinical trials in bone regeneration to optimize the outcomes of MSFA.

Bone substitutes are considered to be reliable alternatives for autogenous bone. Hydroxyapatite (HA) is a good biomaterial with high biocompatibility and negligible negative reactions. In addition, Hydroxyapatite provides osteoconductivity in bone formation [10]. β-tricalcium phosphate (β-TCP) is another reliable and highly biocompatible biomaterial that uses osteoconductive properties in bone formation [11].

Multiple growth factors have also been used in regenerative alveolar bone grafts, such as recombinant human bone morphogenetic protein-2 (rhBMP-2), platelet-rich plasma (PRP) and platelet-derived growth factor (PDGF). It is believed that these factors might help in osteoinductivity, i.e., the stimulation of osteogenic differentiation of cells to promote bone reconstruction and healing [12-14].

Randomized clinical trials (RCTs) are considered the gold standard for evaluating the effectiveness of medical interventions [15]. Appropriately designed and applied RCTs show maximum validity and the highest level of evidence-based medicine [16].

Nevertheless, an RCT is still susceptible to the risk of bias [17]. One of the definitions of risk of bias is “the risk of a systematic error or deviation from the truth, in results or inferences” [18]. The quality evaluation is an alternative term for bias risk [19]. Inappropriate randomization, blinding, patient withdrawal, and allocation concealment increase the likelihood of bias [20]. Various quality scales are available to evaluate RCTs, such as the Jadad scale [21] and the Delphi list [22]. According to Olivo et al.'s [23] systemic review, these are the most commonly used tools for scoring the risk assessment of a study. Kyzas concluded that the literature on oral and maxillofacial surgery (OMFS) from 2004 to 2006 had low scores for the quality of the evidence [24] after applying the Jadad and Delphi methods to the evaluations of OMFS RCTs.

In this systematic review, we intend to assess the regenerative materials that have been used in control trials in MSFA from 1990 to December 2020, to conduct a meta-analysis of the studies that described the mean and standard deviation, and to assess the quality of the extracted trials using the Jadad scale and the Delphi list.

Materials and Methods

The Trials Register of the Cochrane Oral Health Group, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (PubMed), EMBASE, and Google Scholar were searched to identify existing trials on the topic. The International Journal of Biomaterials and the Journal of the International Society for Preventive and Community Dentistry were manually searched to identify additional trials. Articles published in English from 1 January 1990 to 1 December 2020 are included.

Search Strategy

The search strategy was designed to identify the relevant controlled trials (CTs) comparing two or more groups of different materials for augmenting the maxillary sinus for rehabilitation. We used a combination of controlled vocabulary (MeSH) and free text terms:

#1 Maxillary sinus floor augmentation OR sinus augmentation therapy OR sinus floor elevation OR sinus membrane elevation OR sinus lift OR maxillary sinus lift OR sinus elevation

#2 regenerative OR regenerative medicine OR tissue engineering OR stem cells OR growth factors OR cell therapy OR bone regeneration OR Graft material OR graft OR autologous OR (names of different materials)

#3 Clinical trial terms

#4 Human

#1 AND #2 AND #3 AND#4 [Title/Abstract/Keywords]

Exclusion criteria: Studies conducted in (i) animal models; (ii) adults with ASA 3 and beyond; (iii) adolescents (less than 18 years of age) and elderly people (over 70); (iv) studies with an insufficient description of the number of MSFA procedures performed, the technique used for MFSA, number of patients included, or number of inserted implants; and (v) studies with an insufficient description of the length of the observation period.

Summary Measures

Descriptive continuous data were used to examine new bone formation by regenerative materials vs. autogenous bone graft, i.e., mean, sample size, standard deviation, and weight. The amount of new bone formation was evaluated by the mean difference (MD) and the corresponding 95% confidence interval (CI). The MD values were considered significant when the P-value was < 0.05. Reviewer Manager 5 software (the Cochrane Collaboration) was used for meta-analysis. Statistical heterogeneity among studies was assessed with I^2, and a value greater than 50% will be considered an indicator of substantial heterogeneity between studies, which was classified as follows: I^2 < 30% - low heterogeneity, I^2 = 30-60% - medium heterogeneity, I^2 > 60% - high heterogeneity [26].

Risk of Bias

The selected articles underwent quality assessment using the Jadad scale and the Delphi list (Table 1). Two independent reviewers analyzed and scored the risk of bias. However, the author had the final decision-making responsibility in the areas with contention or disagreement. The Jadad scale comprises five yes/no questions (each

valued 1 point if “yes”), whereby a higher score represents a lower risk of bias. The Delphi list contains nine questions, with answers yes, no, or do not know; 1 point is given for yes, while 0 points are given for either "no or do not know " answers. A higher score is considered a low risk of bias (Table 1). For example, a score of 4-5 on the Jaded scale and 6-9 on the Delphi list is considered a low risk of bias [25].

Table 1. Jadad scale and Delphi list.

Scales	Scores
A- Jadad scale	
1. Randomisation	
Was the study described as randomised (this includes the use of words such as randomly, random, and randomisation)?	0–2
Give 1 additional point if the method used to generate the sequence of randomisation was described and it was appropriate (such as from a table of computer-generated random numbers	Plus 1
Deduct 1 point if the method to generate the sequence of randomisation was described and it was not appropriate (such as if patients were allocated alternately, or according to date of birth or hospital number)	Minus 1
2. Double-blinding	
Was the study described as double-blind?	0–2
Give 1 additional point if the method was described and it was appropriate (such as an identical placebo, an active placebo, or a dummy)	Plus 1
Deduct 1 point if the study was described as double-blind but the method of blinding was not appropriate (such as comparison of tablet and injection with no double dummy)	Minus 1
3. Withdrawals and “dropouts”	0-1
Was there a description of withdrawals and “dropouts”? (the number and the reasons in each group must be stated)	
B- Delphi list	
1a. Was a method of randomisation used?	0-1
1b. Was the method of allocation to treatment concealed?	0-1
2. Were the groups similar at baseline as far as the most important prognostic indicators were concerned?	0-1
3. Were the criteria for eligibility specified?	0-1
4. Was the assessor of outcome aware of the treatment allocated?	0-1
5. Was the provider of care aware of the treatment allocated?	0-1
6. Was the patient aware of the treatment allocated?	0-1
7. Were point estimates and measures of variability presented for the primary measures of outcome?	0-1
8. Did the analysis include an intention-to-treat analysis?	0-1

Questions were answered Yes, No, or Do not know. A score of 1 is given when the answer is ‘Yes’. No points are given if the answer is ‘No’ or ‘Do not know’

Results

The primary outcomes from MEDLINE, EMBASE, Cochrane, and Google Scholar included 122 articles (Figure 1). After screening the titles and abstracts, 35 articles were obtained, and upon applying the eligibility, inclusion, and exclusion criteria, 32 studies were selected and fully evaluated (Table 2).

Figure 1: Literature Search Strategy

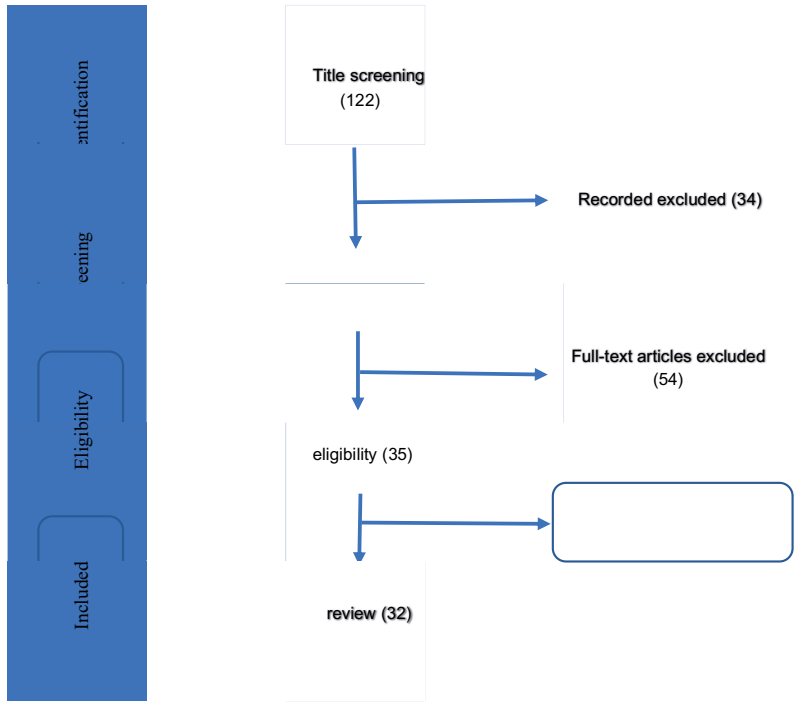


Table 2. List of Clinical trials:

N	Author, year	Country	NOP / NOTS	Mean healing time (months)	Measurement Methods	Comparator/ Control	Graft material /Test / Intervention	Number of Sites	Graft Conclusion	Mean / SD
1.	Boyne et al. (2005) [27]	United States	48 (AB: 13 sinus, rhBMP-2 (0.75); 18 sinus, rhBMP-2 (1.5): 17 sinus).	4-6	Radiographic	AB	rhBMP-2 (0.75 or 1.50 mg/mL) + ACS	Multicenter	At 4 months; rhBMP-2 at 1.5 mg/ml was significantly greater bone density compared with the 0.75 mg/mL, but not to the level of AB. At 6 months; no significant difference.	BD: at 4 months: AB: 350±243, rhBMP-2 /0.75mg/ml +ACS:84±50, rhBMP-2 /1.50 mg/ml +ACS:137±77. At 6 months: AB: 448±213, rhBMP-2 /0.75mg/ml +ACS:456±131, rhBMP-2 /1.50 mg/ml +ACS:508±126. ABH: at 4 months: AB: 11.29±4.12, rhBMP-2 /0.75mg/ml +ACS:9.47±5.72, rhBMP-2 /1.50 mg/ml +ACS:10.16±4.7.
2.	Raghoebar et al. (2005) [28]	Netherlands	5 (5 sinus control, 5 sinus test)	6	Light microscopic, microradiographical and histomorphological	AB	AB with PRP	Monocentric	No significant increase in bone formation by adding PRP.	BD: Premolar side: PRP side 91± 23.1 and non PRP side 84.6±19.6 Molar side: PRP side 71.8±23.8, non-PRP side 90.7± 13.5.
3.	Schaaf et al. (2008) [29]	Germany	34 (34 sinus control, 34 sinus test)	4	Radiographic	AB	AB with PRP	Multicentric	No significant increase when PRP was used in term of bone density in CT evaluation.	NR
4.	Torres et al. (2009) [30]	Spain 74	87 (70 sinus control	6	Histological, histomorphometrical,	BBM	BBM with PRP	Monocentric	Volume of new bone formed was Significantly	NBF: BBM: 21.3 ±4.5
			, 74 sinus test)		densitometric				increased with PRP group.	ABB with PRP 31±5. HAB PRP/ABB groups: 10.4 ± 0.7 and ABB 9.4 ± 0.7.
5.	Bettega et al. (2009) [31]	France	18 (18 sinus control, 18 sinus test	6	Radiological, histological, histomorphometrical.	AB	AB + APC (Small quantity of)	Multicentric	Adjunction of APCs permitted a 60 percent reduction of bone graft required for sinus floor elevation. No significant difference between both groups radiographically and histologically	BD: AP: 72.2±13, AB + APC: 88.9±16.
6.	Rickert et al. (2011) [32]	The Netherlands	12 (12 sinus control, 12 sinus test)	3.5	Histomorphometric	BBM + AB	BBM + Iliac mesenchymal stem cells	Monocentric	BBM+ Stem cell has significantly more bone formation.	NBF: Test group: 17.7 ±7.3, BBM + AB: 12.0 ±6.6.
7.	Sauerbier et al. (2011) [33]	Germany	26 (11 sinus control, 34 sinus test)	3-4	Histological, Histomorphometric	BBM + AB	BBM + BMAC + mesenchymal stem cells.	Monocentric	No significant difference in new bone formation.	NBF: Test group 12.6±1.7. control group14.3±1.8, HAB: Test group 1.74 ±0.69, control group 1.33 ±0.62. Fraction of BBM: Test group 31.3±2.7 control group 19.3±2.5.
8.	Tatullo et al. (2012) [34]	Italy	60	6	Clinical, histological evaluations	BBM	BBM + PRF	Monocentric	PRF helps into production of new bone at 106 days after surgery.	NR

9.	Zhang et al. (2012) [35]	Austria	10 (5 sinus control, 6 sinus test	6	Histological, Histomorphometric	BBM	BBM + PRF	Monocentric	No significant statistical differences in new bone formation And residual bone substitute	NBF: BBM + PRF 18.35±5.62 BBM: 12.95 ± 5.33. RBG BBM + PRF :19.16±6.89, BBM: 28.54±12.01. Bone-to-bone substitute contact in BBM + PRF 21.45±4.57, BBM 18.57± 5.39.
10	Khairy et al. (2013) [36]	Egypt	15 (5 sinus control, 10 sinus tests	6	Histological, Histomorphometric.	AB	AB + PRP	Monocentric	PRP group had statistically significant highest mean bone density at 6 months	BD: AB: 39.5 ±7.4, AB + PRP: 28 ±4.1.
11	Del Fabbro et al. (2013) [37]	Italy	30 (15 sinus control, 15 sinus test)	6-8	Radiological	BBM	BBM + P-PRP	Monocentric	NR	ABH BBM: 4.1 ± 1.1, BBM + P-PRP: 3.9 ±1.3.
12	Pasquali . (2015) [38]	Brazil	8 patients (8 sinus control, 8 sinus test)	6	Histomorphometric	BBM	BBM + BMAC	Monocentric	Significantly higher amount of vital mineralized tissue in when using BMAC and less resorption rate.	RBG: BBM: 22.79 ± 9.60, BBM + BMAC: 6.32 ± 12.03. TBV: BBM + BMAC: 61.47 ± 24.2; BBM: 50.09 ± 11.0. Vital mineralized tissue BBM: 27.30 ± 5.55, BBM + BMAC: 55.15 ± 20.91 Nonvital mineralized tissue BBM: 22.79 ± 9.60, BBM + BMAC: 6.32 ± 12.03
13	Kılıç et al. (2017) [39]	Turkey	26 (9 sinus control, 9 sinus test G1, 8 sinus test G2)	6	Histological, Histomorphometric	β-TCP	P-PRP-mixed b β-TCP (P-PRP group), and PRF-mixed β-TCP (PRF group).	Monocentric	No significant difference in term of new bone formation and residual graft particles.	NBF: Control group 33.40 ± 10.43, P-PRP group 34.83 ±10.12, and PRF group 32.03 ± 6.34. RBG: Control group 30.39 ± 10.29, PRP group 28.98 ±7.94, and PRF group 32.66 ± 7.46. STA: Control group 36.21±10.6, PRP group 36.19±13.9 and PRF group 35.31±10.8 NBF: Test group; 21.38 ± 8.78 and control group; 21.25 ± 5.59.
14	Nizam et al. (2017) [40]	Turkey	13 (13 sinus control, 13 sinus test)	6	Histological, Histomorphometric	BBM	BBM + L-PRF	Monocentric	No significant difference in term of new bone formation residual graft particles.	RBG: Test group; 25.95 ± 9.54 and control group; 32.79 ± 5.89. STA: Test group; 52.7 ± 12.5 and control group; 45.9 ± 8.4. BBGC: Test group; 47.33 ± 12.33 and Control group; 54.04 ± 8.36.
15	Márton et al. (2018) [41]	Hungary	18 (18 sinus control, 18 sinus test)	6	Histomorphometric, radiographic	BBM	Albumin Impregnated BBM	Monocentric	Percentage of the residual graft in the Bone Albumin group was Significantly less and underway towards complete remodeling resembling the maxillary bone.	NR
16	Pichotano et al. (2018) [42]	Brazil	12 (12 sinus control, 12 sinus test)	4, 8	Histological, Histomorphometric, Radiographic, RFA	BBM	BBM + L-PRF	Monocentric	Significant increase of newly formed bone for the BBM + L-PRF group.	NBF: Test group 44.58 ± 13.9, control group 30.02 ± 8.42. RBG: Test group 3.59 ± 4.22, Control group 13.75 ± 9.99.

17	Corinaldesi et al. (2013) [43]	Italy	18: (9 sinus control, 9 test group)	4	Histological, Histomorphometric, radiographic	BBM	rhBMP-7 and BBM;	Monocentric	significantly more new bone on the control group	STA: Test group 26.6 ± 1.1, control group 30.6 ± 12.5. NBF: rhBMP-7 and BBM: 6.55± 4.75; BBM: 19.88± 6.79. RBG: rhBMP-7 and BBM: 27.66 ± 4.74; BBM: 43 ±4.89; TBV: rhBMP-7 and BBM: 34.21 ± 6.7; BBM: 62.88 ±8.4.
18	Kaigler et al. (2015) [44]	USA	12 (12 sinus control, 11 test group)	4	Histological, Histomorphometric, radiographic	ß–TCP	Stem cell therapy + ß -TCP	Monocentric	BVF was significantly higher in the stem cell therapy group in treating severe defects.	TBV: Stem cell + ß -TCP: 49 ± 0.72; ß -TCP: 43 ±0.81. Later bone height: Stem cell + ß -TCP: 12.2 ± 3.3; ß -TCP: 12.8 ± 2.8.
19	Payer et al. (2014) [45]	Austria	6 (6 sinus control, 6 test group)	3,6	Histological, Histomorphometric, radiographic	BBM	BMAC+ BBM	Monocentric	No significant difference in amount of new bone formation	NBF: 3 months: BMAC+BBM: 10.36± 11.83. BBM: 9.45 ± 4.15. 6 months: BMAC+BBM: 14.17 ± 3.59; BBM: 10.41 ± 5.25. BBGC: At 3-month: BBGC 16.40 ± 18.59 at test sites and 15.06 ± 12.52 at control sites At 6 -month: BBGC of 20.26± 11.32 at test sites and 17.89± 9.63 at control sites.
20	Wildburger et al. (2014) [46]	Austria	7 (7 sinus control, 7 test group)	3 and 6	, Histomorphometric	BBM	MSCs from BMAC + BBM	Monocentric	No significant difference in new bone formation between the test and control group	NBF: 3-month, Control group 11.8± 6.2, Test group 7.4± 4.1. at 6-month, control group 13.9± 8.5, test group 13.5±5.4.
21	Gassling et al. (2013) [47]	Germany	6 (6 sinus control, 6 test group)	5	Histomorphometric	AB+ BBM +membrane	AB+BBM+PRF	Monocentric	Bone quality, mean vital bone formation and residual bone substitute not much difference.	NR
22	Olgun et al. (2018) [48]	Turkey	18 (8 sinus control, 10 test group)	4-6	Histological, Histomorphometric, radiographic	Allograft	Titanium-PRF	Monocentric	Radiographically, allograft showed better result in density, volume, and bone height.	NBF: Control group 17.28±2.53, Test group 16.58±1.05. BAH: Control group 19.89±7.41 Test group 11.73±2.37. TBV: Control group 246.6±70.2, Test group 172.7±82.6. BD: Control group 160.8±63.6 Test group 86.7±43.6.
23	Kassolis. (2013) [49]	USA	10 (10 sinus control, 10 test group)	4.5-6	Histological, Histomorphometric, radiographic	FDBA + membrane	FDBA + PRP	Monocentric	FDBA + PRP showed significant higher difference in NBF, and vital tissue compared to control group.	Vital tissue: Test group 78.8±8.8, control group 63.0±15.7. NBF: Test group 33.3±11.3, control group 26.5±6.8. RBG: Test group 21.2±8.3, control group 37.0±15.7.
24	Consolo et al. (2007) [50]	Italy	16 (16 sinus control, 16	4-7	Histological, radiographic	AB	AB+PRP	Monocentric	Histology documents enhanced bone activities in sites treated with PRP, 4	TBV: at 4 months: Test group 43.3±9.1, control group 26±5.2. At 5 months: Test group 39.3±5.7, control group 29.2±4.
21	Gassling et al. (2013) [47]	Germany	6 (6 sinus control, 6 test group)	5	Histomorphometric	AB+ BBM +membrane	AB+BBM+PRF	Monocentric	Bone quality, mean vital bone formation and residual bone substitute not much difference.	Fraction of bovine bone material: 3 months Test group 42.6±3.5 and control group 34.9±11.8. 6 months Test group 36.2±7.8; control group 39.5±9.3.
22	Olgun et al. (2018) [48]	Turkey	18 (8 sinus control, 10 test group)	4-6	Histological, Histomorphometric, radiographic	Allograft	Titanium-PRF	Monocentric	Radiographically, allograft showed better result in density, volume, and bone height.	NR
23	Kassolis. (2013) [49]	USA	10 (10 sinus control, 10 test group)	4.5-6	Histological, Histomorphometric, radiographic	FDBA + membrane	FDBA + PRP	Monocentric	FDBA + PRP showed significant higher difference in NBF, and vital tissue compared to control group.	NBF: Control group 17.28±2.53, Test group 16.58±1.05. BAH: Control group 19.89±7.41 Test group 11.73±2.37. TBV: Control group 246.6±70.2, Test group 172.7±82.6. BD: Control group 160.8±63.6 Test group 86.7±43.6.
24	Consolo et al. (2007) [50]	Italy	16 (16 sinus control, 16	4-7	Histological, radiographic	AB	AB+PRP	Monocentric	Histology documents enhanced bone activities in sites treated with PRP, 4	Vital tissue: Test group 78.8±8.8, control group 63.0±15.7. NBF: Test group 33.3±11.3, control group 26.5±6.8. RBG: Test group 21.2±8.3, control group 37.0±15.7.

			test group)						months after surgery. No statistical significance in long term.	
25	Kim et al. (2015) [51]	Korea	127 (74 sinus control, 73 test group)	3	Histomorphometric, radiographic	BBM	Escherichia coli–derived rh-BMP-2+BMP-2/H	Multicenter	The BMP-2/H group was noninferior to the ABX group	NBF: Test group 16.10 ± 10.52, control group 8.25 ± 9.47, STA: Test group 25.27 ± 10.86, control group 29.44 ± 15.31. RBG: Test group 58.64 ± 14.61, control group 62.31 ± 14.57.
26	Triplett et al. (2009) [52]	UAS	160 (82 sinus control, 78 test group)	6	Histological, radiographic	AB or AB+ Allograft	rhBMP-2/ACS 1.50	Multicenter	No marked differences were found in the histologic parameters evaluated between the 2 groups.	ABH: Test group 7.83 ± 3.52, control group 9.46 ± 4.11
27	Froum et al. (2013) [53]	USA	21 (11 sinus control, 10 test group1, 11 test group2)	6-9	Histological, Histomorphometric	MCBA (allograft)	5.6 ml rhBMP-2/ACS + MCBA (T1) 2.8 ml rhBMP-2/ACS + MCBA (T2)	Monocentric	No statistically significant differences in vital bone and NBF.	RBG: Control group 23.21 ±12.9 TG1; 10.5 ± 12.8, TG2: 22.6±7. NBF: Control group 21.5±11.6, TG1: 25.3±15.3, TG2: 17.5 ±10.9.
28	Kim et al. (2014) [54]	Korea	41 (21 sinus control, 20	6	Histological, radiographic	BBM	ErhBMP-2 0.67 ml in 1.50	Monocentric	No statistically significant in either group	TBV: Control group 279.96±186.76, TG: 205.4±81.2. HAG: Control group 12.39±3.18, TG 13.42±2.26
			test group)				mg/ml buffer + 1 g BCP			
29	Rickert et al. (2013) [55]	The Netherlands	12 (12 sinus control, 12 test groups)	3-4	Radiographic	BBM +AB	BBM+BMAC	Monocentric	No differences in soft tissue parameters or peri-implant bone loss were observed between the control and test sides.	Marginal bone loss in test group 0.47 ±0.31 mm, control group 0.41 ±0.25 mm
30	Whitt et al. (2020) [56]	USA	11 (9 sinus control, 9 test group)	3-4	Histological, Histomorphometric, radiographic	Allograft	Stem cell + allograft	Monocenter	Significant higher vital bone percentage after just 4 months of healing in the posterior aspect.	Vital bone control group (anterior side) 34.14% ± 0.35 Test group (Anterior side) 44.11% ± 0.33. Vital bone control group (posterior side) 24.45% ± 0.23, Test group (posterior side) 50.12% ± 0.35.
31	Koch et al. (2010) [57]	Germany	31 (10 sinus control, 11 test group1, 10 test group 2)	3-4	Histological, Histomorphometric, radiographic	b β-TCP + AB	T1 (3 months healing): rhGDF-5/b-TCP. T2 (4 months healing): rhGDF-5/b-TCP	Multicenter	No differences in NBF	NBF: Control group: 31.8±17.9, test group1, 31.4±17, test group 2, 28±15.5.

32	Froum et al. (2014) [58]	USA	24 (12 sinus control, 12 test groups))	4-5, 7-9	Histological, Histomorphometric, radiographic	BBM	BBM+rhPDGF	Monocenter	Vital bone formation was significantly greater in the 4- to 5-month, but not in 7-9 month.	NBF: At 4-5 month Control group 11.8±9.2, test group 21.1±11.8. At 7-9 months: Control group 21.4±8.6, test group 19.5±10.7. RBG: At 4-5 months: Control group 33.6±12.0, test group 24.8±11.4. At 7-9 months: Control group 40.3±6.7, test group 35.5±9.4.

(NOP) number of patients, (NOTS) number of treated sinuses, (ACS) Absorbable collagen sponge, (PRP) Platelets rich plasma, (P-PRP) Plasma rich in growth factors, (ABC) Absorbable collagen sponge, (BBM) Bovine bone material, (AB) Autologous Bone, (MCBA) Mineralized solvent dehydrated bone allograft, (L-PRF) Leukocyte and Platelet-rich fibrin, (APC) Autologous platelet concentrates, (BMAC) Bone marrow aspirate concentrate, (HA) Hydroxyapatite, (RFA) Resonance Frequency Analysis, (BMAC) Bone marrow aspirate concentrate, (BCS) Biphasic calcium sulfate, (BBS) Biphasic bone substitute, (NHA) Nanohydroxyapatite, (HA) Hydroxyapatite, (β-TCP) Beta-tricalcium phosphate, (EB) Equine-derived bone, (FDBA) Freeze-dried mineralized bone allograft, (BCP) Biphasic calcium phosphate, (BC) Bioapatite-collagen, (CP) Calcium phosphate, (NR) Not reported, (BD) Bone density, (HAB)Height of augmented bone, (TBV) Total bone volume, (RBG) Residual bone graft, (STA) Soft tissue area, (FDBA) Freeze-dried bone allograft, (BMP-2/H) Hydroxyapatite granules, (MCBA) Mineralized cancellous bone allograft, (rhGDF-5) Recombinant human growth and differentiation factor-5. (BBGC) Bone-to-bone graft contact, (rhPDGF) Recombinant human platelet-derived growth factor.

Meta-Analysis

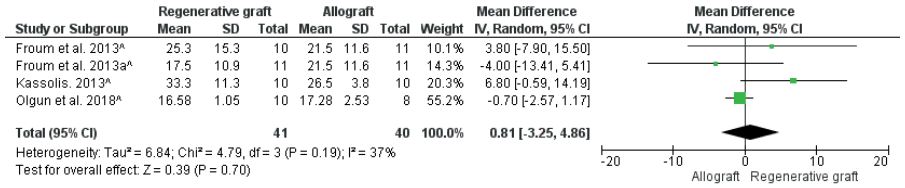
New Bone Formation (NBF)

Sixteen studies (Corinaldesi et al. [43]; Froum et al. [53]; Froum et al. [58]; Kassolis et al. [49]; Kim et al. [51]; Koch et al. [57]; Kılıç et al. [39]; Nizam et al. [40]; Olgun et al. [48]; Payer et al. [45]; Pichotano et al. [42]; Rickert et al. [32]; Sauerbier et al. [33]; Torres et al. [30]; Wildburger et al. [46]; Zhang et al. [35]) reported data on mean and SD of NBF. Three of those sixteen studies had two intervention groups, so the meta-analyses were done in nineteen instead of sixteen.

Three articles used allograft as the control group (Froum et al. [53]; Kassolis et al. [49]; Olgun et al. [48]), in which one study (Froum et al. [53]) had two intervention groups. The meta-analysis showed no significant difference between the allograft and the regenerative groups (containing rhBMP2, PRP and PRF, respectively) regarding new bone formation with P=0,39 and medium heterogeneity = 37% (Figure 2a). Another three articles had a mixture of autologous and bone substitutes as the control group (Koch et al. [57]; Rickert et al. [32]; Sauerbier et al. [33]) in which the study of Koch et al. [57] had two intervention groups. The regenerative treatments consisted of rhGDF-5 and MSCs, respectively. The meta-analysis showed no significant difference in new bone formation with P=0.83 and medium heterogeneity = 55% (Figure 2b).

A third group, consisting of ten studies (Corinaldesi et al. [43]; Froum et al. [58]; Kim et al. [51]; Kılıç et al. [39]; Nizam et al. [40]; Payer et al. [45]; Pichotano et al. [42]; Torres et al. [30]; Wildburger et al. [46]; Zhang et al. [35]) used bone substitute as graft material in their control group, of which one study (Kılıç et al. [39]) had two intervention groups. The regenerative treatments were performed with rhBMP2, rhBMP7, PRP, PRF and BMAC. The meta-analysis showed no significant difference in new bone formation with P= 0,13 and high heterogeneity = 88% (Figure 2c). In an overall meta-analysis comparing the control groups with the regenerative graft intervention groups, a high heterogeneity (I^2= 91%) and no significant difference (P value = 0.13), but still, a trend towards regenerative grafts being in favor of control treatments was found (Figure 2d).

a



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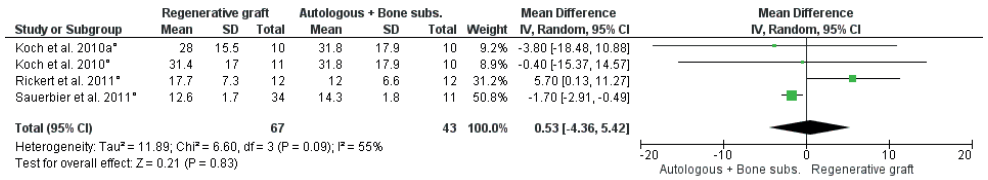

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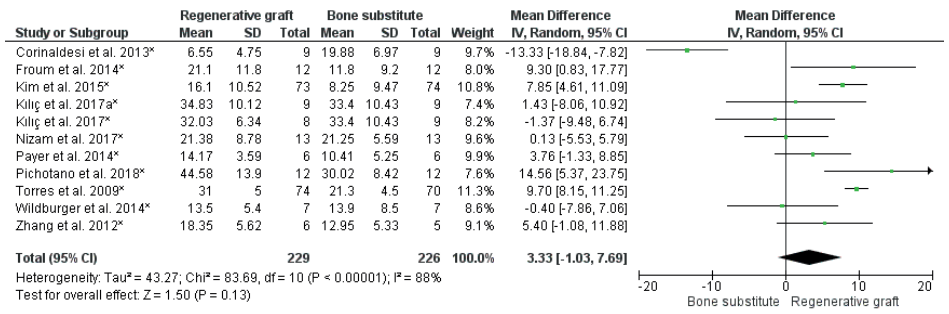

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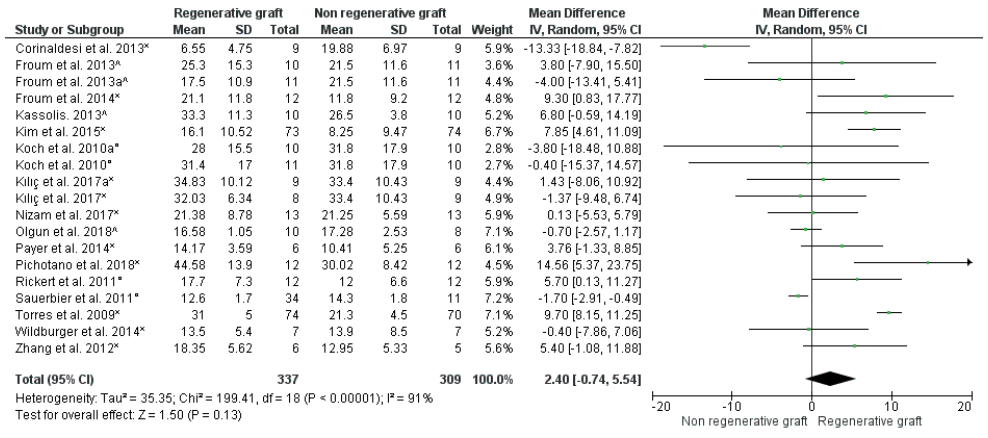


Figure 2a-d: Meta-analysis comparing control groups and regenerative grafts using new bone

formation as the outcome parameter. x the control group was the bone substitute, ^ the control group was allograft, ° the control group was a mixture of autologous and bone substitute.

Bone Density

Five studies (Bettega et al. [31]; Boyne et al. [27]; Khairy et al. [36]; Olgun et al. [48]; Raghoebar et al. [28]) reported data (Mean/SD) on bone density after maxillary sinus augmentation. All but one (Olgun et al. [48], used allograft) studies used autologous graft as a control group. Regenerative treatments comprised autologous platelet concentrate, rhBMP2, PRP and PRF. The meta-analysis revealed no significant difference (P value = 0.54) with high heterogeneity (I^2= 81%) between the regenerative grafts when compared to the control group (Figure 3).

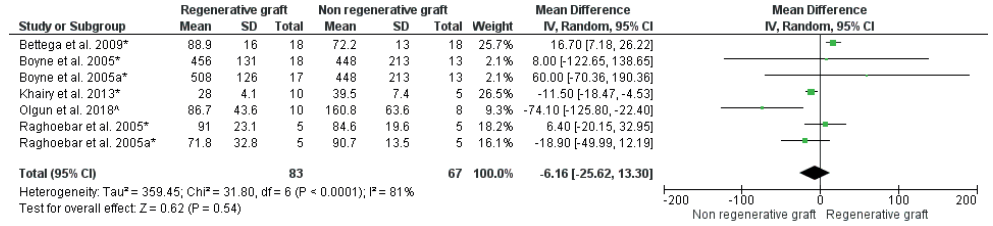


Figure 3: Meta-analysis comparing autologous grafts with regenerative grafts using bone density as the outcome parameter, ^ allograft control group, * autologous bone control group.

Bone Volume

Six studies (Corinaldesi et al. [43]; Consolo et al. [50]; Kaigler et al. [44]; Kim et al. [54]; Olgun et al. [48]; Pasquali et al. [38]) reported data on mean and SD of bone volume. The regenerative grafts contained rhBMP7, PRP, CD90+ BM stem cells, rhBMP2, PRF and BMAC, respectively. The meta-analysis revealed no significant difference (P value = 0.61) with high heterogeneity (I^2= 96%) between the regenerative grafts and the control groups (Figure 4).

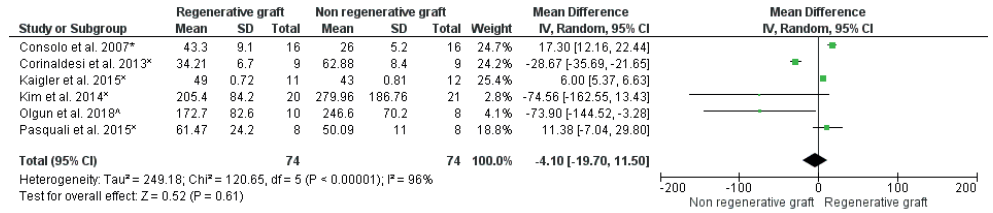


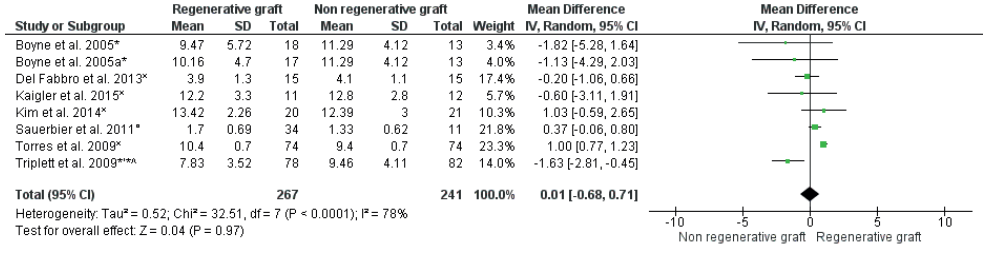
Figure 4: Meta-analysis comparing autologous grafts with regenerative grafts using bone volume as

the outcome parameter. * The control group was autologous graft, ^ the control group was allograft, x the control group was the bone substitute.

Augmented Bone Height

Seven studies (Boyne et al. [27]; Del Fabbro et al. [37]; Kaigler et al. [44]; Triplett et al. [52]; Kim et al. [54]; Sauerbier et al. [33]; Torres et al. [30]) reported data on mean and SD of augmented bone height. One (Boyne et al.[27]) of those seven studies had two intervention groups, so the meta-analysis was done in eight instead of seven. The meta-analysis revealed no significant difference (P value = 0.97) with high heterogeneity (I^2= 78%) between the regenerative graft as intervention groups compared to the control groups (Figure 5a). Four of those seven studies have used bone substitutes as control studies compared to regenerative grafts. The meta-analysis revealed no significant difference (P value = 0.24) with high heterogeneity I^2= 64%) between the regenerative graft as intervention groups compared to the control groups (Figure 5b).

a



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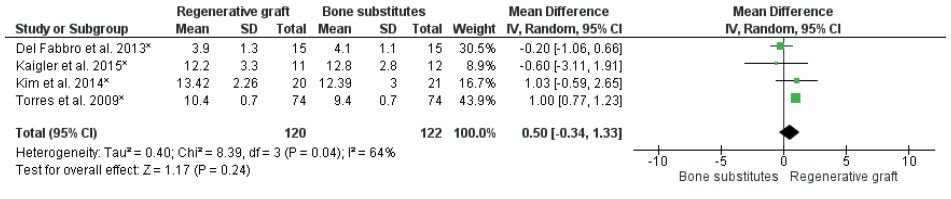


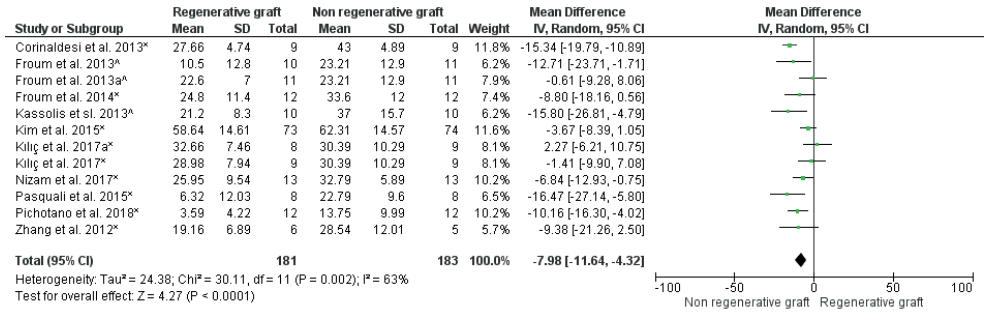
Figure 5 a,b: * the control group was autologous graft, *,*^ the control group either autologous or mixture between autologous and allograft, ° the control group was mixture of autologous and bone substitute; ° the control group was mixture of autologous and bone substitute, x the control group was bone substitute.

Residual Bone Graft

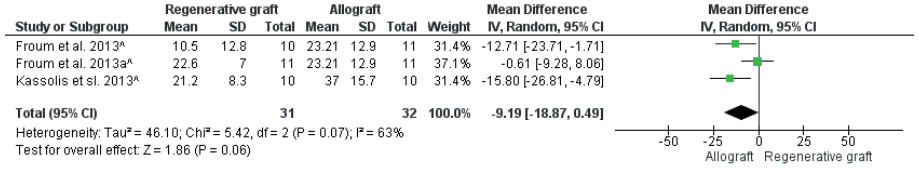
Ten studies (Corinaldesi et al. [43]; Froum et al. [53]; Froum et al. [58]; Kassolis et al. [49]; Kim et al. [51]; Kılıç et al. [39]; Nizam et al. [40]; Pichotano et al. [42]; Pasquali et al. [38]; Zhang et al. [35]) reported data on mean and SD of residual bone graft. Two of those ten studies had two intervention groups, so the meta- analysis was done in twelve instead of ten. The meta-analysis revealed a significant difference (P value = 0.0002) with high heterogeneity I^2= 66%) between the regenerative graft as intervention groups in comparison to the control groups (Figure 6a).

Of the ten studies, two were found to use allograft in their control group, in which Froum et al. [53] had two intervention groups, as mentioned earlier. Even though the result was in favor of allograft, there was no significant difference noticed (P value = 0.06) and high heterogeneity I^2= 63%) (Figure 6b). Eight out of the ten studies (Corinaldesi et al. [43]; Froum et al. [58]; Kim et al. [51]; Kılıç et al. [39]; Nizam et al. [40]; Pichotano et al. [42]; Pasquali et al. [38]; Zhang et al. [35]) were found using a bone substitute, in which Kılıç et al. [39] had two intervention groups in his study. The meta-analysis revealed significant differences (P value = 0.0003) with high heterogeneity I^2= 68%) (Figure 6c).

a



b



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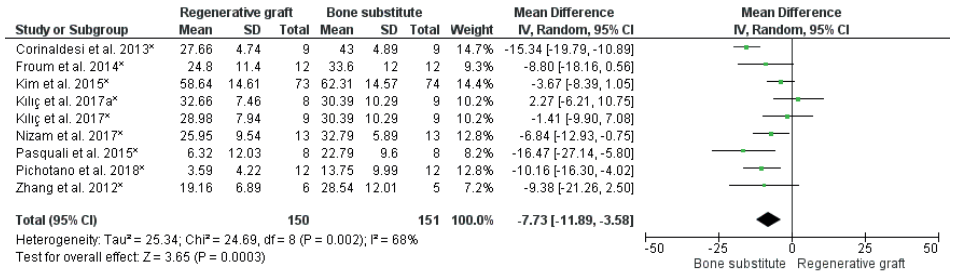

Figure 6 a,b,c: x The control group was autologous graft, ^ the control group was allograft.

Soft Tissue Area

Four studies (Kim et al. [51]; Kılıç et al. [39]; Nizam et al. [40]; Pichotano et al. [42]) reported data on mean and SD of NBF. However, one of those ten studies (Kılıç et al.[39]) had two intervention groups, so the meta-analysis was done in five instead of four. The meta-analysis revealed no significant difference (P value = 0.53) with medium heterogeneity I^2= 32%) between the regenerative graft as intervention groups in comparison to the control groups (Figure 7).

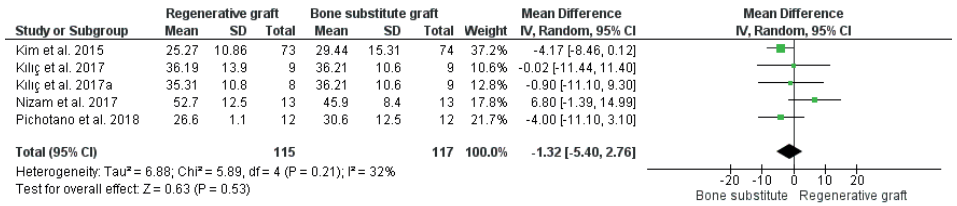

Figure 7: the control group was bone substitute graft, ^ the experiment group was regenerative graft.

Risk of Bias assessment

Table 3 displays that the mean Jadad score was 2.03 (SD: 0.76) while the mean Delphi score was 4.78 (SD: 1.45). In the present assessment, the Jadad quality assessment scale yielded zero articles with a low risk of bias and a score of 4 or 5. 11 out of 32 articles evaluated using the Delphi list received a high score (6) for the risk of bias in their studies (Figures 8,9). We conclude that no papers met the criteria for a low bias risk according to the Jadad scale and the Delphi lists.

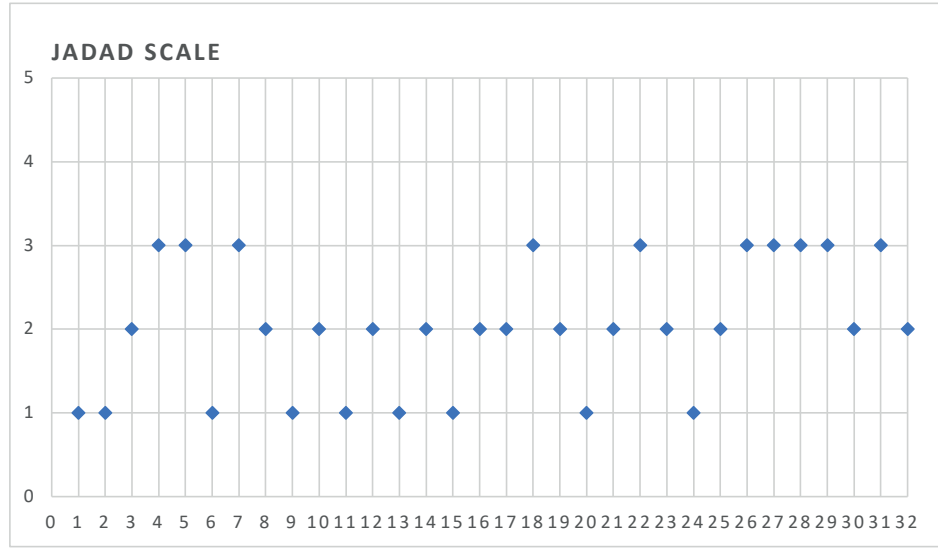

Figure 8: Jadad scale scores

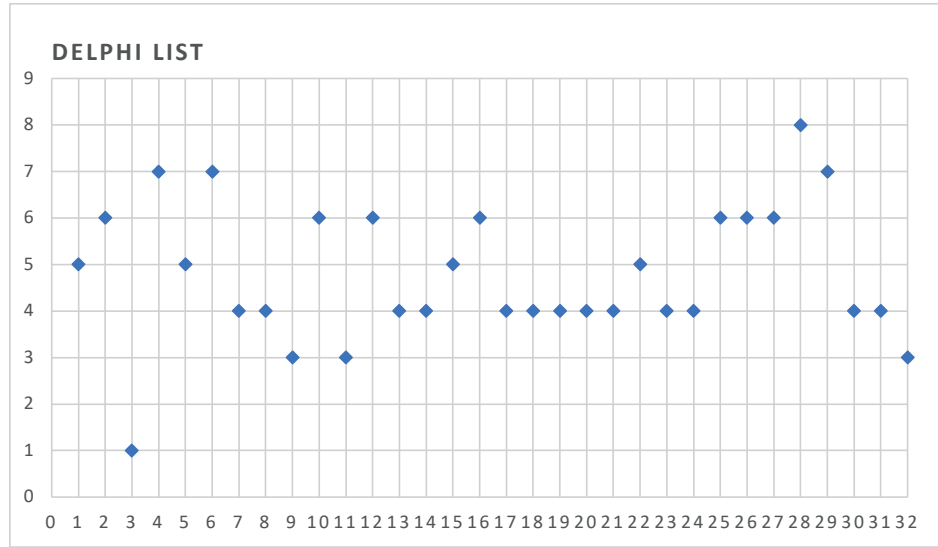

Figure 9: Delphi list scores

Table 3. Scores/risk of bias (n= 32 in each case)

Score	0	1	2	Mean (SD) (range)
Jadad 1	0	32	4	
Jadad 2	23	8	2	
Jadad 3	21	11	-	
Total Jadad				2.03 (0.76) (0-2)
Delphi 1a	0	32	-	
Delphi 1b	28	6	-	
Delphi 2	14	18	-	
Delphi 3	3	29	-	
Delphi 4	10	22	-	
Delphi 5	28	6	-	
Delphi 6	29	3	-	
Delphi 7	3	29	-	
Delphi 8	32	0	-	
Total Delphi				4.78 (1.45) (0-9)

Overall, in the Jadad scale, the most important point in the risk of bias was blinding, whereas, in the Delphi list, more items contributed: intention to treat analysis, concealment of allocation, patient awareness and provider awareness. The mean (SD) score for the randomization was given 1.56 (0.49) with a percentage of 43.75% and 56.25% for scores 1 and 2, respectively, in the Jadad scale, while in Delphi was given 1 (0) with the percentage of 100%. Out of 32 articles, only 18 described the methods of randomization in their clinical trial.

In the Jadad scale, the mean (SD) score for double blinding (range 0-2) was 0.125 (0.33). Four articles (12.5%) (Bettega et al. [31]; Pichotano et al. [42]; Torres et al. [30]; Rickert et al. [50]) indicated a double-blinded method in their clinical trial, with zero articles properly stating the method of blinding.

Delphi items 4, 5 and 6 (assessor, provider, and patient awareness) revealed 22 articles (68.75%) where the assessors were not aware of the allocation, six articles (18.75%) had the care provider blinded of the treatment allocation, and three articles (9.37%) reported that patients were blinded from the treatment received.

Discussion

When evaluating the efficacy of regenerative constructs, multiple factors must be considered; first, the relative contribution of the graft material to the regenerative effect. In short, one may assume that the additive value of the regenerative compound will be greatest when the contribution of the graft material is minimal. In this regard, the strictly osteoconductive bone substitutes and, to a lesser extent, allografts will be the most conclusive. Allografts are typically obtained from a biobank's frozen storage and frequently pre-processed to eliminate cellular activity. Autograft will be the least conclusive because it is typically processed intraoperatively and in a fresh state, containing live osteogenic cells, osteogenic factors in the bone matrix, and bone scaffolding properties.

Another consideration is the outcome evaluation methodology. Two common methods for evaluating bone grafts after MSFA are radiographic analysis and histological/histomorphometric analysis. In maxillary sinus floor augmentation, a two-dimensional radiographic evaluation is the simplest way to assess the vertical bone height of the grafted bone. However, two-dimensional radiographs (dental or panoramic) cannot be used for bone volumetric analysis. Alternative forms of three-dimensional radiography (MRI, CBCT, or CT) should be used to assess the volume of maxillary sinus grafts [74].

However, when radio-opaque scaffold materials such as calcium phosphates (CaP) are used, it is nearly impossible to distinguish the scaffold from the newly formed bone within the material's pores or directly surrounding it. Radiographic analysis alone cannot provide access to more detailed information (including cellular activities) regarding new bone formation, bone resorption, bone structure, osteoid volume, bone mineralization, and other bone modeling/remodeling parameters. Such information can only be obtained through histomorphometric analysis [75,76].

Analyzing the parameters primarily determined radiographically, i.e., bone density, augmented bone height, and bone volume, our meta-analyses revealed no statistically significant differences between regenerative and non-regenerative grafts but a slight preference for the latter. Since it is difficult to distinguish radio-opaque scaffolds from newly formed bone (see above), the higher bone volume may result from the non-regenerative grafts being less actively remodeled and remaining intact for a longer

period. In contrast, the regenerative compounds may rapidly remodel and volume-reduce the scaffold material. Also, since the majority of regenerative grafts consist of autologous bone plus a regenerative compound when compared to autografts alone, the value of the autologous bone (still regarded as the optimal osteogenic graft) is likely to be diminished.

Using histological/histomorphometric analysis, the primary outcome parameters being evaluated are new bone formation and residual bone graft. From our meta-analyses, a clear picture emerges: a clear preference for regenerative grafts with regard to new bone formation, and a more active remodeling when considering the residual graft volume (note: the non-regenerative grafts showing more residual bone graft indicates that the material is less actively resorbed and replaced by new bone).

As stated previously, we believe that histological/histomorphometric analysis is the most conclusive type of analysis, especially when the scaffold materials used are bone substitutes. Based on these considerations, we believe that the subgroups depicted in Figures 2c and 6c provide the most reliable information regarding which compounds possess the greatest regenerative capacity. In the MSFA model, several regenerative compounds have been evaluated. Some of these factors exhibit favorable results for the two most important parameters (new bone formation and residual bone graft).

Platelet-rich fibrin (PRF) is the subject of most studies [35,39,40,42], of which 50% demonstrate enhanced new bone formation and 75% demonstrate accelerated resorption of the graft material, indicating active bone remodeling. PDGF [58], rhBMP-2 (at high concentration) [51], and concentrated bone marrow aspirate [45] are additional factors exhibiting increased osteogenic activity and active bone remodeling. PRP [30,39] and bone marrow MSCs [46] exhibited negligible to negligible positive results. Most notable was the dual negative effect of rhBMP-7, which exhibited active resorption but concurrently significantly reduced new bone formation compared to the control treatment. In conclusion, it appears that PRF, PDGF, and higher concentrations of BMP-2 would be advantageous regenerative agents, whereas rhBMP-7 should be avoided. Due to the high risk of bias determined for the studies included in this review, the aforementioned conclusions should be viewed with caution.

In the field of oral and maxillofacial surgery (OMFS), Verhagen et al. [22] emphasized that adding unbiased quality to any study would result in a more accurate and

trustworthy conclusion. In our study, 32 RCT articles involving MSFA clinical trials exhibited poor design, resulting in a substantial risk of bias. Blinding the outcome assessor from both the group sample and the purpose of the study may help to reduce the possibility of bias [68,69]. Assessment bias may result if the outcome evaluator is aware of the intervention assignment. Blinding surgical trial participants is feasible when two similar surgical procedures are utilized [50]. The generation of random sequences is insufficient to eliminate selection bias. It is necessary to conceal the allocation in order to generate a random sequence [9].

Akl et al. [70] suggested that the central allocation using a sequentially numbered opaque sealed envelope (SNOSE) should be used to maximize the patients' and researchers' privacy. Additionally, the authors stated that opening the envelope in advance poses a risk of bias. For the "Withdrawals" question on the Jadad scale, it is essential that the author explicitly states that either all patients are the same patients from the first day of trial enrollment or that the date and number of patients who dropped out of the study are provided. An outpatient who drops out of a randomized clinical trial can have a significant impact on the values of the results, primarily through the analysis of "intention to treat" (ITT), which involves analyzing all candidates regardless of whether they complete the trial or not, since all participants are subjected to the same protocol [71]. Pre-protocol (PP) analysis, on the other hand, refers to only those patients who completed the trial and strictly adhered to the protocol [21]. According to Pocock and Abdalla [72], ITT should be the primary criterion in a randomized clinical trial, and treatment conclusions should be based on this pragmatic analysis.

How does our research compare to other systematic reviews recently published? Stumbras et al. [59] conducted a systematic review in 2020 evaluating the effectiveness of bone substitute materials used for MSFA. They concluded that autologous bone, either alone or in combination with bone substitutes, continues to have the greatest regenerative potential for sinus floor augmentation. However, they could not conduct the meta-analysis due to the trials' heterogeneity. In 2017, Correia et al. [60] published a systematic review regarding the use of regenerative medicine in sinus augmentation. Although randomized clinical trials were evaluated in the review, no meta-analysis was conducted. Starch- Jensen et al. [61] conducted another systematic review, including a meta-analysis of MSFA. The review's focus was not on regenerative grafts per se, and it included trials in which the autologous graft served as the control group. Three

additional systematic reviews on the effect of a growth factor as regenerative medicine were discovered in the literature [62-64]; each review focused on a single growth factor.

Conclusion

Besides bone substitutes, meta-analyses of various outcome parameters comparing regenerative compound-supplemented grafts to their non-supplemented counterparts did not reveal significant preferences for regenerative over non-regenerative grafts. The high RoB observed in RCTs suggests that CT quality must be enhanced. Based on the data presented in this review, we conclude that the RCTs evaluating maxillary sinus augmentation have a high risk of bias, as measured by the Jadad scale and the Delphi list. Future trials on materials for maxillary sinus augmentation should be designed to adhere to standardized and validated reporting methodologies.

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Abbreviations

(MSFA) Maxillary sinus floor augmentation

(ACS) Absorbable collagen sponge

(PRP) Platelets rich plasma

(P-PRP) Plasma rich in growth factors

(ABC) Absorbable collagen sponge

(BBM) Bovine bone material

(AB) Autologous Bone

(MCBA) Mineralized solvent dehydrated bone allograft

(L-PRF) Leukocyte and Platelet-rich fibrin

(APC) Autologous platelet concentrates

(BMAC) Bone marrow aspirate concentrate

(HA) Hydroxyapatite

(RFA) Resonance Frequency Analysis

(BMAC) Bone marrow aspirate concentrate

(BCS) Biphasic calcium sulfate

(BBS) Biphasic bone substitute

(NHA) Nanohydroxyapatite

(HA) Hydroxyapatite

(β-TCP) Beta-tricalcium phosphate

(EB) Equine-derived bone

(FDBA) Freeze-dried mineralized bone allograft

(BCP) Biphasic calcium phosphate

(BC) Bioapatite-collagen

(CP) Calcium phosphate

(NR) Not reported

(BD) Bone density

(HAB)Height of augmented bone

(TBV) Total bone volume

(RBG) Residual bone graft

(STA) Soft tissue area

(BMP-2/H) Hydroxyapatite granules

(MCBA) Mineralized cancellous bone allograft

(rhGDF-5) Recombinant human growth and differentiation factor-5

(BBGC) Bone-to-bone graft contact

(rhPDGF) Recombinant human platelet-derived growth factor

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Chapter 3

Stem Cell-Based Tissue Engineering of Cleft Defects: Systematic Review and Meta-Analysis

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Stem Cell-Based Tissue Engineering for Cleft Defects: Systematic Review and Meta-Analysis

Running title: Stem Cell-Based Tissue Engineering for Cleft Defects

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Abstract

Objectives: This study aimed to analyze the efficacy of stem cell-based tissue engineering for the treatment of alveolar cleft (AC) and cleft palate (CP) defects in animal models.

Design: Systematic review and meta-analysis.

Setting: Preclinical studies on alveolar cleft repair in maxillofacial practice.

Patients, Participants: Electronic search was performed using PubMed, Embase, and Cochrane databases. Pre-clinical studies, where stem cell-based tissue engineering was used in the reconstruction of AC and CP in animal models were included. Quality of the selected articles was evaluated using SYRCLE (SYstematic Review Centre for Laboratory animal Experimentation).

Interventions: Review of alveolar cleft bone augmentation interventions in preclinical models.

Main Outcome Measures: Outcome parameters registered were new bone formation (NBF) and/or bone mineral density (BMD).

Results: Thirteen large and twelve small animal studies on AC (21) and CP (4) reconstructions were included. Studies had an unclear-to-high risk of bias. Bone marrow mesenchymal stem cells were the most widely used cell source. Meta-analyses for AC indicated non-significant benefits in favor of: (1) scaffold+cells over scaffold-only (NBF p=0.13); and (2) scaffold+cells over empty control (NBF p=0.66; BMD p=0.31). Interestingly, dog studies using regenerative grafts showed similar to superior bone formation compared to autografts. Meta analysis for the CP group was not possible.

Conclusions: AC and CP reconstructions are enhanced by addition of osteogenic cells to biomaterials. Directions and estimates of treatment effect are useful to predict therapeutic efficacy and guide future clinical trials of bone tissue engineering.

Keywords: Alveolar cleft, cleft palate, stem cell, animal study, systematic review

Introduction

Oral clefts consist of heterogeneous congenital malformations that are typically presented as incomplete formation of the upper lip (cleft lip) and/or the roof of the mouth (cleft palate). The malformations occur in about 1 in 700 live births. They can appear individually, or both defects may occur together (cleft lip and palate)[1]. The conditions may develop as a unilateral or bilateral malformation with a wide range of severity [2]. The oral cleft may also occur with other congenital anomalies or be part of a genetic syndrome [2,3]. The malformations are usually associated with the following factors: heredity, genetics, nutritional disturbances, stress during developmental stages, inadequate vascular supply, mechanical disturbances, infections, and teratogens that inhibit the union of nasal process and palatal shelves between the fourth and tenth week of gestation age [4].

One of the crucial steps of oral cleft surgery is the reconstruction of the alveolar cleft and cleft palate by a multidisciplinary team with various approaches depending on the degree of the defect [5,6]. The gold standard for cleft palate surgery is primary palate repair, usually performed around 18 months [6].However, this method is often associated with insufficient tissue to close the defect properly[7] or post-surgical results such as facial growth disturbance and oronasal fistula. As for alveolar cleft surgery, the standard therapy uses autologous bone grafts to replace the lost bone [5]. The timing of alveolar cleft surgery, in general, is divided into three stages: early repair (<5 years old), secondary repair around the canine eruption (>10 years old), and late repair (>13 years old)[6]. The therapy, however, has several side effects, such as growth disturbances6, specific to donor site morbidity such as infection, bleeding, loosening of splint, pain, or sensory deficiency [8,9]. Allograft and synthetic materials as alternatives to autologous bone grafts also have several side effects such as infection, immunologic reaction [5], and reduced bone formation rates[10]. All of these standard approaches may become more complex due to the need for simultaneous repair (e.g., cleft palate and alveolar cleft repair at the same time) in areas where health facilities are limited[11].

These challenges prompted the search for better alternatives for the golden standard procedure. Preferred technologies that are feasible, adaptive, and cost-effective with minimal side effects, and can be implemented even in limited settings. One example is the use of stem cell-based tissue engineering. The technology combines stem cells, biomaterials or scaffolds, and/or biomolecules to regenerate new tissue [12,13]. The combination can be used to replace the harvesting process of autologous bone graft for alveolar cleft repair [12] and to overcome the poor quality or quantity of mucosa for cleft palate repair [13]. The application of stem cell-based tissue engineering for the alveolar cleft is not new several clinical applications have been reported[14,15]. In contrast, the progress of stem cell-based tissue engineering application for palatal bone is still limited to animal studies [16,17].

Many article reviews have discussed the topic of tissue engineering for cleft palate or alveolar cleft. To name a few, Moreau et al. wrote an article review about the general concept of tissue engineering as an alternative way of cleft palate reconstruction [13]. It was Zuk et al. who first wrote an article review focused on possible applications of adipose stem cells for cleft-palate tissue engineering procedure [18]. In 2015, Gladyzs et al. described stem cell-based tissue engineering for alveolar cleft in a narrative review, but only summarized the pre-clinical studies, early case reports, and ongoing trials [19]. Recently, Shanbhag et al (2019) conducted a large systematic review and meta-analysis of cell-based tissue engineering in clinical and pre-clinical studies in a broader manner in all oral and maxillofacial areas [20]. However, none of these reviews focused on stem cell-based tissue engineering for the alveolar cleft and cleft palate.

Therefore, the present study aims to evaluate the efficacy of stem cell-based tissue engineering for cleft palate and alveolar cleft defects by conducting a systematic review and meta-analysis of pre-clinical studies.

Materials and Methods

Protocol and eligibility criteria:

This review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[21]. The protocol was registered on PROSPERO (ID: CRD42021259614). The inclusion criteria were:

1. English language studies.
2. Randomized or non-randomized controlled animal experimental studies with two or more experimental groups.
3. Transplantation of differentiated or undifferentiated mesenchymal stem cells seeded on biomaterial scaffolds in at least one experimental group.
4. A control group receiving “cell-free” biomaterial scaffolds and/or autogenous bone.

5. Reported results quantitative histomorphometric new bone formation/growth (%NBF/NBG), quantitative radiographic assessment of bone formation via computerized tomography (CT) or micro-CT (%NBF/NBG), quantitative histomorphometric assessment of remaining defect (RD), and/or quantitative radiographic assessment of RD or Bone Mineral Density (BMD) using CT or micro-CT.

The exclusion criteria were:

1. In vitro studies.
2. Case reports.
3. Absence of a control group.

Information sources and search:

The electronic literature search was performed using MEDLINE (via PubMed), Embase, and Cochrane for relevant English-language articles until 5 April 2022. Other literature was searched via the Google and Google Scholar search engines. A specific search strategy was developed for MEDLINE and adapted for other databases.

#1 "Mesenchymal Stromal Cells"[Mesh] OR "Mesenchymal Stem Cell Transplantation"[Mesh] OR Mesenchymal Stromal Cell*[tiab] OR Mesenchymal Stroma Cell*[tiab] OR Mesenchymal Stem Cell*[tiab] OR BMSC*[tiab] OR Mesenchymal Progenitor Cell*[tiab] OR Bone marrow stromal cell*[tiab] OR Bone marrow stroma cell*[tiab] OR Bone marrow stem cell*[tiab]

#2 "Adipose Tissue"[Mesh:NoExp] OR "Abdominal Fat"[Mesh] OR ADSC*[tiab] OR ASC[tiab] OR ASCs[tiab] OR Abdominal Adipose Tissue*[tiab] OR Abdominal fat pad*[tiab] OR Adipose Derived Stem Cell*[tiab] OR Adipose Stem Cell*[tiab] OR stromal vascular fraction*[tiab] OR SVF[tiab]

#3 "Cleft Palate"[Mesh] OR OR cleft palate*[tiab] OR palatal cleft*[tiab] OR alveolar cleft*[tiab]

#4 "Alveolar Bone Grafting"[Mesh] OR (Alveolar Bone[tiab] AND (graft*[tiab] OR repair*[tiab] OR transplant*[tiab]))

#5 ((#1 OR #2) AND (#3 OR #4))

Study Selection, Data Collection, and Data Items

Two independent reviewers (DSNK and SAA) performed a title and abstract screening to obtain the full texts of all eligible articles. Disagreements regarding the determination of admissible articles were resolved through discussion. A third reviewer (FNH) was consulted for statistical analysis and to evaluate the articles, if necessary. Based on the inclusion and exclusion criteria, three authors (DSNK, SAA, and NEN) examined the full-text articles and selected the final eligible studies. Figure 1 provides an overview of the complete screening procedure.

Author, publication year, subjects/models, number of subjects, age, stem cell criteria (source, expanded/non-expanded, osteogenic medium usage, cell dose/density), scaffold criteria (type, size), growth factor criteria (type, concentration), control group, observation time, method, and result (histomorphometry, CT, and others) were extracted from the eligible articles. Tabular descriptions of the included studies were maintained. For the potential meta-analysis, quantitative data on histomorphometric new bone formation (%NBF), radiographic assessment of bone formation using computerized tomography (CT) or micro-CT (%NBF), histomorphometric assessment of remaining defect (RD), and radiographic assessment of RD or BMD using CT or micro-CT were extracted.

If data were only presented graphically, numerical values were requested from the authors; if no response was received, digital ruler software (ImageJ; National Institutes of Health, Bethesda, Maryland, United States) was used to measure graphical data. When studies reported outcomes at some time points, the most recent outcomes were extracted. DSNK and NEN performed a meta-analysis of the outcome data from multiple studies at comparable time points. When studies reported outcomes of more than one experimental group, meta-analysis was performed by “including each pair-wise comparison separately, but with shared intervention groups divided out approximately evenly among the comparisons” (Cochrane Handbook Chapter 16.5)[22].

Risk of Bias

The risk of bias (RoB) of animal studies was assessed using SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) [23,24]. The results were

presented in the risk of bias graph and summary using RevMan 5.4 program (Review Manager. The Cochrane Collaboration, 2020).

Meta-analysis

The data were analyzed using Review Manager Software version 5.4 (Review Manager. The Cochrane Collaboration, 2020). Meta-analysis was performed by comparing the standardized mean difference of outcome measures for new bone formation and remaining defects after using differentiated or undifferentiated mesenchymal stem cells for cleft palate and alveolar cleft defects. Subgroup analyses were performed at the level of animals. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed for an evaluation period of at least 6 weeks (42 days). Statistical heterogeneity was analysed using Cochrane's Q test and the inconsistency I^2 test, in which value higher than 50% were considered indicative of substantial heterogeneity. Publication bias was not assessed using the symmetry of funnel plots because there were less than 10 studies thus the assessment methods are not very reliable)[25,26].

Results

Initially, 365 articles were identified from MEDLINE (via PubMed), Embase, and Cochrane databases. No studies were identified from other sources. Of 365 articles, only 25 studies were included for qualitative analysis, and only 10 of the 25 studies were eligible for quantitative analysis. All articles were in vivo studies in an animal model that investigated the alveolar cleft (21 studies) or cleft palate (4 studies) using cell-based tissue engineering. The maximum follow-up time ranged from 6 weeks (42 days) to 6 months (180 days). The characteristics of the included studies were summarized in Tables 1 and 2.

In the sections below, we will discuss outcomes of individual studies and group meta-analyses sometimes in terms of "better, more, or higher levels." These statements should be regarded as qualitative and indicative, but certainly not as being statistically relevant. Nevertheless, we thought it important in which direction the differences between cell-based and control reconstructions headed, even though we realize ourselves that this is maybe not scientifically correct, but rather "telling."

Synthesis of Results

a. Alveolar Cleft:

A total of 21 articles have provided information on cell-based tissue engineering in the alveolar cleft animal model. Six types of animals were used namely rat, rabbit, pig, dog, goat, and monkey. Genetically, cell transplantation was comprised of 4 types (autologous, allogenic, syngenic, and xenogenic). Cell sources were bone marrow mesenchymal stem cells from animals ((rat[27–33], dog[34–37], pig[38], monkey[39]) n= 13), umbilical cord mesenchymal stem cells from human[40–42] (n= 3) or animal (pig[43], n= 1), human differentiated gingiva derived mesenchymal stem cells44 (n= 1), dog adipose stem cells[45,46] (n= 2), and finally human mesenchymal stem cell from orbicularis oris muscle[47] (n= 1).

Five types of scaffolds were applied, namely ceramics, synthetic polymers, natural polymers, autologous bone, or without any scaffold. Four articles used a single type of ceramics scaffold[28,34,36,37]. Four articles used a single type of synthetic polymer scaffold[38,39,43,44]. Two articles used a single-type natural polymer scaffold30,33, one article used autologous bone35, and one article did not use any scaffold in its study[31]. Five articles used a combination of ceramics and natural polymers[27,29,40–42], 3 articles used a combination of at least two types of ceramic scaffolds[32,45,46], and only one article used three types of ceramic scaffolds separately[47].

Two types of growth factors were applied, namely BMP-2 and PRF. Two articles used BMP-2[40,44], 1 article used BMP-2 gene-loaded nanoparticles[39], 1 article used adenovirus BMP-2[38], and 1 article used PRF[35]. The remaining 16 articles in this group did not use growth factor in their study[27–34,36,37,41–43,45–47].

All studies reported the osteogenic potential as an outcome parameter. Still, we only focused on the outcome results based on histology, histomorphometry, CBCT, and/or CT-Scan analysis. One study expressed a higher level of bone formation with the cell-only application for alveolar cleft reconstruction[31]. Nine studies showed a trend towards higher bone formation for alveolar cleft reconstruction with cell + scaffold combination[27,30,32,33,36,41–43,47]. Five studies showed that the combination of cell + scaffold showed similar levels of alveolar cleft reconstruction

compared to the control group[28,29,34,37,45]. Five studies expressed more bone formation for alveolar cleft reconstruction with cell + scaffold + GF combination compared to control conditions[35,38–40,44].

b. Cleft Palate:

A total of four articles have provided information on cell-based tissue engineering in cleft palate animal models. Three animal groups were used, namely rat[17,48], dog[49], and rabbit[16]. Cell transplantation was comprised of only two types (autologous and allogenic). Bone marrow was the sole source of mesenchymal stem cells in dogs and rats (n= 3)[17,48,49], whereas the rabbit model applied MSCs from adipose tissue (n= 1)[16]. Four types of scaffolds were applied, namely calcium phosphate (n= 1)[49], alginate-based hydrogel scaffolds (n= 1)[48], poly(lactic-co-glycolic acid) (n= 1)[17], and fibrin-agarose (n= 1)[16]. In this group, there were no growth factors applied.

The osteogenic potential was assessed as the primary outcome parameter in all studies. One study expressed more bone formation clinically with cell + scaffold application for cleft palate reconstruction[17]. Three studies described higher bone formation levels for cleft palate reconstruction with cell + scaffold combination compared to scaffold only conditions[16,48,49].

Table 1. Animal studies of the alveolar cleft defect

No	Study	Model	Number	Cell Source	Scaffold	Growth Factor	Experimental group(s)	Control Group	Results
1.	Ahlfeld 2021	Lewis rats (alveolar cleft)	16 (4 dropouts, total of 12)	Syngenic (rMSC)	CPC and cell-laden fibrin hydrogel	n.a	2)CPC/fibrin/rMSCs	1)Historical data obtained with scaffold alone	Histomorphometry (12 weeks) Group 2: NBF 13.7 ± 12.1%. Micro-CT (12 weeks) Lamellar and cancellous bones were isodense with the 3D plotted CPC structure.
2.	Bangun 2021	Goats (alveolar cleft)	24	Xenogenic (hUMSC)	HA/Chitosan/Gelatin	BMP-2 (Novosys)	2) HA/Chito-san/Gelatin + rhBMP-2 3) HA/Chito-san/Gelatin + hUMSC	1) ICABG	Histology using Mankani Score (12 weeks) Group 1: 3.2 (3;3-4) Group 2: 2 (1.5;1-4) Group 3: 3.2 (3.5;2-4) 3D CT-Scan (12 weeks) Group 1: NBF 22.53% (20.6; 13.9-35) Group 2: NBF 19.05% (16.2; 10.8-33.1) Group 3: NBF 38.3% (39.8; 0.9-72.8)

3.	Kandalam 2021	athymic nude rats (bilateral alveolar bone defect)	30	Xenogenic (dGMSC)	Hydrogel PuraMatrix ™ (PM)	BMP-2	2) PM/BMP-2 3) PM/dGMSC 4) PM/dGMSC/ BMP-2	1a) Empty defect 1b) PM alone	Histology (8 weeks) Group 1b: supported new bone formation Group 2: showed lamellar bone formation followed by mature bone Group 3: showed viable osteocytes with lacunae, osteoblasts, and immature blood vessels within the new bone Group 4: more matured bone with reversal lines and neovascularization with osteocytes Micro-CT (8 weeks) Group 1a: BV 22.40 ± 0.59 % Group 1b: BV 22.75 ± 3.08 % Group 2: BV 27.10 ± 3.08 % Group 3: BV 26.22 ± 1.22 % Group 4: BV 39.98 ± 7.73 %
4.	Toyota 2021	Rats (alveolar cleft)	15	Xenogenic (UC-MACS)	ReFit (HA+Collagen 80:20)	n.a	2) Scaffold + UC-MACS	1a) Empty control 1b) Scaffold only	Histology (8 weeks) Group 1a: NBF 24.5% ± 7.6% Group 1b: NBF 44% ± 5.7% Group 3: NBF 60.5% ± 6.9% Micro-CT (8 weeks) Group 1a: a small number of island-shaped opacities Group 1b: a thin bone bridge between the bone defect Group 2: a bone bridge between the defect with a higher CT value than immediately after the implant
5.	Korn 2020	Lewis rat (alveolar cleft)	80	Syngenic (donor adult Lewis rats) (rMSC)	CPC	n.a	2) Scaffold A + rMSC 3) Scaffold B + rMSC	1a) empty control 1b) Scaffold A (60º rotated layers) only 1c) Scaffold B (30º rotated layers) only	Histology (12 weeks) Group 1a: NBF 22.5 ± 1.8% Group 1b: NBF 19 ± 1.8% Group 2: NBF 8.7 ± 1.8% Group 1c: NBF 10.2 ± 2.0% Group 3: 10.8 ± 1.9%
6.	Shahnaseri 2020	Mongrel dogs (alveolar cleft)	4	Autologous (MSCs subcutaneous adipose stem cell)	HA/TCP	n.a	2) Tissue-engineered MSCs with HA/TCP	1)Autograft group (Tibia)	Digital radiography (90 days) Group 1: bone density 100.32 ± 41.17 Group 2: bone density 93.77 ± 29.73
7.	Sun 2020	JW Rabbits (alveolar cleft)	24	Xenogenic (hUMSC)	Bone collagen particles (HA and collagen 1)	n.a	2) Bone collagen particles (Collagen+HA) + hUMSC	1) Empty control 1b) Bone collagen	Histology (6 months) Group 1a: no NBF Group 1b: Small amount of Group 3: Large portion of NBF Micro-CT (6 months)

					+ collagen membrane			particles (HA and collagen)	Group 1: BT 11.05 ± 1.23% Group 1b: BT 25.29 ± 2.53% Group 2: BT 31.18 ± 2.12%
8.	Wang 2019	Rhesus monkey (alveolar cleft)	4	Syngenic (rBMSC)	3D-BG microspheres	BMP/CS nanoparticles	2)3D-BG + BMP 3)3D-BG+BMP/CS + BMSCs	1a) SO group 1b) 3D-BG	Histology (12 weeks) Group 1a: no sign of osteogenensis Group 1b: has undegraded scaffolds, new bone tissue, and massive connective tissue in bone defect Group 2: has a small number of undegraded scaffolds, surrounded by bone-like tissue around the unabsorbed scaffold material Group 3: scaffolds were all absorbed, and a large number of new bones and new blood vessels were formed.
9.	Caballero 2017	Yorkshire pigs (alveolar cleft)	22	Autologous (UC-MSCs)	PLGA	n.a	2) Undiff - MSCs 3) Diff - MSCs	1a) Empty control 1b) Autologous ICG	CT (30 days) Group 1a: NBG 1.94 ± 1.35 mm^3/kg Group 1b: NBG 2.58 ± 2.83 mm^3/kg Group 2: NBG 7.23 ± 4.99 mm^3/kg Group 3: NBG 5.82 ± 4.48 mm^3/kg
10.	Korn 2017	Lewis rats (alveolar cleft)	84	Syngenic (BMSC-donor adult Lewis rats)	bHA	n.a	2) bHA 3) Undiff -MSCS + bHA 4) Diff - MSCs	1)Empty control	Histomorphometry (12 weeks) Group 1: NBF 43 ± 13.3 % Group 2: NBF 30.8 ± 12.1 % Group 3: NBF 20.5 ± 10.9 % Group 4: NBF 11.5 ± 7.3 % CT (12 weeks) Group 1: RD 11.07 ± 2.32 mm3 Group 2: RD 12.57 ± 1.17 mm3 Group 3: RD 13.25 ± 1.48 mm3 Group 4: RD 14.08 ± 1.36 mm3

11.	Wen 2016	Sprague-Dawley rats (alveolar defect)	4	Allogenic (MSC and EPC from femur and tibia of rats)	FG; Pasteurized FG	n.a	2) MSCs/EPCs (coMSCs)	1) MSCs (monoMSCs)	Histology (6 weeks) Group 2 presented better healing conditions, with a large amount of BMSC and osteogenetic cells in the center of the defect. Micro-CT (6 weeks) Group 1: 362.67 ± 27.65 HU Group 2: 527.78 ± 23.37 HU
12.	Liang 2016	Sprague-Dawley rats (alveolar bone defect)	27	Allogenic (MSC and EPC from 2-week-old rats)	n.a	n.a	2)EPC + MSC 3) MSC	1) Empty control	Histology (8 weeks) Group 1: NBF 28.53 ± 2.81 % Group 2: NBF 44.72 ± 5.96 % Group 3: NBF 70.28 ± 8.3 % Micro-CT (6 weeks) Group 1: TMD 503.66 ± 29.58 mg/cc Group 2: TMD 546.62 ± 34.67 mg/cc Group 3: TMD 609.88 ± 48.01 mg/cc
13.	Huang 2015	Beagle dogs (alveolar cleft)	14	Autologous (BMSC)	β-TCP	n.a	2) Autologous ICG/ No RME 3) Autograft ICG /RME 4) BMSCs/ β-TCP/RME	1) Empty control /No RME	Histomorphometry (12 weeks) Group 1: NBF 13.11 ± 1.72% Group 2: NBF 55.74 ± 9.26% Group 3: NBF 79.51 ± 4.92% Group 4: NBF 78.69 ± 6.39% Radiography (12 weeks) Group 2: VH 4.85 ± 0.4 mm Group 3: VH 5.85 ± 0.48 mm Group 4: VH 5.97 ± 0.48 mm
14.	Yuanzheng 2015	Beagle dogs (alveolar cleft)	20	Autologous (BMSC)	Autologous bone	PRF	2) Autologous graft (ICG)/BMSC/PRF 3) Autologous graft (ICG)/BMSC 4) Autologous graft (ICG)/PRF	1) Autologous graft (ICG)	CT (6 months) Group 1: BMD 733.56 ± 69.31 mg/cc K_2HPO_4 Group 2: BMD 1233.56 ± 94.93 mg/cc K_2HPO_4 Group 3: BMD 1182.47 ± 83.97 mg/cc K_2HPO_4 Group 4: BMD 1142.33 ± 80.27 mg/cc K_2HPO_4
15.	Korn 2014	Female Lewis rats (alveolar cleft)	72	Syngenic (BMSC-donor female Lewis rat)	HA ceramics/β-TCP/silica matrix	n.a	2) Scaffold only 3) Scaffold/Undiff -MSCs 4) Scaffold/Diff -MSCs	1) Empty control	Histomorphometry (6 weeks) Group 1: RD width 2.63 ± 0.52 mm Group 2: RD width 2.70 ± 0.66 mm Group 3. BMSC group: RD width 2.39 ± 0.23 mm Group 4: RD width 2.53 ± 0.22 mm

									CT (6 weeks) Group 1: RD-volume 6.86 ± 3.21 mm^3 Group 2: RD-volume 5.50 ± 1.05 mm^3 Group 3: RD-volume 4.08 ± 1.36 mm^3 Group 4: RD-volume 5.00 ± 0.84 mm^3
16.	Raposo-Amaral 2014	Wistar rats 28 (alveolar cleft)		Xenogenic (human MSCs from orbicularis oris muscle of cleft patients)	1. Bio-Oss collagen 2. α-TCP-matrix	MSCs	2)Bovine bone mineral free of cells 3)Bovine bone mineral loaded with MSCs 4)α-tricalcium phosphate free of cells 5) α-tricalcium phosphate loaded with MSCs	1)Autogenous bone grafts	Histomorphometry (8 weeks) Group 1: NBF: 60.27 ± 16.13%, Fibrosis Volume: 3.89 ± 10.24% Group 2: NBF: 23.02 ± 8.6%, Fibrosis Volume: 19.85 ± 7.04 Group 3: NBF: 38.35 ± 19.59%, Fibrosis Volume: 18.55 ± 12.41% Group 4: NBF: 51.48 ± 11.7%, Fibrosis Volume: 13.24% ± 12.07% Group 5: NBF: 61.80 ± 2.14%, Fibrosis Volume: 0.64 ± 1.56%
17.	Pourebrahim 2013	Mongrel dogs (alveolar cleft)	4	Autologous (ASC)	HA/β-TCP	n.a	2)Adipose tissue (MSCs)/Scaffold	1)Autologous graft (Tibia)	Histomorphometry (15 days) Group 1: NBF 45 ± 14.14% Group 2: NBF 5 ± 1.75% Histomorphometry (60 days) Group 1: NBF 96 ± 3.55% Group 2: NBF 70 ± 16.41%
18.	Chung 2012	Swine (alveolar bony defects)	9	Autologous (BMSC)	PF127	AdvBMP-2	2)AdvBMP-2 infected MSC/PF127	1)MSC/PF127	Histology (3 months) Group 2: Considerably more bone was formed than in group 1, extending from the apical aspect of the defect through the coronal extension In group 1, only small amounts of immature bone were formed. The new bone contained some fatty marrow space and extended from the apical aspect of the defect to the middle of the root 3D CT (3 months) Group 1: TBV 690.55 ± 119.84 mm^3 Group 2: TBV 1824.84 ± 14.36 mm^3

19.	Yoshioka 2012	Beagle dogs (jaw cleft)	3	Autologous (BMSC)	CAP particles	n.a	2) CAP/BMSC	1) Scaffold (CAP particles)	Histology (6 months) Group 1: NBF was present in the transplanted area, but fibroblastic cells were still located around CAP particles Group 2: NBF was observed in almost the whole area, and CAP particles had almost disappeared X-Ray (6 months) Group 1: Radio-opacity NB 0.35±0.15 Grroup 2: Radio-opacity NB 0.75±0.2
20.	Zhang 2012	Sprague-Dwayley rats (alveolar defect)	15	Allogenic (BMSC from femora of 2-week-old rat	FG	n.a	2)FG only 3) BMSC + FG	1)Empty control	Histology (6 weeks) Group 1: poor NBF Group 2: poor NBF Group 3: good healing conditions Micro-CT (6 weeks) Group 1: BMD 669.04 ± 6.72 HU Group 2: BMD 668.80 ± 6.70 HU Group 3: BMD 682.96 ± 6.70 HU
21.	Zhang 2011	Beagle dogs (alveolar cleft)	7	Autologous (BMSC)	β-TCP	n.a	2) BMSC 3) β-TCP 4) BMSC/ β-TCP	1)Empty control	Histomorphometry (20 weeks after orthodontic treatment is completed) Group 1: no data Group 3: NBF 54.98±9.22% Group 4: NBF 70.79±7.02% Ratio Residual Alveolar Height (X-Ray 20 weeks) Group 1: no data Group 2: 72.42±8.72% Group 3: 56.31±7.72% Group 4: 73.6±6.51%

rMSC: rat mesenchymal stromal cells; CPC: calcium phosphate cement; NBF: new bone formation; hUMSC: human umbilical cord mesenchymal stromal cells; HA: hydroxyapatite; BMP-2: bone morphogenetic protein 2; ICABG: iliac crest bone graft; dGMSC: differentiated gingiva derived mesenchymal stem cells; BV: bone volume; UC-MACS: enzymatic digested human umbilical cord MSC using magnetic-activated cell sorting; n.a.: not applicable; TCP: tricalcium phosphate; BT: bone trabeculae; rBMSC: rhesus marrow bone MSC; 3D-BG: 3D printed bioglass; BMP/CS: BMP-2 gene loaded nanoparticles; SO: sham-operated; UC-MSCs: umbilical cord mesenchymal stem cells; PLGA: poly(lactic-co-glycolic acid); NBG: new bone growth; Undiff : Undifferentiated ; Diff : Differentiated; ICG: iliac crest cancellous bone graft; bHA: bovine hydroxyl apatite/collagen; RD: remaining defect; β-TCP: Beta tricalcium phosphate; RME: rapid maxillary expansion; VE: vertical height; PRF: platelet rich fibrin; BMD: bone mineral density, K_2HPO_4: Dipotassium hydrogen phosphate; EPC: endothelial progenitor cell; FG: fibrin glue; co-MSC: co-cultured MSC; monoMSC: mono-cultured MSC; TMD: tissue mineral density; PF127: pluronic F127; advBMP-2: Adenovirus BMP-2; TBV: total bone volume; NB: new bone; CAP: calcium phosphate;

Table 2. Animal studies of cleft palate defect

No	Study	Model	Number	Cell Source	Scaffold	Growth Factor	Experimental group	Control Group	Results
1.	Abe 2020	Beagle dogs (cleft lip and palate)	1	Autologous (BMSC)	CAP	n.a	2) CAP/BMSC	1)Scaffold (CAP)	X-Ray (3 months) Group 1: residual CAP was still detected Group 2: almost no granular opacity, bone bridge structure was present
2.	Naudot 2020	Sprague Dawley rats (critical sized-cleft palate)	27	Allogenic (BMSC)	Alginate-based hydrogel scaffolds	n.a	2)Scaffold 3)Scaffold/ BMSC	1) Empty control	Histology (12 weeks) Group 1: nonmineralized healing connective tissue, a few blood vessels, and mature bone at defect margin. Group 2: Defect full of fibrous tissue. Group 3: New bone formation in the center of the defect Micro-CT (12 weeks) Group 1: NBF 36.91 ± 5.132%. Group 2: NBF 61.01 ± 5.288 %. Group 3: NBF 17.24 ± 6.886 %.
3.	Amalraj 2017	Wister albino rat pups (cleft palate induced by Triamcinolone acetonide	12	Allogenic (BMSC donor Wister albino female rat)	PLGA	n.a	2) PLGA 3)PLGA/BMSC	1)No cell trasplant	Group 3: Complete reconstruction of the cleft palate in the group 3 of rat pups which received BMSCs along with PLGA scaffold. Bone growth in the cleft defect was faster
4.	Liceras-Liceras 2017	New Zealand white rabbits (cleft palate defect)	12	Autologous (ASC)	Fibrin-agarose	n.a	2)ASC/ Fibrin-agarose	1a) Positive control (no surgical procedure of the palate) 1b)Negative control/Blank control (cleft palate was left untreated)	CT Palate bone length (right side) Group 1a: 50.15±0.41 % Group 1b: 38.57±1.74 % Group 2 :(1 month): 48.21±0.44 % Group 2: (4 months): 47.21±0.52 % Palate bone width (right side) Group 1a: 51.22±0.02 % Group 1b: 46.15±0.15 % Group 2: (1 month): 50.55±0.24 % Group 2: (4 months): 52.51±1.12 % Right side = operated side

BMSC: bone marrow stem cells; CAP: calcium phosphate; n.a: not applicable; PLGA: poly (lactic-co-glycolic acid); ASC: adipose stem cell

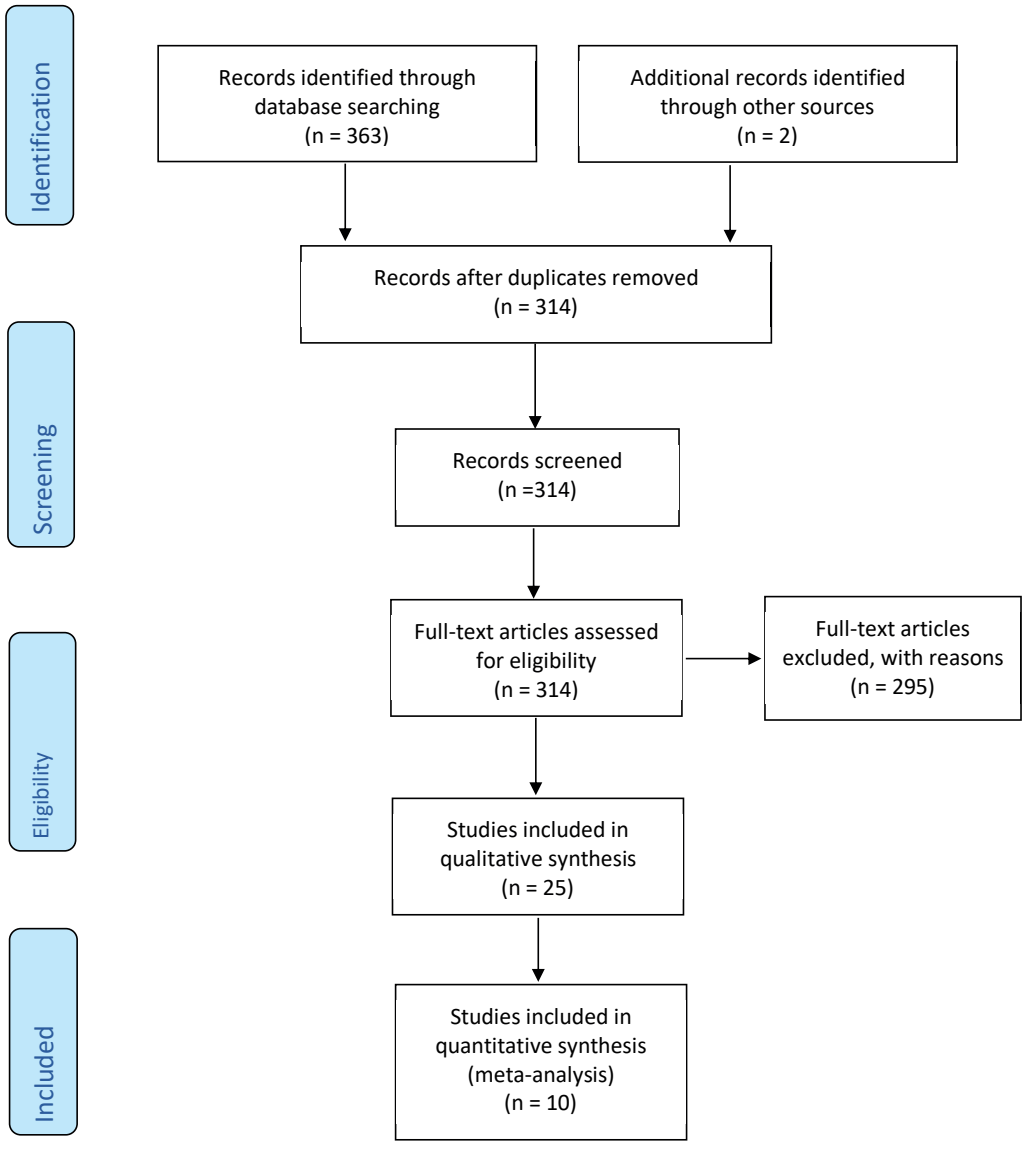

Figure 1. Flowchart of the study selection process

c. Meta-analysis:

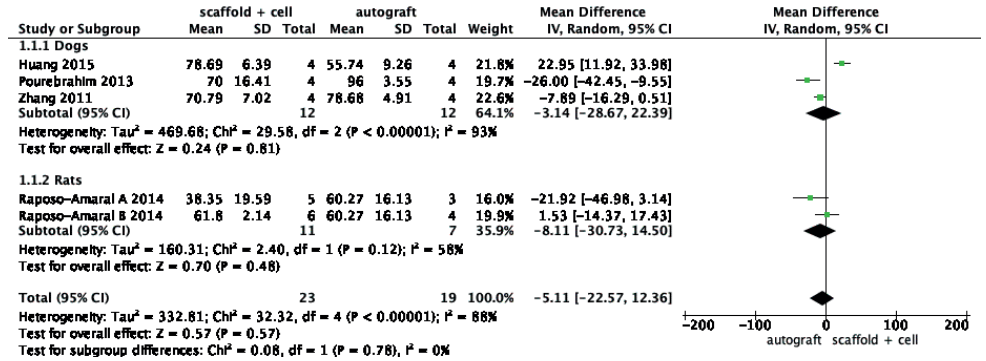

Figure 2. Forest plot for the new bone volume formation (%) histomorphometry analysis of autograft vs. cells-loaded scaffold group in alveolar cleft dog and rat models.

Figure 2 is the forest plot of the meta-analysis of the percentage of new bone volume formation as assessed with histomorphometry analysis of autograft vs. cells-loaded scaffold group in alveolar cleft dog and rat models. One study in the dogs' group reported higher new bone formation in the scaffold + cell group compared to the autograft group[34]. Two studies reported higher new bone formation in the autograft group compared to the scaffold + cell group[37,46]. These studies showed a standard mean difference (SMD) of -3.14 [95%CI (-28.67,2.39), P=0.81, with heterogeneity I^2=93%]. In the rats' group, one study reported higher new bone formation in the autograft group[47], and one study reported similar bone formation results of autograft and scaffold + cell group[47] SMD of -8.11 [95%CI (-30.73,14.50), P=0.48, with heterogeneity I^2=58%]. Although far from significant, autograft was favoured over scaffold+ cell combination with a SMD of -5.11 [95% CI (-22.57,12.36), P=0.57, with heterogeneity I=88%]. There was no statistically significant difference after subgroup analysis, indicating that the subgroup did not contribute to heterogeneity.

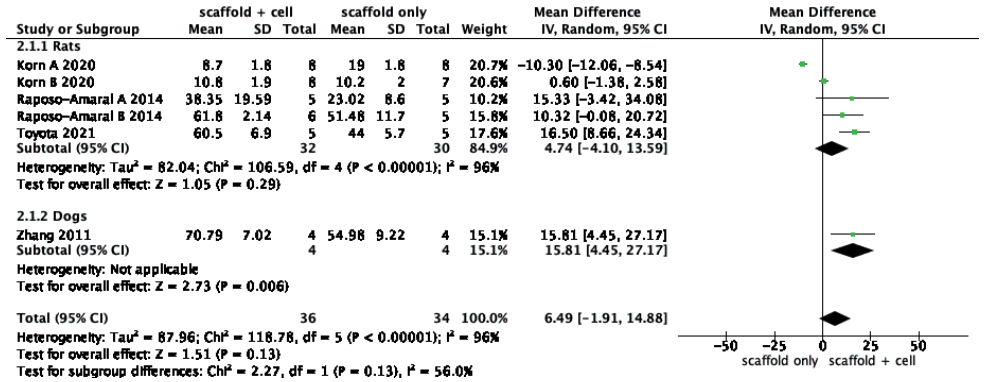

Figure 3. Forest plot for the new bone volume formation (%) histomorphometry analysis of scaffold-only group vs cells-loaded scaffold group in alveolar cleft dog and rat models.

Figure 3 depicts the forest plot of the meta-analysis comparison of the scaffold-only group vs. the cell-loaded scaffold group in alveolar cleft dog and rat models, again as histomorphometrically assessed with % new bone volume formation as the outcome parameter. In the rat subset, four studies reported higher new bone formation in the scaffold + cell group compared to the scaffold-only group[28,41,47], whereas 1 study reported higher new bone formation in the scaffold-only group compared to the scaffold + cell group[28]. These studies showed a standard mean difference (SMD) of 4.74 [95%CI (-4.10,13.59), P=0.29, with heterogeneity I^2=96%]. In the dogs' group, one study reported the higher new bone formation of the scaffold + cell group compared to scaffold only group[37] SMD of 15.81 [95%CI (4.45,27.17), P=0.006]. Although insignificant, the overall result favoured scaffold + cell over scaffold-only with an SMD of 6.49 [95% CI (-1.91,14.88), P=0.13, with heterogeneity I=96%]. There was no statistically significant difference after subgroup analysis, indicating that the subgroup did not contribute to heterogeneity.

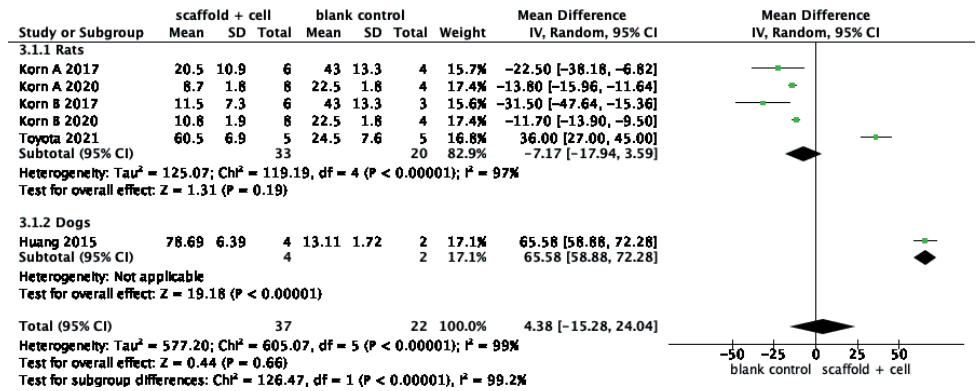

Figure 4. Forest plot for the new bone volume formation (%) histomorphometry analysis of blank control group vs cells-loaded scaffold group in alveolar cleft dog and rat models.

In Figure 4, the meta-analysis of the histomorphometry assessment of the new bone formation of a blank control group vs cells-loaded scaffold group in alveolar cleft dog and rat models is depicted. In the rat subset, two studies reported the higher new bone formation of blank control compared to the scaffold + cell group[28,29]. One study reported the higher new bone formation of the scaffold + cell group compared to the blank control group[41]. These studies showed a standard mean difference (SMD) of -7.17 [95%CI (-17.94,3.59), P=0.19, with heterogeneity I^2=97%]. In the dog subgroup, one study reported the higher new bone formation of the scaffold + cell group compared to the blank control group[32] with an SMD of 65.58 [95%CI (58.88,72.28), P<0.00001]. Although insignificant, the overall result favored scaffold + cell over blank control SMD of 4.38 [95% CI (-15.28,24.04), P=0.66, with heterogeneity I^2=99%]. After subgroup analysis for animal species, a statistically significant difference was discovered, indicating that species subgroups contributed to heterogeneity.

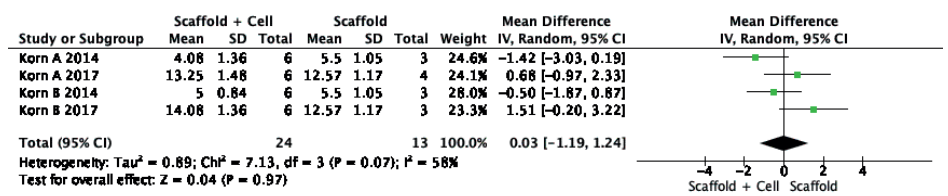

Figure 5. Forest plot for the remaining defect volume CT-scan analysis of scaffold-only group vs. cells-loaded scaffold group in the alveolar cleft rat model.

Figure 5 depicts the forest plot of the meta-analysis addressing the remaining defect volume CT-scan analysis of the scaffold-only group vs. the cells-loaded scaffold group in the alveolar cleft rat model. One study reported less remaining defect volume of scaffold + cell compared to the scaffold group[32]. The other study reported the opposite[29]. Overall, scaffold + cell and scaffold only showed similar remaining defect volumes with an SMD of 0.03 [95%CI (-1.19,1.24), P=0.97, with heterogeneity I^2=58%].

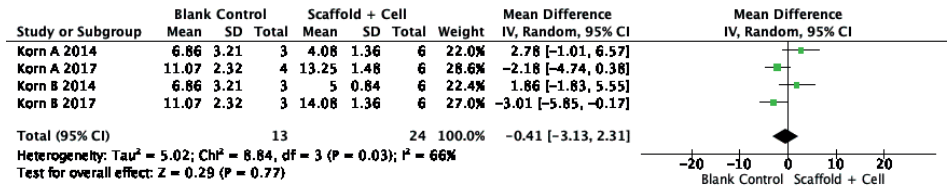

Figure 6. Forest plot for the remaining defect volume CT-scan analysis of blank control group vs cells-loaded scaffold group in the alveolar cleft rat model.

The meta-analysis of the remaining defect volume CT-scan analysis of the blank control group vs. cells-loaded scaffold group in the alveolar cleft rat model is given in Figure 6. One study reported less remaining defect volume of scaffold + cell compared to the blank control group[32]. The other study showed the reverse effect[29]. Overall, the blank control had a slightly higher remaining defect volume than the scaffold + cell group, with an SMD of -0.41 [95% CI (-3.13,2.31), P=0.77, with heterogeneity I^2=66%].

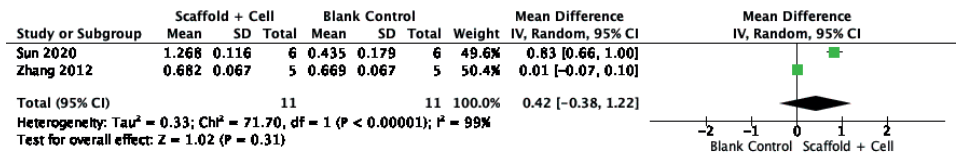

Figure 7. Forest plot for bone mineral density CT-scan analysis of blank control group vs cells-loaded scaffold group in the alveolar cleft rat model.

The meta-analysis evaluating the bone mineral density CT-scan analysis of the blank control group vs. cells-loaded scaffold group in the alveolar cleft rat model is shown in Figure 7. One study reported higher bone mineral density in the scaffold + cell group compared to the blank control group[42]. In contrast, the other study reported similar bone mineral densities in both groups. The overall result showed a somewhat higher bone mineral density in the scaffold + cell group with an SMD of -0.42 [95%CI (-0.38,1.22), P=0.31, with heterogeneity I^2=99%].

d. Risk of bias within and individual studies

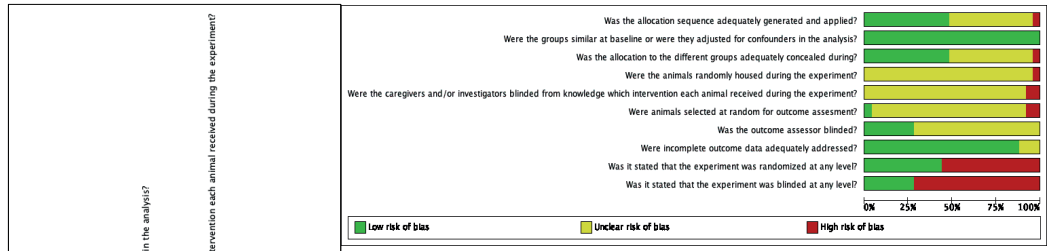

Figure 8. Risk of bias graph & summary: review authors' judgments about each risk of bias item presented as percentages across all included studies and as items for each included study.

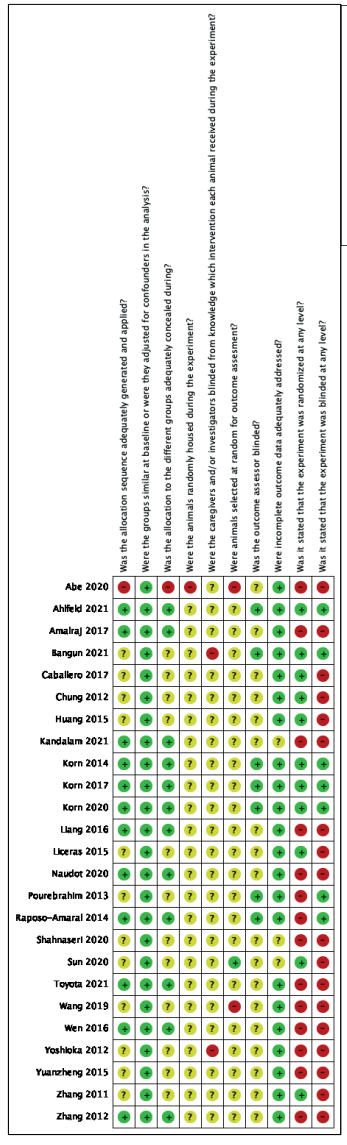

Figure 8 shows the overall results of the risk of bias assessment of the 25 studies included in this systematic review. Regarding the selection bias item “sequence

generation", 48% of the studies were scored as "unclear risk", 48% of the studies were scored as "low risk of bias", and only 4% of the studies were scored as "high risk of bias". All studies described that intervention and control groups were similar at the start of the experiment. Regarding the selection bias item "allocation concealment", 48% of the studies were scored as "unclear risk", 48% of the studies were scored as "low risk of bias", and only 4% of the studies were scored as "high risk of bias". In addition, 96% and 92% of the included studies were scored as unclear risk of bias concerning performance bias items 'random housing' and 'blinding', respectively. For the detection bias item 'random outcome assessment', 88% of the studies were scored as "unclear risk".

Only 28% of the included studies were scored as "low risk of bias" by outcome assessor-blinded. For attrition bias, 88% of the included studies scored as low risk of bias, as they adequately addressed incomplete outcome data. Overall, only 44% of the included studies were achieved as "low risk of bias" because it was stated in the studies that the experiment was randomized at any level. Only 28% of the included studies were scored as "low risk of bias" because it was stated in the studies that the experiment was blinded at any level.

d. Publication Bias

Since each meta-analysis consisted of less than ten studies and therefore lacked sufficient power to distinguish chance from real asymmetry, an assessment of publication bias via statistical testing or funnel plots was not performed[25].

Discussion

Cleft lip and/or palate is one of the most common congenital malformations in the maxillofacial area and occurs in the setting of genetic and environmental factors [6]. Standard management of oral clefts including cleft palate and alveolar cleft surgery, has side effects that are often associated with post-operative results on the defect site or donor site [50]. Clinicians and researchers have been working together to search for applicable stem cell-based tissue engineering to overcome these challenges [14,15,51]. Unlike alveolar cleft, stem cell-based tissue engineering technology for cleft palate is still in process for future clinical human application [52]. In addition, the application of

new technologies for oral cleft treatments is often hampered by limited healthcare settings where many patients are left untreated until they reach adult age[11].

Recently, a systematic review on alveolar bone tissue engineering in pre-clinical studies by Shanbhag et al. (2017) reported: 1) the addition of osteogenic cells (MSCs or OB) to biomaterial scaffolds can enhance alveolar bone regeneration in small and large animal models; 2) Ex vivo BMP gene-transfer to MSCs and OB can enhance their in vivo osteogenic potential based on small animal models; 3) Bone tissue engineering may result in comparable alveolar bone regeneration as induced by autograft (limited evidence); and 4) Large heterogeneity between studies resulting from biological and methodological variability[53]. However, most of the included studies (83.3%) used critical size defects in the mandible, where alveolar clefts do not occur. Only three included studies reported the use of maxillary cleft models. Therefore, we decided to update the results and focused on alveolar cleft and cleft palate pre-clinical models. A review by Alkaabi et al. (2022) found that regenerative therapies showed better alveolar bone regeneration, although not significantly, compared to autogenous bone grafting on clinical application[54]. However, this review could not conclude which type of regenerative therapy is the most optimal for alveolar bone grafting on clinical application.

In the present study, we performed a systematic review and meta-analysis of pre-clinical studies to evaluate the efficacy of stem cell-based tissue engineering for cleft palate and alveolar cleft defects. Twenty-five studies using stem cell-based tissue engineering technology were included, comprising 21 alveolar cleft animal studies[27–47] and 4 cleft palate animal studies[16,17,48,49]. Of these, 10 studies met the criteria to be included in the meta-analyses[28,29,32–34,37,41,42,46,47]. Although only a relatively small number of studies could be included, it still enabled us to perform the meta-analyses and explore the effect of several subgroup variables. Despite this, there are some potential limitations related to this approach. First, as also addressed above, all experiments should preferably be performed in a similar manner when their results are being combined in a meta-analysis. However, the publications display experimental variability for the utilized animal species, defect type and size, the used cell types, the number of cells per defect, the biomaterials applied as cell carrier, the growth factor, the healing time after cell transplantation, and the result assessment parameters. Not surprisingly, substantial statistical heterogeneity was found. We performed subgroup analyses (animal species) in an attempt to tackle this issue, but this did not notably

reduce the heterogeneity. We also conducted direct comparison of meta-analysis between control group (blank control, autograft, or scaffold without cell) versus stem cell-based tissue engineering group. In addition, we reported applications of stem cell-based tissue engineering for cleft palate reconstruction besides alveolar cleft. In the next paragraphs, these results will be discussed in more detail.

As shown in this systematic review, mesenchymal stem cells from bone marrow are the main used cell type for preclinical trials for both the alveolar cleft and cleft palate model. Another frequently used source of MSCs is adipose tissue. There is still controversy on which cell source has better osteogenic potential. Some say that bone marrow is better (e.g. Musina[55] 2006; Mohamed-Ahmed[56] 2021, Brennan[57] 2017), others state that adipose-derived MSCs may have higher osteogenic potential (Huang[58] 2022; Holmes[59] 2022) and some found similar osteogenic activities (Humenik[60] 2022). In this regard, it should be kept in mind that variations in the distinctive features of both cell sources may depend on the source and method of isolation and epigenetic changes during maintenance and growth (Brown[61] 2019). Nevertheless, it would be worthwhile and fascinating to evaluate adipose stem cells for their efficacy in pre-clinical cleft models and subsequent clinical implementation.

In both the alveolar cleft and cleft palate groups, small and large animals were used. Small animal models can provide “proof of principle” and large animal models can be used to represent the efficacy of pre-clinical testing[53]. In one meta-analysis, greater but not significant bone formation was observed in the cell-loaded scaffold group vs scaffold-only group for the alveolar cleft reconstruction of rats and dogs. Strikingly, the dog studies showed not only more efficient better bone formation compared to scaffold only, but also similar[37] to superior bone formation[34] compared to autografts. These interesting results show, at least preclinically, that regenerative grafts have equal or higher bone regeneration efficacy in comparison with autografts, and imply that regenerative grafts may be full-blown, suitable alternatives for the golden standard, which is still autologous bone.

From our risk of bias assessments, we had to conclude that the animal studies suffered from many unclarities and high risk of bias in their publications. Key measures to avoid bias, such as randomization and blinding, were infrequently reported. For example, only 44% of the studies provided sufficient details to judge the adequacy of the method of randomization, and only 28% of the studies reported that the outcome assessment was blinded. Moreover, the results of the meta-analyses may be subject to publication

bias from non-publication of negative results, true study heterogeneity or differences in study quality, which unfortunately statistical assessment with funnel plots was not conducted in this study because meta-analysis was consisted of less than 10 studies to confirm this. Nevertheless, the combined analysis of the included studies still generated extra and valuable information that could not be derived from individual studies[24]. To generate reliable and unbiased data, it is suggested that the standards of animal experiment reporting should be more like the standards routinely applied in human randomized controlled trials[24]. Also, standardization of follow-up periods may help reduce the enormous spread in post-operative monitoring points and maximum follow-up date, which now ranges from 6 weeks (42 days) to 6 months (180 days).

Although histomorphometry is considered the “gold standard” for the evaluation of bone structure[53], our study assessed bone regeneration using histology, histomorphometry, CBCT, and/or CT-Scan analysis with new bone formation, remaining defect or bone mineral density as outcome parameters. Recently, micro-computed tomography (micro-CT) has been proposed as an alternative method for assessing three-dimensional bone microarchitecture with high resolution and accuracy, in a fast and nondestructive manner[53]. However, care should be taken when interpreting outcomes of CT or micro-CT because of the difficulties in differentiating between mineralized scaffolds and newly formed bone[53]. In this regard, Prins and coworkers[62] showed that by varying threshold values in CT evaluations, it may still be possible to distinguish between both mineralized entities. In addition, this publication showed that it may be very useful to combine both methods, since it offered a mutual confirmation of the one method by the other[62].

Defect size also influences the clinical application of cell-based tissue engineering. Unlike calvarial critical-size defects, alveolar critical-size defects models have not been well characterized in the literature regarding defect location, size, and morphology. Defect dimensions varied between studies for the same animal model/species. In many cases, the selection of a particular model appeared to be based on one previously established by the same or related research group(s)[53].

It is tempting to compare data obtained from pre-clinical and clinical studies to conclude the validity and feasibility of extrapolation of pre-clinical outcomes for the prediction of efficacy in clinical models. However, clinical studies employing cellular therapies for alveolar cleft are scarce. This scarcity of pediatric cell-based studies is a more general phenomenon, which has been covered extensively by Nitkin et al[63]. The

most important issue is, and should be, thorough consideration of the ethical aspects for this vulnerable population. As also indicated above, a recent review by Alkaabi and co-workers addressed the use of regenerative grafts for alveolar cleft repair, including cell-based therapies[54]. Still, unfortunately, the studies listed there used different cell preparations than those addressed in this review[54]. So, for cleft studies, extrapolation from pre-clinical results to clinical implementation remains an issue nowadays.

Despite the limitations mentioned above, the results of this systematic review and meta-analysis revealed that cell-based approaches are favorable for alveolar cleft and cleft palate reconstructions. These are displayed by the positive effect of cell-based approaches on new bone formation, remaining defect volume, and bone mineral density. The meta-analysis did not show a statistically significant difference in osteogenic potential between the control group (blank control, autograft, or scaffold without cell) versus the stem cell-based tissue engineering group for in vivo alveolar cleft reconstruction. As for cleft palate reconstruction, limited result data hampered the meta-analysis to be performed.

In perspective, meta-analyses of animal studies tend to be exploratory rather than confirmatory. Standardization of alveolar cleft and cleft palate models to better represent the clinical scenario and standardization of study reporting should be essential considerations in future studies of alveolar and palate bone tissue engineering. Another issue, although slightly beyond the scope of this review, is that in most of the included preclinical studies also osteogenic peptides and recombinant growth factors are being used in combination with the regenerative cell populations, whereas in particular in pediatric cleft repair these stimulatory compounds are still not clinically implemented except in clinical trials. For example, the application of BMP-2 is still debated: In recent reviews Fisher et al.[64] advocate the use of BMP-2 to decrease donor site morbidity or when alternatives are contraindicated, whereas Sales et al.[65], in particular based on high risk of bias in studies, conclude that recommendations to use BMP-2 in pediatric populations should be treated with caution. In our view, given the data presented in the latter review showing equal bone formation in BMP-2 vs. autologous bone treatment, avoiding iliac crest surgeries may be an important factor in reducing pediatric patients risks, as long as the high dosages causing major adverse events like in spinal surgeries[66] are not applied. An alternative from our own experience may be ex vivo stimulation of regenerative (stem) cells with physiological dosages of rhBMP-2, thus avoiding body exposure to BMP-2 at all[67]. Nevertheless, we advocate well-designed studies with cell-growth factor combinations to be evaluated for alveolar cleft repair, to accelerate clinical implementation of these potent candidates.

Further more extensive and prospective studies with greater methodological aspects and rigor in data collection, analysis, and reporting, as well as long-term post-operative follow-up periods with information on complications, are needed. Most importantly, the animal models presented in this systematic review were all fresh acute models, except for one study was conducted in rabbit models by creating a pseudo-cleft palate defect[16] and one study was conducted by injecting Triamcinolone acetonide (TAC) in pregnant rats[17]. In our view, the latter model properly reflects the real situation appropriately by creating chronic alveolar cleft/cleft palate defects, proper for regenerative medicine.

Conclusion

Alveolar cleft and cleft palate reconstructions using regenerative grafts are currently still in its infancy, and have so far not resulted in clear data about efficacy, in contrast to other craniofacial bone defect areas. The models used seem inadequate to reflect the human situation due to their non-chronic induction of the clefts, and uncertainty about whether critical size defects are being created. The Triamcinolone acetonide model is very promising in that regard and should probably be used as the new standard model for pre-clinical studies on cleft defects.

Competing interests

None declared.

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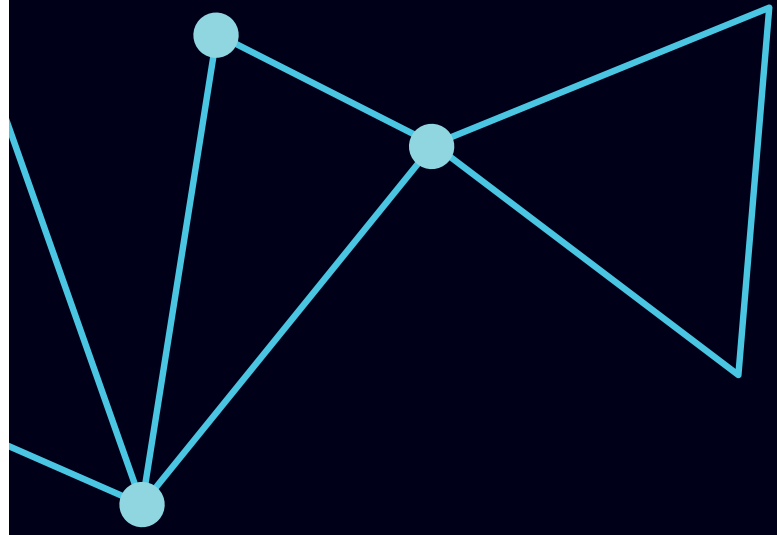
Chapter 4

A Systematic Review on Regenerative Alveolar Graft Materials in Clinical Trials: Risk of Bias and Meta-Analysis



Alkaabi SA, Alsabri GA, NatsirKalla DS, Alavi SA, Mueller WEG, Forouzanfar T, Helder MN. A systematic review on regenerative alveolar graft materials in clinical trials: Risk of bias and meta-analysis. J Plast Reconstr Aesthet Surg. 2022 Jan;75(1):356-365. doi: 10.1016/j.bjps.2021.08.026. Epub 2021 Sep 17. PMID: 34642060.

A systematic Review on Regenerative Alveolar Graft Materials in Clinical Trials: Risk of Bias and Meta-Analysis.

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Abstract:

Background:

Alveolar cleft grafting is a necessary procedure to restore the bone defect. Randomized clinical trials (RCT) are considered a golden standard for investigating the efficacy of treatments. Nevertheless, the risk of bias (RoB) can still affect the validity of these trials. We aimed to systematically review all control trials (CT) CTs using regenerative materials for alveolar cleft reconstructions, evaluate their RoB, and perform a meta-analysis of new bone formation.

Methods:

The Cochrane Oral Health Group's Trials Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (PubMed), EMBASE, AND Google Scholar were searched until October 2020. To evaluate the RoB, the articles were subjected to quality assessment methods (according to the Jadad scale and the Delphi list).

Results:

None of the 15 trials that met the inclusion criteria received a full score. 20% did not randomize the trials, 73.33% did not describe the randomization method, and none reported double-blind criteria. In addition, concealment of allocation (99.9%), intention to treat (100%) and patient awareness (100%) were not adequately described. In the meta-analysis, there was no significant difference between regenerative materials and iliac crest grafts.

Conclusion:

This review showed high RoB in CTs, implying that quality improvement of CTs is necessary. Meta-analysis showed no significant difference between the regenerative materials and autogenous grafts.

Keywords:

Alveolar bone grafting; Tissue engineering; Bone regeneration; Regenerative medicine; Cell transplantation; Evidence-based medicine; Adequacy of method; Risk of bias.

Introduction:

Cleft lip and palate (CLP) are congenital deformities that affect the orofacial region due to fusion failure between the nasal process and the oropalatal shelves.[1-3] Bone grafting is a well-known surgical procedure to rehabilitate alveolar cleft defects.[4,5] It is essential for alveolar cleft reconstruction to be scheduled after the cleft lip and palate repair and before the rhinoplasty and orthognathic surgery.[6] This procedure has different goals, such as closing the oronasal fistula,[7] stabilizing the maxillary segments in the unilateral/ bilateral clefts,[8] and reconstructing the alar base structure.[9] In addition, alveolar bone grafting can play an important role in teeth stability and eruption, as well as periodontal support to the adjacent teeth at the site of the bone graft.[10,11] Autogenous bone is still considered the gold standard for grafting procedures. Several factors should be considered when choosing a grafting source, such as the bone volume available, the surgeon's experience, and postoperative donor site morbidity.[12]

Over the last few years, a major effort has been made in regenerative medicine to offer reliable alternatives, i.e., bone substitutes for autogenous bone grafts.[13,14] Hydroxyapatite (HA) is a good biomaterial with high biocompatibility and negligible negative reactions. Hydroxyapatite provides osteoconductivity in bone formation.[15] β-tricalcium phosphate (β-TCP) is another reliable and highly biocompatible biomaterial that uses osteoconductive properties in bone formation.[16]

Collagen is a natural polymer and an important element in several bone substitutes used in tissue engineering and repair. The main advantages of collagen are; easy degradability and simplicity of attachment from the cells.[17,18]

Moreover, stem cell therapy showed a promising alternative method to promote and accelerate bone regeneration.[19,20] Multiple growth factors have also been used in regenerative alveolar bone grafts, such as recombinant human bone morphogenetic protein-2 (RhBMP-2), platelet-rich plasma (PRP) and platelet-derived growth factor (PDGF). It is believed that these factors might help differentiate osteogenic cells to promote bone reconstruction and healing.[21-23] Quality is difficult to assess as a term. It has been defined in RCTs as "the likelihood of the trial design to generate unbiased results".[24] Application of proper quality assessment methods in RCTs shall enhance the validity of the trial results. To assess the quality of controlled clinical trials (CTs),

various scales are available, such as the Jadad scale[24] and the Delphi list.[25] These scales are being used to evaluate the methodology of the RCTs.

According to our knowledge, an up-to-date review of regenerative materials in CTs of the alveolar cleft defect using an appropriate quality assessment method is currently lacking.

In this review, we aim to conduct the following:

- A systematic review of the regenerative materials that have been used in CTs in alveolar cleft defect up to October 2020.
- Quality assessments of the extracted trials using the Jadad scale and the Delphi list.
- Meta-analysis of the studies that described the mean and the standard deviation of the new bone formation in comparison to the autogenous bone graft.

Materials and Methods

Study Design

This study considered all controlled CTs referencing the use of regenerative materials in the treatment of alveolar bone defects in the title or abstract. All studies must include both control and intervention groups. Regenerative medicine includes tissue engineering, cell therapy, growth factors, or their combination. Only human studies published in English through October 2020 were included. Experimental studies, such as animal studies, were excluded.

Search strategy

Cochrane Oral Health Group's Trials Register, the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (PubMed), EMBASE, AND Google Scholar were searched to identify the existing trials on the topic. International Journal of Biomaterials and Journal of International Society of Preventive and Community Dentistry were hand-searched simultaneously to identify additional trials. The

bibliographies of review articles were checked, and personal references were searched.

#1 alveolar cleft OR alveolar defect OR cleft palate OR alveolar grafting

#2 regenerative OR regenerative medicine OR tissue engineering OR stem cells OR growth factors OR cell therapy OR bone regeneration

#3 Human

#4 Control trial (CT)

#1 AND #2 AND #3 AND #4 CT [Title/Abstract/Keywords]

Risk of bias assessment:

All extracted articles were then subjected to a quality assessment using the Jadad scale and the Delphi list (**Table 1**). A total of five questions (yes or no questions) should be answered on the Jadad scale. Each question is scored 1 point for a “yes” or 0 points for a “no”. An accumulative high score represents a low risk of bias. While in the Delphi list, a total of 9 questions should be answered by (yes, no, or do not know); 1 point is given for a “yes”, while 0 points are given for either "no" or "do not know" answers. A higher score also indicates a low risk of bias. A score of 4-5 on the Jaded scale and 6-9 on the Delphi list is considered a low risk of bias.[26]

Table 1. Jadad scale and Delphi list.

Scales	Scores
A- Jadad scale	
1. Randomisation	
Was the study described as randomised (this includes the use of words such as randomly, random, and randomisation)?	0–2
Give 1 additional point if the method used to generate the sequence of randomisation was described and it was appropriate (such as from a table of computer-generated random numbers	Plus 1
Deduct 1 point if the method to generate the sequence of randomisation was described and it was not appropriate (such as if patients were allocated alternately, or according to date of birth or hospital number)	Minus 1
2. Double-blinding	
Was the study described as double-blind?	0–2
Give 1 additional point if the method was described and it was appropriate (such as an identical placebo, an active placebo, or a dummy)	Plus 1
Deduct 1 point if the study was described as double-blind but the method of blinding was not appropriate (such as comparison of tablet and injection with no double dummy)	Minus 1
3. Withdrawals and “dropouts”	0-1
Was there a description of withdrawals and “dropouts”? (the number and the reasons in each group must be stated)	
B- Delphi list	
1a. Was a method of randomisation used?	0-1
1b. Was the method of allocation to treatment concealed?	0-1
2. Were the groups similar at baseline as far as the most important prognostic indicators were concerned?	0-1
3. Were the criteria for eligibility specified?	0-1
4. Was the assessor of outcome aware of the treatment allocated?	0-1
5. Was the provider of care aware of the treatment allocated?	0-1
6. Was the patient aware of the treatment allocated?	0-1
7. Were point estimates and measures of variability presented for the primary measures of outcome?	0-1
8. Did the analysis include an intention-to-treat analysis?	0-1
Questions were answered Yes, No, or Do not know.A score of 1 is given when the answer is ‘Yes’. No points are given if the answer is ‘No’ or ‘Do not know’	

Summary Measures:

Descriptive continuous data were utilised for the meta-analysis of new bone formation by regenerative materials versus autogenous bone graft, including mean, sample size, standard deviation, and weight. The amount of new bone formation was assessed

using the mean difference (MD) and the 95% confidence interval (CI). The MD values were deemed significant if the P-value was less than 0.05. The Cochrane Collaboration's Reviewer Manager 5 software was utilized for meta-analysis. I^2 was used to evaluate statistical heterogeneity between studies. A value greater than 50% will be interpreted as an indicator of substantial heterogeneity between studies, which was classified as follows: I^2 < 30% - low heterogeneity, I^2 = 30-60% - medium heterogeneity, I^2 > 60% - high heterogeneity.[27]

Result:

Search Results and Study Characteristics

Figure 1 illustrates 112 primary Medline, Embase, Cochrane, and Google Scholar results. After the title and abstract screening, a total of 19 articles were obtained. After applying the eligibility, inclusion, and exclusion criteria, 15 studies were obtained and comprehensively evaluated (**Table 2**).

Figure 1: Literature Search Strategy

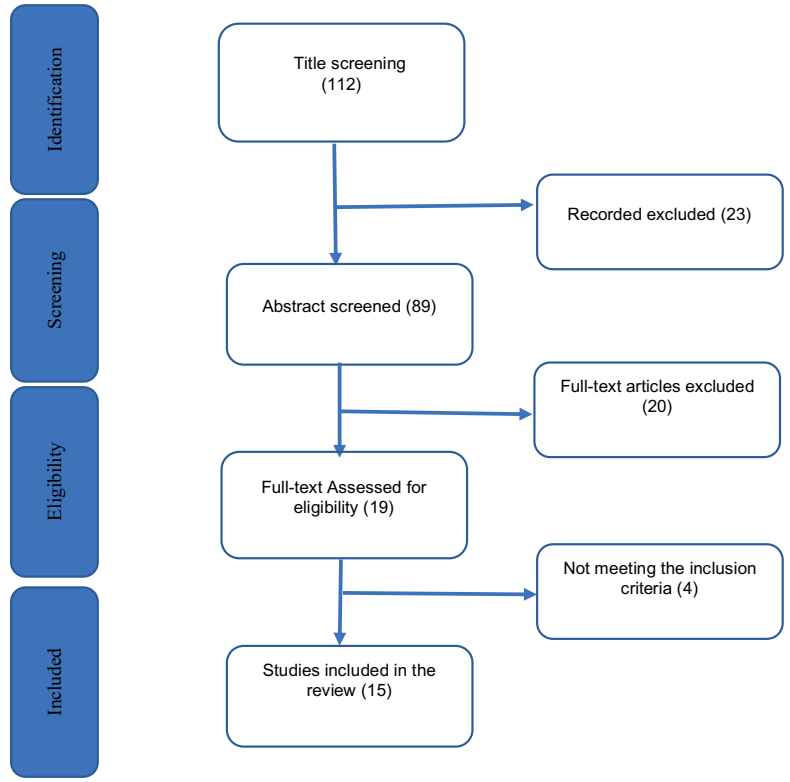


Table 2: List of the studies

Study Id	Author, year	Study type	Number of patients	Control	Graft material/Test/ Intervention	Regeneration type	Result
1.	Herford et al. 2007 [28]	CT	12	ICBG	rh-BMP-2 combined with Type 1 bovine Collagen sponge	GF	The study group had comparable bone volumes. Considered as an alternative method for alveolar grafting. rh-BMP2 can accomplish an effective bony repair. Extra swelling with no pain.
2.	Dickinson et al. 2007 [29]	CT	21	ICBG	rh-BMP-2 + Collagen sponge	GF	BMP-2 95% bone formation compared to 63% for ICBG. rh-BMP-2, enhance mineralization. Improve bone healing and reduce morbidity.
3.	Alonso et al. 2010 [30]	CT	16	ICBG	rh-BMP-2 + Collagen matrix	GF	Alveolar bone reunion in all groups has similar (satisfactory) results after 12 months.
4.	Marukawa et al. 2011 [31]	CT	20	ICBG	ICBG + PRP	GF	ICBG+PRP group significantly has less bone resorption.
5.	Canan et al. 2012 [32]	CT	18	ICBG	rh-BMP-2 + Collagen sponge	GF	No significant in bone volume formation, density and height repair.
6.	Shawky & Seifeldin 2016 [33]	CT	24	ICBG	PRF combined with ICBG	GF	Significant increase in the percentage of newly formed bone in the PRF group. Does not enhance bone density
7.	Du et al. 2017 [13]	CT	20	ICBG	BMMCs combined With β-TCP granules	Cell	No significant finding in Chelsea score and bone formation, Safe to use.
8.	Attar et al. 2017 [34]	CT	20	ICBG	Chin graft + allogeneic bone + leukocyte + PRF	GF	No significant difference, proper for small and moderate defects, caution in large defects.
9.	Shirani G et al. 2017 [35]	CT	32	ICBG plus PRGF	FDBA in presence of PRGF	GF	ICBG reinforced with PRGF was more successful as bone regenerative volume (statistically significant) than FDBA plus PRGF. Autografts should still be preferred.
10.	Sakio et al. 2017 [23]	CT	29	ICBG and marrow grafts	ICBG and marrow grafts + PRP	Cell + GF	No significant effect on the bone volume or bone resorption
11.	Khojasteh et al. 2017 [36]	CT	10	AIC bone and a collagen membrane	1. LRCP with BFSCs mounted on a natural bovine bone mineral 2. AIC bone, BFSCs cultured on natural bovine bone mineral	Cell	AIC shows the least new bone formation. (AIC or LRCP) +BFSC shows a higher new bone formation. Differences were not statistically significant in all groups
12.	Bajestan et al. 2017 [37]	CT	18	Block graft harvested from the symphysis +	Ixmyelocel-T (Stem cell) mixed with β -TCP and covered with	Cell	Safe to use stem cell therapy in the alveolar cleft. Limitation in large alveolar defect.

				allograft +collagen membrane	collagen membrane		
13.	Al-Ahmady et al. 2018 [38]	CT	20	ICBG	BMMCs seeded on a collagen sponge in combination with Nanohydroxyapatite and autologous PRF	Cell + GF	Considered as an alternative therapeutic option for alveolar bone cleft
14.	Huang et al. 2018 [39]	CT	20	GBR using acellular dermal matrix (ADM) film + ICBG	CGF + ICBG	GF	Not significant between ADM and CGF in bone resorption. Bone density was better in CGF than ADM.
15.	Omidkhoda et al. 2018 [40]	CT	10	ICBG	ICBG + PRF	GF	No significant difference in mean thickness, bone height reduction and total bone loss.

Three studies reported the use of cell-type therapy for bone regeneration in alveolar bone defects. Two of these studies used a synthetic bone graft [β–TCP] in combination with cell therapy. The β-TCP was used in combination with Bone Marrow Mononuclear Cells (BMMCs) in the study of Du et al. (2017) and in combination with Ixmyelocel-T (Stem cell) in the study of Bajestan et al. (2017). Du et al. showed no significant difference in bone volume outcomes between BMMCs with the β-TCP and control groups (iliac crest bone graft (IC)). They concluded that BMMCs with β-TCP are a safe and reliable alternative for alveolar grafting. On the other hand, Bajestan et al. (2017) did not specify the efficacy of cell therapy in bone formation. They only reported that combining β-TCP with stem cells is safe; however, it should be limited to not too large defects.

The 3rd and only study to use cell therapy in combination with autogenous bone graft was conducted by Khojasteh et al. (2017). They used two intervention groups versus a control group; the first intervention group had the alveolar cleft grafted by using the lateral ramus cortical plate (LRCP) with buccal fat pad derived mesenchymal stem cells (BFSCs) mounted on a natural bovine bone mineral. In contrast, the second group underwent grafting using anterior iliac crest (AIC) bone and BFSCs cultured on natural bovine bone minerals. Khojasteh et al. (2017) revealed no statistically significant differences in bone regeneration rates among all groups. However, bone formation was higher in the group of AIC+BFSCs.

Ten of the 15 articles used growth factors in their studies. Three of these ten studies used platelet-rich fibrin (PRF) as a growth factor source in combination with autogenous bone. Attar et al. (2017) reported no significant difference in bone formation. The study concluded that the combination graft (Chin graft + allogeneic bone + leukocyte + PRF) could be used in small to moderate defects and with caution in large ones. Omidkhoda et al. (2018) compared a combination of PRF with anterior iliac crest bone graft (study group) to anterior iliac crest bone graft only (control group). They found no significant difference in “thickness, height and density” between both groups.

Similarly, Shawky and Seifeldin (2016) compared PRF with anterior iliac crest bone graft (study group) to anterior iliac crest bone graft alone (control group). In this study, the quantity of new bone formation was significantly greater in the study group. In

contrast, the quality of bone was lower in the study group, but the difference was not statistically significant.

One of these ten studies (Huang et al., 2018) compared a CGF (concentrated growth factors) preparation combined with ICBG (CGF+ICBG) to an acellular dermal matrix combined with ICBG (ADM+ICBG) in alveolar grafting. Despite a significant increase in bone density in the CGF+ICBG group, there was no significant difference in bone resorption between the two groups.

In the fifth study by Shirani et al. (2017), plasma-rich growth factor (PRGF) was combined with autogenous graft or allograft in alveolar bone defects, and differences in bone formation were evaluated. They concluded that combining autogenous bone graft and PRGF significantly increased bone regeneration compared to the allograft counterpart.

Four out of ten studies used rh-BMP-2 mixed with collagen compared to ICBG. Three studies showed no significant difference in terms of bone formation between the study and control groups (Alonso et al., 2010; Canan et al., 2012; Herford et al., 2007). In contrast, Dickinson et al. (2007) showed a significantly higher bone formation in the rh-BMP-2 study group.

Marukawa et al. (2011) compared the use of PRP combined with autogenous bone graft in alveolar grafting (interventional group) to standard ICBG (control group). In the PRP study group, less bone resorption was observed. This difference was statistically significant. Two of the studies we included utilized a combination of cells and growth factors. In one study, the effect of the regenerative combination (ICGB + marrow graft + PRP) was compared to the effect of grafting the alveolar cleft with only autogenous bone. The difference between the two groups in resulting bone volume was not statistically significant (Sakio et al., 2017).

The second study compared the regenerative combination (BMMCs + collagen sponge + Nanohydroxyapatite + autogenous PRF) to the iliac crest bone graft (ICBG). Al-Ahmady et al. (2018) discovered that the regenerative combination enhanced bone regeneration. They concluded that using autogenous bone marrow mononuclear cells (BMMCs) in combination with nanohydroxyapatite and PRF is a reliable alternative

treatment for alveolar bone defect and demonstrated a complete bone union in 90% of patients, compared to only 70% in the control group (ICBG).

Risk of assessment result:

This study used the Jaded scale and the Delphi list to assess the trial design quality (**Figure 2,3**). This analysis showed a high risk of bias (RoB) in all the included trials. In general, the mean value of the Jaded score was 1.2, with an SD of 0.909, while the mean value of the Delphi list was 3.13, with an SD of 1.454 (**Table 3**). Overall, the items that showed high RoB were noticed in both the Jadad scale and the Delphi list: blindness, intention to treat analysis, concealment of allocation, patient awareness and provider awareness. The mean (SD) of the randomization score was 1.06 (0.679), with a percentage of 80% on the Jaded scale, while in the Delphi list, it was 0.8 (0.4), again with a percentage of 80%. Out of 15 CTs, twelve trials used any randomization method, while only four of them described their randomization method.

In the Jadad scale, the mean (SD) score for double blinding (range 0-2) was 0 (0). None of the studies was double-blinded. Furthermore, the Delphi list assessment (items 4,5 and 6; assessor, provider, and patient awareness) revealed that in eight studies (53.3%), the assessors were not aware of the allocation, in none of the studies (0%) were the care provider blinded to the treatment used. None of the studies (0%) reported that the patients were blinded to the treatment allocation.

Figure 2: Jadad scale scores

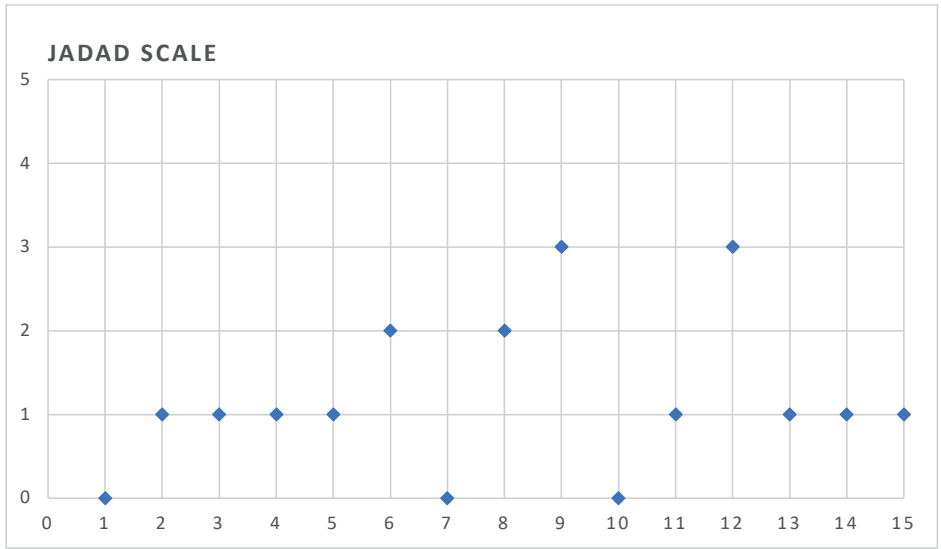


Figure 3: Delphi list scores

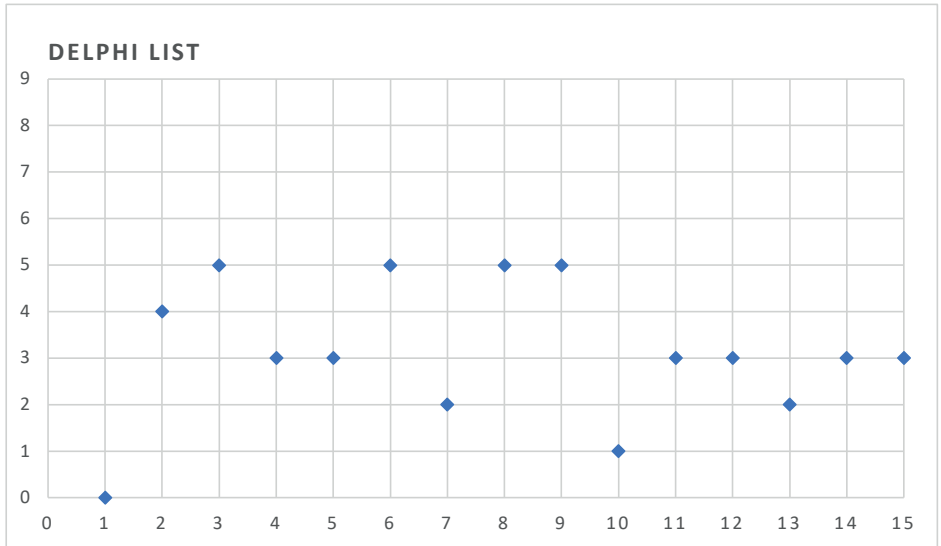


Table 3: Scores/risk of bias (n= 15 in each case)

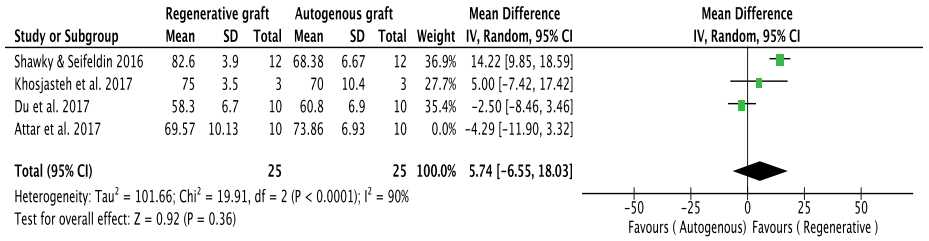
Score	0	1	2	Mean (SD)	(range)
Jadad 1	3	8	4		
Jadad 2	0	0	0		
Jadad 3	12	3	-		
Total Jadad				1.2 (0.909)	(0-2)
Delphi 1a	3	12	-		
Delphi 1b	14	1	-		
Delphi 2	8	7	-		
Delphi 3	6	98	-		
Delphi 4	7	8	-		
Delphi 5	15	0	-		
Delphi 6	15	0	-		
Delphi 7	4	11	-		
Delphi 8	15	0	-		
Total Delphi				3.13 (1.454)	(0-9)

Meta-analysis:

Three of the fifteen controlled CTs were admissible for inclusion in the meta-analysis. These studies have measured and compared the formation of new bone using regenerative techniques and autogenous bone. After six months post-operatively, three studies [Du et al. (2017), Khojasteh et al. (2017), and Shawky & Seifdin (2016)] evaluated new bone formation. Du et al. (2017) and Khojasteh et al. (2017) used BMMCs, and BFSCs stem cells, respectively, while one study (Shawky & Seifeldin, 2016) used PRF as a source of growth factors.

However, the difference was insignificant (P = 0.36), and heterogeneity was high (Figure 4a, I^2 = 90%). In contrast, Attar et al. (2017) compared new bone formation rates by regenerative methods and autogenous bone. However, they favored autogenous bone grafts over regenerative methods. Notably, the evaluation was conducted after 12 rather than six months. Despite the inclusion of this study in the meta-analysis, the overall result indicated that bone formation following the use of regenerative methods was still superior to autogenous bone. Again, the difference was not statistically significant (P = 0.55), and the heterogeneity was high (I^2 = 89%) (Figure 4b). The P-value and heterogeneity results indicate that the studies cannot be compared or utilized.

a:



B:

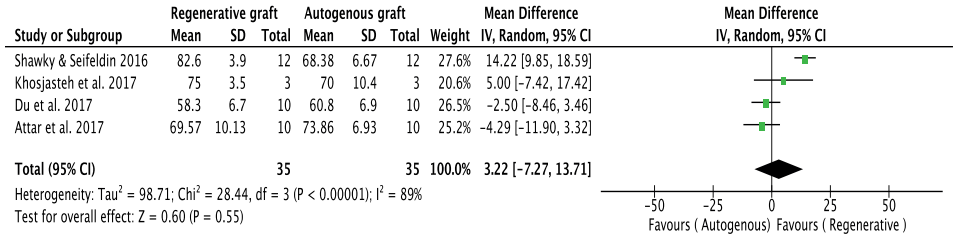


Figure 4 a, b: a: Three studies forest plot for cumulative weighted of the new bone formation rate in regenerative materials compared to control autogenous bone (Iliac crest graft), b; four studies forest plots for cumulative weighted of the new bone formation rate in regenerative materials compared to control autogenous bone (Iliac crest graft).

Discussion

The overall reason for considering regenerative medicine approaches over autogenous bone, particularly iliac crest bone, is to avoid a second surgical site and, thereby, the risk of co-morbidity. However, to be regarded as a feasible, safe and suitable alternative, it is also essential to determine whether the alternative approach is at least equal but preferably better than the standard of care (which is now still autogenous bone). Although many regenerative approaches have been described in the literature, not many were performed in a controlled trial design or RCT, even though this type of trial is considered the most optimal format for drawing conclusions based on evidence-based medicine.

Recently, multiple systematic reviews have been conducted on alveolar bone grafting. Kamal et al. (2018) published an interesting study in alveolar cleft tissue engineering, in which the study reviewed all the retrospective and prospective clinical trials [42]. To evaluate the risk of bias, we decided to update and confirm the result of this study and to focus solely on prospective studies. Osorio et al. (2020) have also discussed bone substitutes in comparison to autogenous bone grafts in a general sense. Although the review concluded that rh-BMP-2 demonstrated satisfactory results, the study did not use meta-analysis to evaluate the regenerative bone graft material's efficacy[43].

Another three systematic reviews focused on using rh-BMP-2. However, we wanted to address all alternatives to autogenous used in the alveolar cleft reconstruction. All these reviews have shown no significant difference between rh-BMP-2 and autogenous graft in terms of volume in alveolar cleft reconstruction [44-46]. This review aimed to comprehensively analyze all CTs utilizing regenerative materials for alveolar cleft reconstructions. In most of these studies (10 out of 15), growth factors were used as the regenerative method. Four of these studies demonstrated significant differences in outcomes regarding regenerative materials [29, 31, 33, 35]. The growth factors used in those five studies were rh-BMP-2 [29], PRP [31], PRF [33] and PRGF [35].

In the study by Shirani G et al. (2017), the PRGF was used in both the intervention (FDBA) and control groups (ICBG); therefore, a definitive conclusion cannot be drawn regarding the effect of the regenerative factor. Collectively, these studies demonstrate that different types of growth factors can be used and have been applied in CTs to

repair alveolar cleft defects, with rh-BMP-2 appearing to be the most preferred and promising due to its comparable efficacy in three studies and superior bone formation in one study compared to autogenous bone.

Three out of 15 used cellular therapies only. [13, 36, 37] Du and colleagues discovered comparable effects of the cellular therapy compared to autogenous bone, Khojasteh and colleagues analyzed two different bone sources both seeded with the same type of stem cells, and Bajestan's report only stated that their cell therapy was safe but did not report efficacy data. This virtually precludes the ability to draw conclusions. Only two out of fifteen studies combined cell-growth factors. The results demonstrated that regenerative therapies demonstrated similar (Sakio et al., 2017) [23] or slightly better (90% bone unions for the regenerative group vs. 70% for the autogenous bone group) (Al-Ahmady et al., 2018) [38] results when compared to the autogenous bone counterpart.

From the studies presented, can we deduce which type of regenerative therapy, i.e. growth factor-mediated, cell-mediated, or combinations thereof, are the most optimal alternative? The short answer is: no. This was confirmed by our meta-analysis, which revealed that regenerative tissue engineering methods resulted in better new bone formation compared to autogenous bone graft. However, the difference was insignificant (P value = 0.36) with high heterogeneity (I^2= 90%). Moreover, in the 15 studies, we identified and presented in this review, our RoB analysis demonstrated that the alveolar cleft repair-controlled trials still encompassed some and, in other cases, even many flaws in the trial design or their reporting of the results, hampering sound and reliable conclusions.

The high RoB in studies addressing regenerative methods for alveolar cleft repair was also reported in 2015 in an earlier review by Khojasteh and coworkers.[42] Due to a lack of evidence and controlled CTs, it was determined that the treatment efficacy of tissue engineering in alveolar cleft bone defects could not be determined and that well-designed controlled studies were required to compare outcomes accurately. Unfortunately, the current study reveals that no substantial progress has been made to date. The studies conducted since then also suffered from a high RoB and inadequate design quality, making it impossible to draw evidence-based conclusions. Thus, there is a persistent call for significantly improved clinical trials.

Conclusion

According to our review, alveolar cleft grafting CTs using regenerative materials have a high risk of bias. Although the results showed better new bone formation in alveolar cleft defects using the regenerative materials compared to the iliac crest graft, the meta-analysis of the available data showed no statistically significant difference. Upcoming CTs should consider improving the quality to avoid the risk of bias.

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This article is free of conflict of interest.

Ethical approval:

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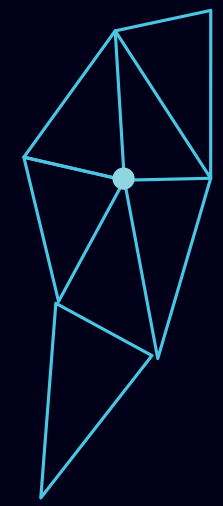
Chapter 5

Polyphosphate (PolyP) for alveolar cleft repair, study protocol for a pilot randomized controlled trial



Alkaabi SA, Natsir Kalla DS, Alsabri GA, Fauzi A, Tajrin A, Müller WEG, Schröder HC, Wang XG, Forouzanfar T, Helder MN, Ruslin M. Polyphosphate (PolyP) for alveolar cleft repair: study protocol for a pilot randomized controlled trial. Trials. 2021 Jun 14;22(1):393. doi: 10.1186/s13063-021-05325-2. PMID: 34127045; PMCID: PMC8201927.

Polyphosphate (PolyP) for alveolar cleft repair, study protocol for a pilot randomized controlled trial.

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Abstract

Objective

Bone grafting is an important surgical procedure to restore missing bone in patients with alveolar cleft lip/palate, aiming to stabilize either side of maxillary segments by inducing new bone formation and, in bilateral cleft cases, also to stabilize the pre-maxilla. Polyphosphate (PolyP), a physiological polymer composed of orthophosphate units linked together with high-energy phosphate bonds, is a naturally existing compound in platelets which, when complexed with calcium as Ca-polyP microparticles (Ca-polyP MPs), was proven to have osteoinductive properties in preclinical studies. **Aim:** To evaluate the feasibility, safety and osteoinductivity of Ca-polyP MPs as a bone-inducing graft material in humans.

Methods

This prospective non-blinded first-in-man clinical pilot study shall consist of 8 alveolar cleft patients of 13 years or older to evaluate the feasibility and safety of Ca-PolyP MPs as a bone-inducing graft material. Patients will receive Ca-polyP graft material only or Ca-polyP in combination with biphasic calcium phosphate (BCP) as a bone substitute carrier. During the trial, the participants will be investigated closely for safety parameters using radiographic imaging, regular blood tests, and physical examinations. After six months, a hollow drill will be used to prepare the implantation site to obtain a biopsy. The radiographic imaging will be used for clinical evaluation; the biopsy will be processed for histological/histomorphometric evaluation of bone formation.

Discussion

This is the first-in-man study evaluating the safety and feasibility of the polyP as well as the potential regenerative capacity of polyP using an alveolar cleft model.

Trial registration

Indonesian Trial Registry under number INA-EW74C1N. The clinical trial protocol received written approval by the ethical committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, with code number 1063/UN4.6.4.5.31/PP36/2019. The results on safety, feasibility, and bone formation with polyP as graft material will be published upon completion of the trial.

Keywords:

Polyphosphate**,** Alveolar bone grafting, Bone regeneration, Regenerative medicine.

Administrative information:

Trials guidance:

Title	Polyphosphate (PolyP) for alveolar cleft repair, study protocol for a pilot randomized controlled trial. For a total of eight patients, four patients (randomized) will receive Ca-PolyP MP as bone graft, and the other fourour patients will receive a combination of PolyP/BCP as graft material
Trial registration.	Indonesian Trial Registry under number INA-EW74C1N. The ethical committee of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia 1063/UN4.6.4.5.31/PP36/2019. .
Protocol version	Version 1.0, dated 28 May 2019
Funding	No funding was received
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	Amsterdam, Amsterdam Movement Sciences, Amsterdam, The Netherlands. Role: Methodology and supervision.
Name and contact information for the trial sponsor	Muhammad Ruslin Department of Oral and Maxillofacial Surgery Faculty of Dentistry Hasanuddin University Kode Pos 90425 Makassar Indonesia Tel: +62-41-158-6012 Fax: +62-41-143-3015
Role of sponsor	There was no sponsor.

Introduction

Background

Alveolar cleft is a defect occurring as a result of the failure of regular development during frontonasal prominence growth, which mostly affects the site between the lateral incisor and the canine (Von Eiselsberg F., 1901). In 1901, the alveolar bone cleft defect was first reconstructed by von Eiselsberg using an autogenous bone graft, while Lexer published in 1908 the first reconstruction with nonvascular graft material [1, 2]. The autogenous bone most often derived from the cancellous iliac crest is still considered as a golden standard for the grafting procedure. Other sources such as the tibia, mandibular symphysis, rib, and the cranium are still being used by surgeon preference [3-7]. However, the drawback of autogenous graft is that it requires another surgical site, which may be associated with post-operative complications [8]. Consequently, the development of effective bone graft substitutes is cur- rently being given high priority and attention [9, 10].

Müller and colleagues identified a new bone graft based on polyphosphate (polyP) [11, 12]. PolyP is a naturally existing compound in the platelets [13]; a physiological polymer composed of orthophosphate units linked together with high-energy phosphate bonds similar to ATP [14]. Complexed with calcium as Ca- polyP microparticles (Ca-polyP MPs), it was proven to have osteoinductive properties in preclinical studies [14- 16]. PolyP is also used as a food additive (E 452) and in cosmetics [17]. As such, polyP is considered a safe ma- terial in current human applications [18].

Biphasic calcium phosphate (BCP) is a mixture of hydroxyapatite (HA) and β-tricalcium phosphate (β- TCP) with different ratios [19]. BCP in some reports showed intrinsic osteoinductive properties causing ectopic bone formation [20, 21]. While other reports such as de Lange et al. showed that BCP has osteocon- ductive properties facilitating the bone formation and re- modeling in a maxillary sinus lift model [22].

The aim of the current phase I clinical protocol study is to test the safety and feasibility of amorphous Ca- polyP MPs as a graft material.

Objective

The protocol of this study as presented here is first-in- human.

Primary objective

The primary objective is to assess the safety of amorphous Ca-polyP MPs as a graft material in the hu- man alveolar cleft reconstruction model.

Secondary objective

The secondary objective is to evaluate the feasibility and the potential regenerative capacity of polyP using an alveolar cleft model amorphous Ca-polyP MPs.

We hypothesize that the bony reconstruction with osteoinductive Ca-polyP MPs, either or not in combin- ation with BCP granulate, will accelerate the quantity and quality of bone formation in a timely manner. Fur- ther, it will reduce the surgical time and morbidity by the absence of a donor site, thereby increasing the cost- effectiveness and quality of care.

Methods and design

Ethics

The clinical trial was approved by the Ethics and Research Committee of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, with code number 1063/UN4.6.4.5.31/PP36/2019. Participants will be recruited from general practices of Hasanuddin Dental Hospital and in the area around Makassar. The trial will be conducted in Hasanuddin Dental Hospital. All participants shall be asked to sign an informed consent. This study complies with the principles of the Declaration of Helsinki.

Study design

This is a single-center prospective control clinical trial that will be conducted in Hasanuddin University, Hasanuddin Dental Hospital, to assess the safety and feasibility of calcium-polyphosphate microparticles (Ca- polyP MPs, CAS No.: 13477-39-9, EC No.: 236-769-6) as a bone graft material in an alveolar cleft model. The average MP particle size diameter is 280 ± 120 nm [12]. A total of 8 patients will be included in the trial using a parallel assignment intervention. Four patients (random-ized) will receive Ca-PolyP MP as a bone graft, and the other 4 patients will receive a combination of PolyP/BCP as a graft material. The primary endpoint will be set at 6 months. At each follow-up visit, AE and/or SAEs will be documented, and clinical

assessments will be per- formed at time points specified in the “Intervention” sec- tion. All patients will be monitored closely using lab tests (complete blood count (https://doi.org/10.1053/ jpan.2003.50013), others if needed), radiographs, and periodic physical examination (Table 1). After these 6 months, a bone biopsy will be taken during dental implant preparation and processed for histological/histo-morphometric analysis. Finally, a report on safety, feasi- bility, and potential efficacy with regard to bone formation will be made and will, irrespective of the out- comes, be published in a peer-reviewed journal.

Table 1: Assessment table:

	Consent form	Panorama	CBCT or CT	Physical examination	CBC	Thermometer	Biopsy
Preoperatively	✓	✓	✓	✓	✓	✓	
Operative day				✓		✓	
Post-op day1		✓		✓	✓	✓	
Post-op day 8		✓	✓	✓	✓	✓	
Post-op day14				✓		✓	
Post-op day 30				✓	✓	✓	
Post-op day 90		✓		✓		✓	
Post-op day 180		✓	✓	✓	✓	✓	✓

Eligibility criteria

Inclusion and exclusion criteria

After written informed consent will be obtained by a research team member, the participant will be screened further for eligibility. Patients should be ≥ 13 years old, healthy male or female patients with an alveolar cleft bone defect, non-smoker, with no history of previous grafting procedure(s), with a normal blood count, and with an ASA1 regarding anesthetic risks.

Patients will be excluded when they have poor oral hygiene with mouth plaque, are over 70 years old, are classified as ASA3 and beyond, have local infection and active systematic disease, or received radiotherapy, chemotherapy, immunosuppressive, or anticoagulant therapy recently. Other exclusion criteria comprise having received bone morphogenetic protein (BMP) growth factors or other bone growth-promoting factor therapy, obvious malnutrition, and active influenza.

Withdrawal of participants

Participants can leave the study at any time for any reason without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. When participants withdraw prior to grafting intervention, they will be replaced. Furthermore, if a membrane has been used for any reason, the patient will be considered as a dropout and will be replaced.

Intervention

Under general anesthesia, and after local infiltration with adrenaline 1:100,000, an incision will be made at the cleft margin to create a pocket-like tissue towards the nose and the mouth in order to reconstruct the nasal floor as well as the palatal tissue. The goal of this ap- proach is to get rid of the oro-nasal fistula and to expose the bony edges on both sides of the cleft. Under sterile conditions, either Ca-polyP MP alone (NanotecMARIN GmbH, Mainz, Germany) or a combination of BCP (Straumann Bone Ceramic, Villeret, Switzerland) and PolyP will be mixed with normal saline in a ratio of 1 g: 1.5ml and 1g:2g:3–5ml, respectively. A homogenous mixture should be reached before placing the graft ma- terial into the cleft defect. A good adaptation of bone graft material should be considered while placing it in the cleft defect. No membrane will be used. A different graft quantity will be considered for larger defects, how- ever, with

the same mixing ratios. Absorbable sutures with 3/0 Vicryl for the mucosa and 4/0 Vicryl for the nasal reconstruction will be used for closure.

Post-operative, suitable antibiotics and painkillers will be prescribed to all patients.

Adverse event (AE) and serious adverse event (SAE)

Any adverse event will be graded with respect to intensity and classified as either serious or non-serious according to the World Health Organization classifica- tion. Any change in health which occurs between screening examination and first administration of amorphous Ca-polyP microparticles or related proce- dures will be recorded as part of the subject's medical history, and full medical care will be given to all partici- pants. In the case of a SAE, the sponsor will be notified within 24 h from the onset. If the SAE concerns severe toxicity or infection associated with the graft site, the trial will be terminated immediately.

Sample size

Since this is a first-in-man trial, the current trial sample size has been limited to only 2 × 4 patients, with the pri- mary goal to gain a first insight on the safety and feasibility of the treatment with Ca-polyP. It is assumed that no SAEs or AEs will occur, and then, an n = 4 for each group should therefore be sufficient.

Recruitment

Prior to recruitment, an audit will be carried out by the surgical and ethical team to evaluate the safety measurements at the research site in the Hasanuddin Dental Hospital. Patients will be recruited from an existing database of patients eligible for the proposed treatment available from the Hasanuddin University, Hasanuddin Dental Hospital.

Randomization and treatment allocation

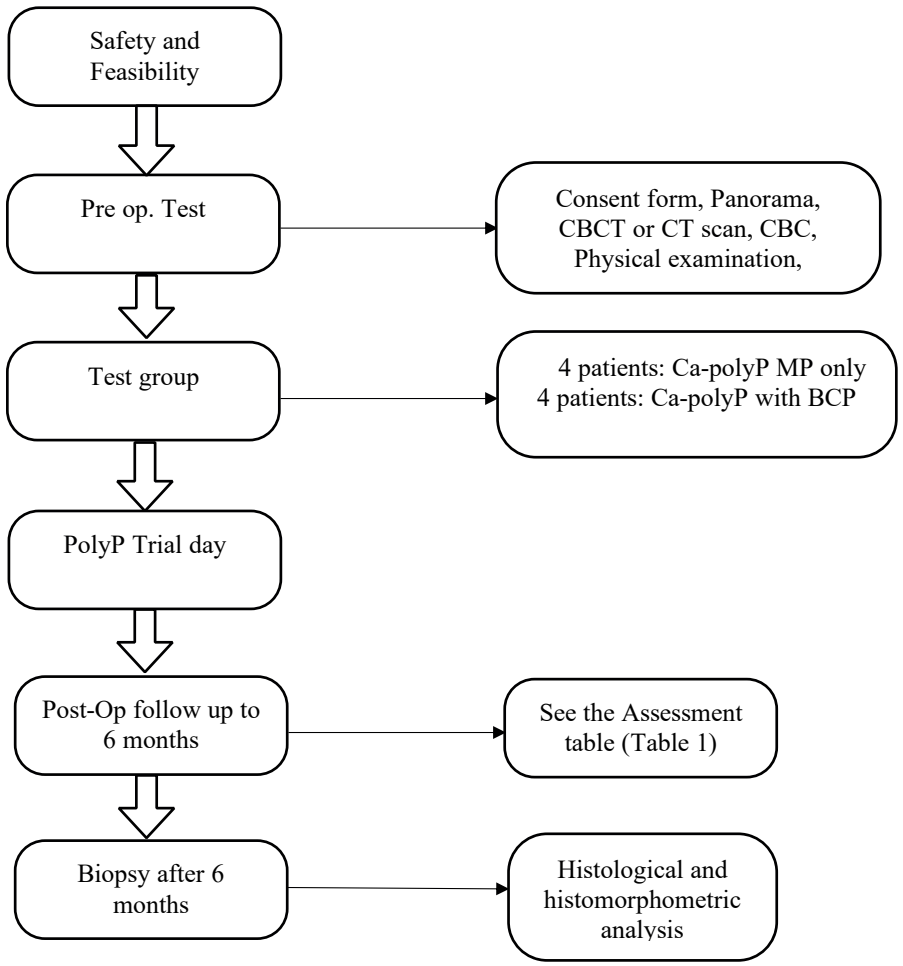
Because this is a first-in-human study, it is not possible to keep all personnel blinded to the assignment group. After written informed consent will be obtained by the main surgeon, randomization will be performed with re- gard to the treatment group. Central randomization using a randomization program on a secure computer will be used after the completion of patient enrollment. Patients will receive a unique study code, and

their data will be provided to the clinical and research evaluators in a patient-coded manner.

Blinding

The radiologist and the histopathologist will be kept blinded to the treatment when evaluating the data (Figure 1).

Figure 1: Protocol flowchart



Data collection and access

The rules and responsibilities will be provided to the research team. The doctors and nurses of the research team will collect the data according to the evaluation (Table 1). All research team members will receive training on how to collect data at all study visits. The patient-coded data will be then handed over to the clinical evaluators and investigators. Each patient will be followed up for up to 6 months. The confidentiality of the participant's data will be well protected by the data manager.

Outcomes

Safety assessment based on physical examination and laboratory measurements When a SAE occurs, it will be concluded that polyP is not (yet) safe in the current setting. For AEs, if they do not occur at a higher frequency than in patients treated with standard care (autologous bone) and/or can be resolved by non-invasive conventional methods (e.g., analgesics, antibiotics), the polyP product will be considered safe. In all other cases, polyP will not be considered safe (yet).

Radiographic evaluation

The Chelsea scale will be used to evaluate the bone graft and the level of the bone in comparison with the adjacent teeth. This scale starts with drawing an imaginary midline between the two teeth on either side of the cleft site. Each of those teeth (mesial and distal roots) will be divided starting from the cemento-enamel junction to the root apex in four parts. A 0 score is given when no bone is present up till the midline; a 0.5 score is given when there is bone, but it fails to reach the mid- line; and a 1 score is given when the bone extends from the root surface to the midline [23].

Histological and histomorphometric analysis

The histological and histomorphometric analysis will be performed in at least 3 patients from each group. In those patients, the dental implant site will be prepared using a trephine burr (⌀ 2.0 mm × 10.0 mm in length) instead of a normal drill, thereby being able to collect a biopsy from the treated site without interfering with the normal procedure. The biopsies will be fixed in 10% formalin and processed for embedding in methylmethacrylate for the evaluation of hard tissue formation. After sectioning, different staining's (Goldner's trichrome, Toluidine blue, tartrate-resistant acid phosphatase (TRAP)) will be used, and histomorphometric parameters for bone

formation will be analyzed. Two trained examiners, blinded for the treatment modality, will evaluate the images, and intra- and inter- observer reliabilities will be determined. In case of dis- agreement between the observers, the specimen will be re-evaluated to reach a consensus.

Monitoring

Monitoring will be done constantly by internal monitors of the Ethics and Research Committee of Faculty of Medicine, Hasanuddin University. Since there is a negligible risk, a data safety monitoring board will not be formed. A safety report will be provided to the Medical Research Ethics Committee of the Ethics and Research Committee of Faculty of Medicine, Hasanuddin University, every year. An interim analysis will not be conducted.

Statistical analysis

A SPSS power analysis for parameter comparisons between the groups will be performed. A p value less than 0.05 will be considered statistically significant.

Amendments

All substantial amendments will be notified to the ethical committee and competent authority to ensure the safety and integrity of participants as well as the scientific value of the trial.

Post-trial care

All participants will be kept in secondary follow-up for a period of 3 years to ensure their safety and to record any delayed side effects of the Ca-polyP graft material.

Discussion

This is the first-in-man study evaluating the potential re- generative capacity of polyP using an alveolar cleft model. PolyP represents a completely novel type of re-generative compound, since it can be considered as a rich energy source for tissue repair, which may be as pivotal for the bone regeneration process as the osteogenic factors, which are generally believed to be the primary active compounds [14]. The high-energy phosphate bonds of polyP are identical to those present in the “common” cellular energy molecule ATP, and both serve as substrates for the enzyme alkaline phosphatase (ALP), a well-known marker for active bone formation [12]. PolyP has also

been reported to promote mineralization [24] and to increase progenitor cell differentiation into osteoblasts [15, 25]. PolyP is present in platelets, which play an essential role in early wound repair. Interestingly, platelet-rich plasma (PRP), a concentrate of platelet-rich plasma protein derived from the whole blood and often used in bone repair strategies, therefore will also contain polyP. However, the efficacy of PRP to promote bone re- pair is nowadays questioned, since both positive and neutral/negative effects have been published recently [26, 27]. We speculate that the much higher dose of polyP present in our preparations will be well above the bone regeneration threshold, and thus may have a positive effect on the bone repair process.

Calcium phosphate ceramics including biphasic calcium phosphates (BCPs) have been widely used as bone substitutes and tissue engineering scaffolds. Calcium phosphates are highly biocompatible, proven to be safe, and successfully used in many different clinical treatment modalities such as bone augmentation in spinal arthrodesis, maxillo- and craniofacial surgeries, orthopedics, periodontal treatment, and metallic implant coatings [28–33]. Some reports describe that BCP may also have osteoinductive properties [34], which implies that BCP may add to the osteoinductivity as well. Moreover, a recent clinical study applying micro structured β-TCP for alveolar cleft repair demonstrated that calcium phosphate could be used safely and effectively for this purpose as well [35]. We are there- fore convinced that the Straumann Bone Ceramic used in the current study will be a safe-to-use scaffold and may have a supportive or even synergistic effect on the bone formation when combined with the bioactive polyP.

For the clinical evaluation of bone formation, radiographic imaging will be applied. We are well aware that this will likely be relatively reliable in the case of the group that is treated only with the (radiolucent) polyP microparticles but will not be easy with the BCP/polyP treatment group. The BCP scaffold will be radiopaque and cause signal scattering, which will preclude accurate visualization of new bone formation within the scaffold material. We will circumvent this limitation by our histological and histomorphometrical analysis of the biopsies taken at the 6-month follow-up time point, during dental implant placement. This will enable us to still evaluate the bone formation at the micro-scopic level and to quantify multiple bone formation- related parameters

and cellular activities as demonstrated before in other bone regeneration studies performed by our group [29, 30, 36, 37].

Conclusion

With this protocol, we summarized how we intend to evaluate the safety and feasibility of Ca-polyP MP as a new grafting material in an alveolar cleft model.

Trial status

Recruitment started in November 2019 and is planned to end in September 2020, with 8 patients randomized. The current protocol version is 1.0, dated 28 May 2019.

Authors' contributions

The authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare that they have no competing interests.

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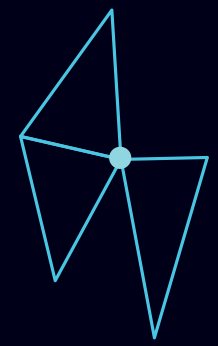
Chapter 6

Safety and feasibility study of using Polyphosphate (PolyP) in alveolar cleft repair, A Pilot study.



Alkaabi SA, Kalla DSN, Alsabri GA, Fauzi A, Jansen N, Tajrin A, Nurrahma R, Müller W, Schröder HC, Xiaohong W, Forouzanfar T, Helder MN, Ruslin M. Safety and feasibility study of using polyphosphate (PolyP) in alveolar cleft repair: a pilot study. Pilot Feasibility Stud. 2021 Nov 8;7(1):199. doi: 10.1186/s40814-021-00939-4. PMID: 34749808; PMCID: PMC8573762.

Safety and feasibility study of using Polyphosphate (PolyP) in alveolar cleft repair, A Pilot study.

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ABSTRACT

Background:

Bone grafting is an important surgical procedure to reconstruct alveolar bone defects in patients with cleft lip and palate. Polyphosphate (PolyP) is a physiological polymer in blood, primarily in platelets. PolyP plays a role as a phosphate source in bone calcium phosphate deposition. Moreover, the cleavage of high-energy bonds to release phosphates provides the local energy necessary for regenerative processes. In this study, polyP is complexed with calcium to form Calcium polyP microparticles (Ca-polyP MPs), shown to have osteoinductive properties in preclinical studies. This study aimed to evaluate the feasibility, safety and osteoinductivity of Ca-polyP MPs, alone or in combination with BCP, in-first human clinical trial.

Methods:

This single-blinded, parallel, prospective clinical pilot study enrolled eight adolescent patients (mean age 18.1: range 13 - 34 years) with a residual alveolar bone cleft. Randomization was performed in two groups (four receiving Ca-polyP MPs only and four a combination of Ca-polyP MPs and biphasic calcium phosphate (BCP)). The patient follow-up was six months. Outcome parameters included safety parameters and close monitoring of possible adverse effects using radiographic imaging, regular blood tests, and physical examinations. Osteoinductivity evaluation using histomorphometric analysis of biopsies was impossible due to COVID restrictions.

Results:

Due to surgical and feasibility reasons, eventually, only two patients received Ca-polyP MPs, and the others a combination graft. All patients were assessed up to day 90. Four of eight could continue with the final assessment day (day 180). Three out of eight could not reach the hospital due to Covid-19 restrictions. One patient decided not to continue with the study. None of the patients showed allergic reactions or remarkable local or systematic side effects. Radiographically, patients receiving Ca-polyP MPs only were scored grade IV Bergland scale, while patients who got the BCP/Ca-polyP MPs combination had scores ranging from I to III.

Conclusions:

Our results indicate that Ca-polyP MPs and the BCP/Ca-polyP MPs combination appear to be safe graft materials; however, in the current setting, Ca-polyP MPs alone may not be sufficiently stable defect-filling scaffolds to be used in the alveolar cleft repair.

Trial registration:

Indonesian Trial Registry under number INA-EW74C1N by the ethical committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, with code number 1063/UN4.6.4.5.31/PP36/2019

Keywords:

Polyphosphate, Alveolar bone grafting, Bone regeneration, Regenerative medicine.

INTRODUCTION:

Background

Cleft lip and palate (CLP) are common anomalies in the craniofacial region. They are considered the second most common congenital deformity after the clubfoot.[1] An alveolar cleft is seen in 75% of CLP patients.[2,3] Alveolar bone grafting (ABG) is an essentially functional and esthetic procedure to reconstruct the bony defect in the maxilla and the nasal floor.[4] ABG plays an important role in facilitating teeth eruption and filling the bony defect by closing the oronasal fistula that routinely occurs in alveolar cleft patients.

The alveolar bone grafting can be performed using autogenous bone, allograft bone, or bone substitutes. Autogenous bone graft is still considered the gold standard for any grafting procedure. [5] Nevertheless, numerous studies are employing various bone substitutes or allografts to overcome the risks and complications that could arise from harvesting bone at the donor site.[6-8] Risks such as gait disturbance, hematoma, donor site morbidity and other concerns associated with the growth (through harvesting from the rib or the iliac crest) could be avoided if a good allograft or bone substitute material were used.[9]

Polyphosphate (polyP) is a molecule naturally present in the bloodstream platelets. Müller and his colleagues have been able to structure a new graft material by precipitation of polyP with calcium, thus forming Ca-polyP microparticles (Ca-polyP MPs).[10-12] The Ca-polyP MPs were proven to have bone osteoinductive characteristics in preclinical studies.[12-14] It has been shown that the Ca-polyP MPs can accumulate and concentrate at the site of the new bone formation. PolyP polymer elicits both the anabolic signals and the fuels due to energy-rich phosphate anhydride linkages as well as the metabolic process in the cells. Such signals could accelerate cell growth and differentiation.[15]

On the other hand, Biphasic calcium phosphate (BCP) is another type of graft that contains a phosphate molecule mixed with Hydroxyapatite (HA) in different ratios. Different specification outcomes have been reported to the BCP as graft material, and some stated that the BCP has osteoconductive characteristics,[16,17] while others concluded that it also could be osteoinductive in nature.[18,19]

Objective:

This first-in-human study evaluates the safety, feasibility and osteoinductivity of Ca-polyP MPs, alone or combined with BCP, as a graft material in alveolar cleft patients.

MATERIAL AND METHODS:

Ethics

This single-blinded, prospective clinical trial, a pilot study, was approved by the ethical committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia, with code number 1063/UN4.6.4.5.31/PP36/2019. It was registered in the Indonesian Trial Registry under number INA-EW74C1N. The study protocol complies with the principles of the Helsinki Declaration. Patients and legal guardians of the patients signed an informed consent.

No special ethical approval was required for this study.

Patients and randomization:

Eight patients with residual alveolar bone cleft participated in this study. The inclusion criteria were the inclusion criteria for non-syndromic, non-smoker, age 13 and above, no history of previous grafting procedure(s), and ASA1 regarding anesthetic risks. The exclusion criteria were systemic diseases, patients with syndromic conditions, localized infections, active influenza, obvious malnutrition, and patients receiving any active medical treatment. Four of eight patients were randomly assigned to receive Ca-polyP MPs alone, while the remaining four patients were to receive a mixture of Ca-polyP MPs and BCP as graft material. Two patients eventually received Ca-polyP MPs alone, while six received the mixture (see results). The surgeon and patients were informed of the graft type, but the assessor remained completely blind to the patient grouping. The surgical procedure and follow-up times are detailed in Table 1.

Table 1: Treatment time schedule

	Consent form	Panorama	CBCT or CT	Physical examination	CBC	Thermometer	Biopsy
Preoperatively	✓	✓	✓	✓	✓	✓	
Operative day				✓		✓	
Post-op day1		✓		✓	✓	✓	
Post-op day 8		✓	✓	✓	✓	✓	
Post-op day14				✓		✓	
Post-op day 30				✓	✓	✓	
Post-op day 90		✓		✓		✓	
Post-op day 180		✓	✓	✓	✓	✓	✓

OPG: Orthopantomogram; CT: computed tomography; CBCT: Cone Beam CT

Sample size

Since this is a first-in-human trial, the number of patients was kept low to minimize the risk of graft exposure in case of any adverse effect. The current trial sample was limited to only 2x4 patients, with the primary goal of gaining a first insight into the feasibility and safety of the treatment with the polyp.

Randomization and treatment allocation

After written informed consent, randomization was performed with regard to the treatment group. However, all patients were aware of the fact that their treatment comprised Ca-polyP MPs.

Blinding

The radiologist remained blind to the treatment when evaluating the data.

Data Collection

The doctors, nurses, and other members of the research team were provided with a list of rules and responsibilities. Doctors and nurses collected data in accordance with Assessment Table 1. All members of the research team were instructed on how to collect data during all study visits. Each patient was followed for six months. The confidentiality of patient information was protected by the data manager.

Polyp and BCP preparation:

PolyP graft comes in the form of Ca-polyP MPs powder produced by NanotecMARIN GmbH (Mainz, Germany). At the same time, the BCP consists of a mixture of 60% hydroxyapatite and 40% of beta-tricalcium phosphate (Straumann Bone Ceramic, Villeret, Switzerland). Under sterile conditions, either Ca-polyP MPs or a mixture of Ca-polyP MPs and BCP was prepared using normal saline at a ratio of 1g: 1.5 ml and 1g:2g:3-5 ml, respectively. The components were mixed until a homogenous mixture was obtained (Figure 1).

Figure 1: Ca-polyP MP + BCP mixed with normal saline.

Surgical procedure:

Under general anesthesia and full aseptic conditions, the oral cavity was rinsed with 0.1% chlorhexidine gluconate solution. A local anesthesia infiltration using lidocaine

with epinephrine 1:100,000 was given. A full mucoperiosteal flap was reflected from the first molar to the central incisor on the contralateral side of the defect. The tissue was dissected carefully to separate the oral mucosa from the nasal layer. A palatal mucoperiosteal flap was reflected from either side of the cleft, followed by elevation of the palatal tissues. The nasal mucosa was cranially elevated and sutured cranially to repair the oronasal fistula (Figure 2a). A Ca-polyP MPs preparation or the Ca-polyP MPs and BCP mixture was applied to the alveolar cleft defect (Figure 2b). Tension-free closure was realized in all wounds.

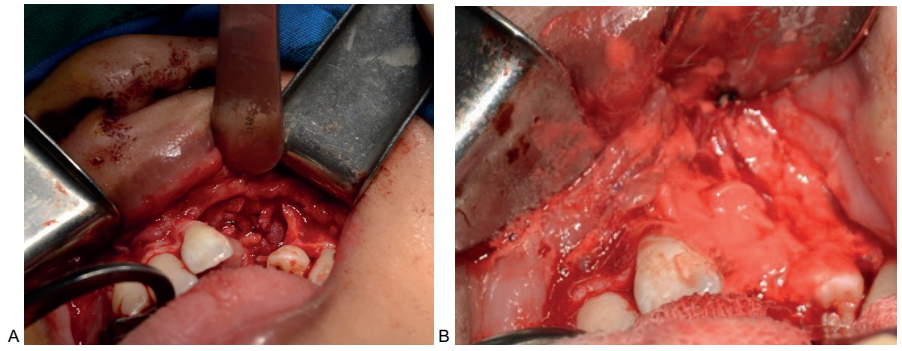

Figure 2: a: nasal floor reconstruction and exposing the bony edges, b; ca-polyP graft placed in the defect.

Post-operative care:

Oral hygiene instructions were given to all patients, including mouth rinsing with 0.12% Chlorhexidine. Antibiotics (Amoxicillin/Clavulanic acid) and painkillers were prescribed for seven days according to the standard of care. During the hospital stay, follow-up examinations of all patients were meticulously performed to report any adverse reaction to the grafting materials locally or systemically. After patient discharge, all patients followed an assessment timetable.

Orthopantomogram (OPG):

Bergland scale:

OPGs were taken one day preoperatively (X-Mind Pano D+ Satelec- Digital panoramic with teleradiography - Satelec) and subsequently after 8, 30, 90 and 180 days. The OPGs were used to assess the vertical graft formation employing the Bergland scale, the gold standard used to evaluate the integrity and height of the alveolar bone graft.[20] The Bergland scale is classified into four grades; grade I: bone height is almost normal

height; grade II: a bone height of at least 75% of the interalveolar septum; grade III: the bone height is less than 75%, grade IV: no evidence of bone integration.[21]

CT scan:

The CT scans (Siemens SOMATOM Definition Flash CT Scanner) were performed pre-operatively at postoperative days 8 and 180. The data were processed by OsiriX (Pixmeo, Switzerland), an open-source Digital Imaging and Communications in Medicine (DICOM).

RESULTS:

All patients could comply with the study requirements up to assessment day 90. Unfortunately, four out of eight patients could not continue with the final assessment (day 180). One patient decided not to continue with the study. In contrast, the other three patients could not approach the hospital due to the COVID-19 lockdown in their towns/villages (Table 2).

Table 2: Demographic and assessment data:

	Pt.1	Pt.2	Pt.3	Pt.4	Pt.5	Pt.6	Pt.7	Pt.8
Gender	F	F	M	F	F	F	F	F
Age	18	13/14	13	15	13	15	24	34
Affected side	Left	Left	Bilateral	Left	Right	Left	Right	Left
Graft type	Ca-polyP MPs	Ca-polyP MPs	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP
Assessment day 30	Completed	Completed	Completed	Completed	Completed	Completed	Completed	Completed
Assessment day 90	Completed	Completed	Completed	Completed	Completed	Completed	Completed	Completed
Assessment day 180	Missed follow-up, Covid-19 lockdown	Completed	Completed	Completed	Missed follow-up, Covid-19 lockdown	Missed follow-up, Covid-19 lockdown	Drop-out	Completed

Pt.: patient; F: female; M: male; Ca-polyP: Calcium polyphosphate microparticles; BCP: biphasic calcium phosphate

The same surgeon performed bone grafting surgery on all eight patients. Local or systematic postoperative complications were not reported in either study group. All patients were closely monitored from day 1 until discharge (day 3). The patients were then monitored according to Table 2. Despite not being part of the original trial design, all patients were contacted via video or telephone for a one-year follow-up. No adverse events were reported, and all patients were satisfied with the treatment.

Feasibility:

Two different application modes of Ca-polyP-MP should have been tested randomly. However, due to the difficulty of handling Ca-polyP microparticles when not complexed with BCP, we had to abandon the randomization of graft type and only apply the BCP-polyP graft type. Thus, feasibility appeared valid for the combination graft but not (in the current setting) for applying Ca-polyP MPs only.

Safety:

Adverse events

This study's primary objective was to assess the safety of Ca-polyP MPs, alone or in combination with BCP, in terms of adverse events (local or systematic) using clinical assessment, radiographic, and laboratory investigations (among others, white blood cells, neutrophil, lymphocyte, and if necessary, C-reactive protein) (Table 3). All patients were hospitalized for 72 hours after surgery to ensure close monitoring. The trial will be terminated immediately if a serious adverse event involves severe graft-site toxicity or infection.

Osteoinductivity:

Since the acquisition of biopsies was impossible due to COVID-19 restrictions, this aspect could not be evaluated as planned.[27]

Table 3: Safety assessments

	Pt.1	Pt.2	Pt.3	Pt.4	Pt.5	Pt.6	Pt.7	Pt.8
Graft type	Ca-polyP MPs	Ca-polyP MPs	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP	Ca-polyP MPs + BCP
Pain	Mild	Mild	Minimum pain/pressure	Mild	Mild	Minimum pain/pressure	Mild	Moderate
Fever	No	No	No	No	No	No	No	No
Allergic reaction	ND	ND	ND	ND	ND	ND	ND	ND
Remarkable local inflammation/ infection	No	No	No	No	No	No	No	No
Systematic adverse effect	ND	ND	ND	ND	ND	ND	ND	ND
Lab tests	WNL	WNL	WNL	WNL	WNL	WNL	WNL	WNL

Ca-polyP MPs: Calcium polyphosphate microparticles, BCP; Biphasic calcium phosphate, ND; nothing detected; WNL: Within normal limit.

Radiographic evaluation:

Orthopantomogram:

The Bergland scale was used in this study to investigate the result of the secondary bone grafts in alveolar defects. This scale is considered the gold standard to assess the post-alveolar graft height of the interdental septum. Although OPG is more susceptible to distortions, it was chosen because it is more patient-friendly when compared to the other intra-oral X-rays, especially when taken postoperatively.

Table 4: Bergland scores based on OPGs

Bergland scale	**Ca-Polyp MPs graft**		**Ca-Polyp MPs + BCP**					
	Pt.1	Pt.2	Pt.3	Pt.4	Pt.5	Pt.6	Pt.7	Pt.8
Day 1	IV	IV	I	I	II	I	I	I
Day 8	IV	IV	I	I	II	I	I	I
Day 90	IV	IV	III	III	III	III	II	III
Day 180	ND	IV	III	III	ND	ND	ND	III

ND: No data

In the Ca-polyP MPs group (patients 1 and 2), bone levels were unsuitable for analysis with the Bergland scale. We scored them as grade IV bone level on all assessment days (Table 4). One of the patients could not attend the last follow-up session (day 180). The bone level in the Ca-polyP MPs-BCP group ranged from grade I to III on assessment days 1, 8 and 90. Only three patients could be assessed at day 180; all had grade III bone levels (Table 4).

CT scan evaluation:

As indicated above, the bone levels in the Ca-polyP MPs group could not be analyzed with the Bergland scale. The material had a ground glass appearance (scattered light radiopaque). Since no bone level could be identified, we classified them as grade IV on both day 8 and day 180. Likewise, the CT scans in the Ca-polyP MPs-BCP group showed a differential bone level from grade I to grade III per patient (Table 5). For the

last three patients who could be scanned at day 180, bone levels were found to coincide with those of the OPG, grade III Bergland scale.

Table 5: Bergland scores based on CTs scan.

Bergland scale	Ca-polyP MPs graft		Ca-polyP MPs + BCP					
	Pt.1	**Pt.2**	**Pt.3**	**Pt.4**	**Pt.5**	**Pt.6**	**Pt.7**	**Pt.8**
Day 8	IV	IV	I	II	I	III	I	II
Day 180	Missed follow-up, Covid-19 lockdown	IV	III	III	Missed follow-up, Covid-19 lockdown	Missed follow-up, Covid-19 lockdown	Drop-out	III

Ca-polyP MPs: Calcium polyphosphate microparticles, BCP; Biphasic calcium phosphate

Complications:

No complications were reported intra- or post-operatively in both study groups.

Discussion:

In the current trial, we found that Ca-polyP MPs appear safe: no unusual adverse reactions were reported, such as infection, severe pain, swelling, allergic reaction, or any other local or systemic adverse effects. Regarding the feasibility, the microparticles may need a stable graft material such as BCP for appropriate alveolar reconstruction.

The optimum age for alveolar bone grafting is between 9 -11 years old.[20,22] Since we did not want to enroll children in a safety study with this novel material in clinical practice, we chose only to include older adolescent and adult patients capable of being involved in decision-making. We performed this study in Indonesia because non-operated patients in this age group are difficult to find in Europe.

The main challenge in the Ca-polyP MPs group was handling and applying the material in the alveolar defect. The characteristics of the Ca-polyP MPs can be

determined by the Pi: Ca+2 molar ratio. In our trial, we used a paste-like mixture formed by mixing fine Ca-polyP MPs graft with normal saline as described in the materials and methods. However, the Ca-polyP MPs graft material was easily lost from the surgical sites once it became saturated with blood, making it virtually impossible to maintain a space-occupying scaffold within the alveolar defect. Therefore, we were compelled to conclude that the physical properties of the Ca-polyP MPs used as a stand-alone scaffolding material were insufficient and not feasible. As a result, we had to reduce the number of patients in the Ca-polyP MPs-only group from four to two instead of the four patients originally envisioned in the study protocol. The microparticles were implanted in a subcutaneous pocket, as opposed to a large, poorly contained void such as the alveolar cleft, which may have contributed to their demonstrated effectiveness in bone formation in preclinical studies.[23,24]

Combining the Ca-polyP MPs with BCP considerably improved the consistency, ease of handling, stability of the graft, and clinical outcome. BCP and calcium phosphates have been used as graft material several times in craniofacial surgery. For example, Levitt et al. had already used calcium phosphate in 1969 for this purpose, and calcium phosphates were subsequently used in dental implants, alveolar ridge augmentation, periodontal treatment, and other maxillofacial surgeries. Biphasic calcium phosphate (BCP) has been proven to be biocompatible, and exhibit osteoconductive as well as osteoinductive characteristics in bony defects reconstruction,[16,17,19] and calcium phosphate was also recently applied in alveolar cleft surgery.[25] Based on our results, we recommend that to achieve the feasibility of applying bioactive Ca-polyP MP, it should be combined with a stable carrier such as BCP or bioresorbable polymers to ensure proper reconstructive activity. Likely, special attention should be paid to the sequestration of the polyP on or within the carrier, which we could not be sure about in the current study.

Our study was limited by several aspects, the most severe being the COVID-19 pandemic allowing only four patients to be evaluated after 180 days of follow-up, resulting in a rather short postoperative follow-up period. Another limitation was the rather radiolucent characteristic of the Ca-polyP MPs, which considerably hampered visualization of the graft in radiographic images and made evaluation with the Bergland scale virtually impossible. We also tried the Chelsea scale[26], which analyzes the bone

position in relation to the adjacent teeth on the grafting site radiographically. However, this did not result in any additional outcomes, so we omitted these results. Therefore, we cannot say with certainty whether the defect filling was sufficient and whether initial bone regeneration events occurred. However, at least no solid bone formation was observed after three months, nor in the one patient evaluated after six months. Lastly, excluding prepubescent children from our study and including only adolescents and adults may have affected the treatment's efficacy. Bone formation activity typically reaches its highest point during puberty; consequently, our post-pubescent patient population may have diminished bone formation capacity. In addition, most cleft defects in our patients were large, reducing the likelihood of successful bone regeneration.

This is the first clinical trial to investigate the safety and feasibility of polyP in humans, either as Ca-polyP MPs alone or in combination with BCP. Due to the COVID-19 restrictions in Indonesia, which significantly impeded osteoinductivity assessment, a histological examination of the bone at six months was not performed. Now, this aspect could be evaluated based solely on the radiographic findings.

Despite this limitation, since we have now conducted video/telephone calls at 1 year post-operatively and all patients reported no adverse events and satisfaction with the treatment, this suggests that the treatment with polyP-containing grafts may be safe and that the combination with BCP appears feasible for alveolar cleft repair. However, new studies with a larger group of patients, biopsies, and formulations of polyP containing appropriate carriers such as BCPs or polymeric scaffold materials are required for sound conclusions regarding their regenerative capabilities. The eruption of teeth through the site, the periodontal and health of the root surface of adjacent teeth, and the orthodontic movement of adjacent teeth to the grafted site must also be considered.

Conclusions

We concluded that Ca-polyP MPs and the Ca-polyP MP/BCP composites appear to be safe graft materials despite the small sample size and some missing data points resulting from the COVID-19 pandemic. However, Ca-polyP MPs alone may not be stable enough to be used in alveolar cleft repair as defect-filling scaffolds.

List of abbreviations

PolyP: Polyphosphate
Ca-polyP MPs: Calcium polyP microparticles
BCP: biphasic calcium phosphate
CLP: Cleft lip and palate
ABG: Alveolar bone grafting
HA: Hydroxyapatite

Declarations

Ethical Approval and Consent to participate

The trial protocol has been evaluated by the Ethical Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia has approved the trial with code number 1063/UN4.6.4.5.31/PP36/2019.

Consent for publication

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Availability of supporting data

Availability of data and materials: Original data is stored securely within the Hasanuddin University, Makassar, Indonesia. Scored data and output for analyses are available upon request from the study.

Competing interests

This article is free of conflict of interest, and no funding was received.

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WEGM and HCS are owners of NanotecMARIN GmbH, Mainz, Germany, and the provider of the polyP graft materials for the clinical trial. SAA received a scholarship from the Ministry of Health of the UAE. DSNK received a scholarship from the LPDP of Indonesia. The remaining authors declare that the research was conducted without any commercial or financial relationships that could affect the study result or any other potential of interest.

Authors' contributions

Alkaabi SA & Natsir Kalla DS: Main author and Conceptualization and Writing.:

Ruslin M: Correspondance.

Alsabri GA & Jansen NA: Reviewer and editing.

Ruslin M, Fauzi A & Tajrin A: Surgical procedures.

Müller WEG, Schröder HC & Wang XG: PolyP Inventor.

Forouzanfar T& Helder MN: Methodology and supervision.

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Müller WEG, Schröder HC & Wang XG: Institut für Physiologische Chemie, Angewandte Molekularbiologie, Universitätsmedizin, Johannes Gutenberg-Universität Mainz, Mainz, Germany.

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Chapter 7

Influence of patient-related factors on intraoperative blood loss during double opposing Z-plasty Furlow palatoplasty and buccal fat pad coverage: A prospective study

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Influence of patient-related factors on intraoperative blood loss during double opposing Z-plasty Furlow palatoplasty and buccal fat pad coverage: A prospective study.

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Abstract

Objective: Surgical procedures, including palatoplasty, have a risk for complications. This study investigated intraoperative and early postoperative blood loss using the buccal fat pad (BFP) during cleft lip and cleft palate (CL/P) surgery.

Material and methods: This prospective study included 109 patients with cleft palate (CP) during three months of treatment at Hasanuddin University Dental Hospital (permanent center) and charity trips in rural parts of Eastern Indonesia. All patients were treated with the DOZ Furlow technique and a BFP graft. Before and after surgery, the total amount of intraoperative blood loss was calculated by measuring the weight differences of the gauze swabs used to control the surgical bleeding, followed by a complete blood count at three days postoperatively.

Results: The difference in the amount of blood loss based on age categories in charity groups was found to be significant ($P<0.05$). We found that high body weight and operation time significantly contributed to increased blood loss ($P<0.05$).

Conclusion: Weight and operative time can contribute to more blood loss during palatoplasty.

Keywords: Buccal fat pad, complication, cleft lip, cleft palate, palatoplasty.

Introduction

Indonesia has many CL/P cases, some left untreated, specifically those living in rural areas [1,2]. In developing and low-middle-income countries (LMC), charity events are a common model for providing cleft treatment in remote sites [3]. However, there are sometimes many barriers in presenting treatments for patients in this area, including lack of safe operating facilities, lack of equipment, lack of well-trained surgeons, and lack of associated specialists and anesthesia providers who can undertake the surgical treatments [4]. Consequently, these various shortages cause the surgical capacity in remote areas to be generally inadequate [3].

During CL/P repair, the surgery is usually carried out when the patients are within the first 12 months of life [5]. At this age, patients with a body weight between 5 kg and 10 kg and a blood volume between 400 mL and 700 mL can have more blood loss, which should be taken seriously [5,6]. Furthermore, when patients present at an older age, complex cleft may also result in a higher risk of bleeding [5]. This patient's characteristic was, in fact, commonly presented among patients with CL/P in Indonesia due to the limited healthcare setting that contributed to the treatment delay [2].

Although the topic of blood loss during palatoplasty is much discussed in the literature, there is not much consensus between these articles [5,7]. A study discussing the relationship between intraoperative blood loss and patient-related factors is lacking in the literature. This study aims to identify the factors that influence the amount of blood loss during palatoplasty using the DOZ Furlow technique in conjunction with BFP as graft material.

Methods and Materials

Study design and patient recruitment

This prospective study was approved by the ethical committee of Hasanuddin University Makassar, Indonesia. Informed consent was obtained from candidates and/or their parents or legal guardians willing to join the study after being fully educated about the procedure. Patient data were collected for three months (March-May 2017) of five charity trips to different regions in Eastern Indonesia and at Hasanuddin University Dental Hospital. The team of the charity trips consisted of oral and maxillofacial surgeons, anesthesiologists, surgical assistants, general dentists, and medical or dental students. Hasanuddin University Dental Hospital is a secondary referral hospital staffed by a team of multidisciplinary cares.

Sample size determination

The number of patients in the hospital group was then adjusted in accordance with the number of patients in the charity group. This was because charity surgeries were performed in confined settings, making them less adaptable than hospital procedures.

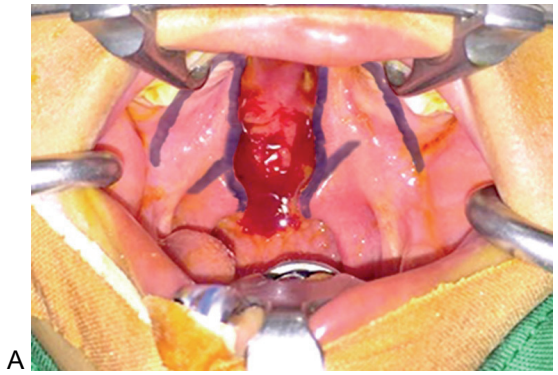
Study Inclusions and Exclusions

Before beginning this study, some inclusion and exclusion criteria were established: patients with cleft palate requiring primary palatoplasty were included, whereas patients with syndromic clefts, fistula after palatoplasty requiring reconstruction, cleft after trauma, patients with multiple syndromes, and patients with a family history of blood loss conditions were excluded. In addition, procedures using techniques other than the DOZ Furlow technique combined with BFP graft to close palatal clefts were excluded.

Operation technique

Patients were administered general anesthesia and prepared for surgery. The patients were injected with a local anesthetic solution of 2-5cc lidocaine 2% with epinephrine 1:100.000 alongside the incision line [8]. The first incision was made five minutes later, and the operative time was measured from that moment onward. Figure 1 depicts the operation procedure for cleft palate closure. Initially, the oral flaps were created by making an incision along the cleft margin, continuing into a relaxing incision along the processus alveolar, and terminating posteriorly at the hamular processes of the palatinal bone [9,10] (Figure 1a).

With a raspatorium, the mucosa was lifted off the hard palate and elevated with a thick suture; these mucoperiosteal flaps were used to close the hard palatal defect. To close the nasal cleft, the surgeon would make a mirrored DOZ-plasty: the nasal mucosal flap anteriorly and the nasal myomucosal flap posteriorly (Figure 1b). The DOZ flaps on the nasal side were transposed and inset. Then the oral flaps were transposed and closed at the midline [9,11]. To prevent scarring and post-operative complications, the surgeons transplanted the BFP into the open, relaxing incisions [12,13] (Figure 1c).



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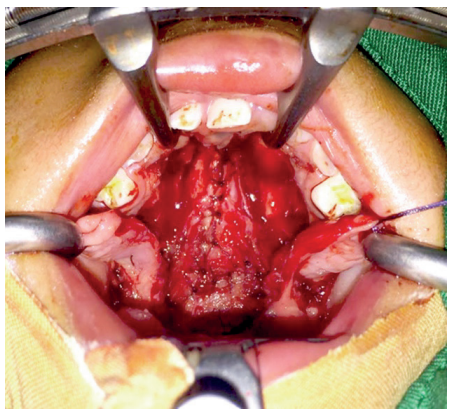

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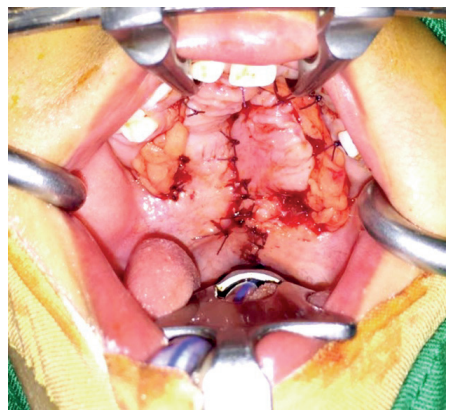

C

Figure 1. Operation procedure: (a) Flap design; (b) Bisection of mucosal and muscle layers, then suturing the nasal mucosa lining with the z-plasty technique; (c) Suturing the oral mucosal and placing the BFP towards the defect area with the interrupt technique.

Data collection

The intraoperative surgical form was completed with the following data recorded: width of cleft, type of cleft, technique used, and operative time. The patients' ages were categorized into four groups according to the previous study with some adjustments: young child (<6 years), child (6-12 years), adolescent (12-18 years), and adult (>18 years) [14]. The intraoperative width of the cleft was measured using a Castroviejo caliper [15], and the type of cleft was noted using the Veau classification [16].

Before the surgery, a blood count was performed as a preoperative screening method. During the surgery, intraoperative blood loss was calculated by measuring the difference in weight of the gauze swabs before and after surgery. The blood-soaked gauze swabs were collected in a metal container lined with a plastic bag to prevent vaporization. After surgery, the swabs would be weighed on an electrical analytical balance (PT. Kenko Electric Indonesia), and the results would be noted on the intraoperative form. The patients got admitted to the hospital postoperatively for three days. On the third day of their stay, another blood count would be taken from each patient to analyze any relationship between the amount of blood loss and values.

Statistical Analysis

The database was created on Microsoft Excel for Mac 2011 version 14.1.0. The statistical analysis was performed using IBM SPSS Statistics 24. In order to identify the difference in blood loss between charity trips and permanent hospitals, a nonparametric Mann-Whitney test was used. Nonparametric Kruskal Wallis was done to find the difference between the amount of blood loss from the two groups based on age category and cleft type. The linear regression with the backwards method was used to evaluate a potential relationship between the amount of blood loss and the numeric and categorical variables. P-values <0.05 were considered statistically significant.

Ethical Consideration

The Health Research Ethics Committee of Hasanuddin University's Faculty of Medicine in Makassar, Indonesia, granted ethical approval (approval number: UH14060319). Due to the average age of the patients, their parents or legal guardians were required to sign the consent form for the study.

Results

The estimations of blood loss were made on a total of 109 patients. As seen in Table 1, a total of 50 patients (29 male and 21 female) were treated during charity trips (group 1), and 59 patients (26 male and 33 female) were treated in the permanent hospital (group 2). The mean age of patients in group 1 was 90.52 months (about seven years), while the mean age of patients in group 2 was 118 months (about nine years). The mean weight of the 50 cases was 22.94 kg, and the mean weight of the 59 cases was

21.96 kg. The mean operation time in group 1 was 90.93 minutes (SD 38.72), and 94.48 minutes (SD 26.37) in group 2. No significant differences were found between the hemoglobin and hematocrit values before and three days after surgery.

Table 1. Patient characteristics in the pre-and post-operation period.

Variables	Group 1 (N=50)		Group 2 (N=59)	
	Mean	SD	Mean	SD
Age (months)	90.52	89.69	118	74.16
Weight (Kg)	22.94	18.37	21.96	13.68
Preoperation:				
- **HGB (g/dL)**	12.39	1.52	12.91	1.58
- **HCT (%)**	37.91	3.90	37.97	3.48
Operation time (min)	90.93	38.72	94.48	26.37
Postoperation:				
- **HGB (g/dL)**	10.79	1.48	10.98	1.36
- **HCT (%)**	32.98	4.32	32.77	4.12
Reduced:				
- **HGB (g/dL)**	1.59	1.00	1.93	5.20
- **HCT (%)**	4.93	3.73	5.20	3.51

Group 1: Charity Trips; Group 2: Permanent Hospital

Table 2 compares the total amount of blood loss between patients treated during charity trips and permanent hospitals. It was seen that the measured blood loss differed much between patients. Even though the mean blood loss was lower in group 1 (98.69 mL) compared to group 2 (106.39 mL), there is no significant difference in the amount of blood loss between the two groups (P >0.05). Of the total included patients, only one patient who was treated during charity trips suffered a postoperative complication, i.e., active bleeding. On further inspection, it was due to a ruptured suture. None of the 109 patients needed any blood transfusion during or after surgery. Furthermore, wound closure was performed in all surgeries using a BFP graft. No other techniques or additional relaxing incisions were used.

Table 2. Comparison of the total amount of blood loss between the two groups.

Area	N	Mean	SD	Mean Difference	95% Confidence Interval		P-value
					Lower	Upper	
Group 1	50	98.687	70.063	-7.706	-35.437	20.025	0.549
Group 2	59	106.394	75.835				

*Mann Whitney test; *P-value* <0.05 is statistically significant

Group 1: Charity Trips; Group 2: Permanent Hospital

The results of the Kruskal-Wallis test are presented in Table 3, which compares the amount of bleeding between each age group and cleft type. In group 1, the difference between age categories was statistically significant (P <0.05). Thus, Pairwise Comparisons were conducted using superscript code to investigate the partial difference in this category. According to the analysis, there is a significant difference in the amount of blood loss between age groups of patients treated on charity trips. Compared to the child, adolescent, and adult age groups, the young child age group experiences significantly less blood loss.

Table 3. Difference between age categories and cleft types based on the total amount of blood loss (mL) from two groups.

Area	Variables	Categories	N	Mean	SD	P-value
Group 1	Age	Young Child	28	71.291[b]	42.916	0.006*
		Child	9	117.169[a]	57.621	
		Adolescent	8	162.256[a]	119.804	
		Adult	5	117.128[a]	37.598	
	Type of cleft	Type 1	6	72.467[a]	49.225	0.654
		Type 2	17	90.570[a]	55.005	
		Type 3	15	112.004[a]	76.350	
		Type 4	12	106.651[a]	90.320	
Group 2	Age	Young Child	16	100.769[a]	78.319	0.087
		Child	25	95.786[a]	55.974	
		Adolescent	11	89.502[a]	53.052	
		Adult	7	183.680[a]	122.430	
	Type of cleft	Type 1	11	100.510[a]	68.200	0.505
		Type 2	25	124.608[a]	91.538	
		Type 3	18	83.863[a]	52.699	
		Type 4	5	109.374[a]	72.237	

* Kruskal Wallis test; p-value <0.05 is statistically significant

A linear regression test has been performed to analyze patient-related factors that may influence intraoperative bleeding during palatoplasty procedures in 109 subjects. In this study, the independent variables are age, gender, weight, the width of the gap, type of cleft, operation time, and surgery location. From Table 4, it can be deduced that weight and operation time have significant effects (P<0.05). This implies that the higher the weight and the longer the operation time, the more at risk the patients are for increased blood loss during the palatoplasty procedure.

Table 4. Regression linear test between variables and the amount of blood loss.

	β	SE	P-value
Width of gap (mm)	-0.006	1.909	0.954
Gender	0.007	13.857	0.941
Age (month)	-0.020	0.169	0.920
Type of cleft	-0.015	7.384	0.874
Location of surgery	0.058	13.228	0.525
Operation time (minutes)	0.199	0.203	0.029*
Weight (kg)	0.306	0.400	0.001*

*P-value <0.05 is statistically significant

Discussion

This study aimed to identify patient-related factors that affect the amount of blood loss during palatoplasty using the DOZ Furlow technique in combination with BFP. To the best of our knowledge, previous studies have only discussed the amount of blood loss that can be expected during DOZ Furlow palatoplasty [5,17].

One hundred and nine CL/P patients were included in this study. Patients' mean ages were 90.52 months (±7 years) and 118 months (±9 years) for charity trips and permanent hospital groups, respectively. In the previous study by Katzel et al. (2009), palatoplasty is performed in patients aged between 6 and 12 months, resulting in satisfying outcomes [18]. This study includes different age ranges because it was performed in a developing country with a poorly developed healthcare system, causing late diagnosis in patients [2,19,20].

Furthermore, this problem is more difficult because those living in rural areas do usually not have money to finance the surgery and extra travelling costs to where the treatment is provided [21-23]. Adeyemo et al. also found that the reasons for late CL/P repair, specifically among rural populations, were lack of awareness of treatment availability (13.3%), lack of health care services nearby (18.4%), and lack of money (56.7%) [20]. This condition seems consistent with the condition in rural areas in

Indonesia; thus, patients have to wait for charity surgery at a nearby village/city, further delaying the CL/P treatment [2]. In contrast, it is a routine procedure in Western countries to prepare pre-and postnatal plans for infants with CL/P [18,24]. Early counseling and treatment planning for CL/P patients were shown to have a better outcome in some aspects, such as speech, cosmetic, and psychological perspectives [25,26].

In the present study, the mean blood loss was 98.69 mL and 106.39 mL for the charity trips and permanent hospital groups, respectively. These were relatively higher than previously published studies [5,17]. Kim et al. (2018) reported a mean blood loss of 16.61 mL. However, their patients had lower mean age, lower mean weight, less severe mean cleft type, and smaller mean palatal gap than the patients in our study [5]. We also found that older children (8–9 years) were at high risk for increased intraoperative blood loss during palatoplasty, especially those performed in rural areas. We must consider that the palate is a highly vascularized structure in the mouth with multiple vessels alongside its width and length that needs to be handled properly [27]. Therefore, early assessments and surgical interventions for patients with CL/P are highly recommended.

Intraoperative time seemed to correlate with blood loss in the present study. Longer operative time will result in a higher amount of intraoperative blood loss. One contributing factor might be that a team of surgeons performed the surgeries. Not all of them were experienced in performing palatoplasty using the DOZ technique with BFP graft. Nevertheless, no blood transfusions were deemed necessary for any of the patients because the pre-operative screening was performed according to the Practice Guidelines for perioperative blood management (2015) as a predictor of perioperative blood loss, risk of transfusion, or other adverse events associated with transfusion [28]. During this screening, the anesthesiologist looked at the blood values and excluded patients with any blood condition present. High amounts of blood loss were also not expected during surgeries because of the procedure type [5,17]. The relatively low intraoperative blood loss, as determined in this study, showed that DOZ palatoplasty is a relatively safe procedure.

This study has several limitations. First, the small sample size prohibited adjudication

of the found statistical significance. Secondly, a difficult point of this study was to measure blood loss as accurately as possible. A previous study by Daabiss et al. measured blood loss using a visual comparative colorimeter [29] which is not applicable in the limited clinical setting of rural areas in Indonesia. In addition, this method is particularly suitable for large amounts of blood loss, unlike the expected low blood loss from palatoplasty procedures. This study measured blood loss using the weight difference between clean and blood-soaked gauze swabs.

The average density of human blood is known to be close to pure water, so weight in mg can be converted into mL [5,17]. Due to technical factors such as a surgeon accidentally using the suction system or the inability to measure the amount of blood loss left on instruments, gloves, and surgical drapes, this method may still underestimate the amount of blood loss. Additionally, postoperative blood loss was not measured because the clinical setting did not permit it. Despite this, we do not believe the final point was detrimental to the study, as our focus was on intraoperative blood loss during palatoplasty.

Conclusion

Our findings indicate that combining DOZ Furlow palatoplasty and BFP graft is a relatively safe procedure. This study found that the procedure resulted in minimal to moderate blood loss but that a patient's weight and the length of the operation significantly increased the amount of blood lost. The first suggestion made by this study is to operate on patients at a younger age. Not only does operating on young children reduce intraoperative blood loss, but it also gives the patients a better start in life. Our second recommendation is to reduce the duration of the operation as much as possible, as this will significantly reduce intraoperative blood loss. In addition, standardized postnatal holistic planning is recommended for the improvement of cleft care in Indonesia.

Conflict of interest

No conflicting relationship exists for all authors.

Ethics approval

Ethical approval was granted by Hasanuddin University Health Research and Ethics

Committee (approval number: UH14060319) in accordance with the Declaration of Helsinki.

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None declared.

Authors' contributions

Diandra Sabrina Natsir Kalla and Muhammad Ruslin: Conceptualization, Methodology, Writing-Reviewing and Editing. **SA Alkaabi:** Data curation, Investigation, Writing-Original draft preparation. **Andi Sitti Hajrah Yusuf:** Software, Investigation, Writing-Original draft preparation and Visualization. **Andi Tajrin:** Data curation. **Siswanto and Hedi Kuswanto:** Data curation, software, investigation, visualization. **Tymour Forouzanfar, Paolo Boffano and Lun-Jou** Lo**:** Writing-Reviewing and Editing.

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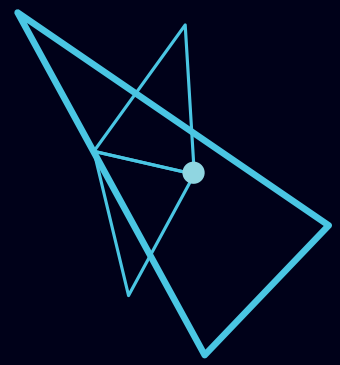
Chapter 8

Microfragmented fat (MFAT) and BCP for alveolar cleft repair: A prospective clinical trial protocol



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Microfragmented fat (MFAT) and BCP for alveolar cleft repair:
A prospective clinical trial protocol

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Abstract

Background

In alveolar cleft reconstructions, inorganic bone substitutes such as osteoconductive biphasic calcium phosphate (BCP) may serve as alternatives for iliac crest-derived autograft bone since the latter are associated with chronic pain and donor site morbidity. To add osteoinductivity, the locally harvested buccal fat pad will be mechanically fractionated to generate microfragmented fat (MFAT), having high regenerative capacity due to high pericyte and MSC content and a preserved perivascular niche. This study aims to evaluate the feasibility and safety of the BCP-MFAT combination.

Methods

This prospective non-blinded first-in-human clinical study will include eight alveolar cleft patients. MFAT will be prepared intraoperatively from the patient's buccal fat pad. Radiographic imaging will be performed before surgery and at regular intervals after placement of the BCP-MFAT combination in the alveolar cleft. Similarly, regular blood tests and physical examinations will be conducted. Radiographic imaging will be used for clinical evaluation, while biopsies obtained after six months with a trephine drill used to prepare the implantation site will be assessed with histological and histomorphometric analyses.

Discussion

In this first-in-human study, not only safety but also the regenerative potential of the BCP-MFAT combination will be evaluated in the alveolar cleft model.

Ethics, trial registration and dissemination

The clinical trial protocol was approved by the ethical committee of Hassanudin University-Makassar, Indonesia [protocol number 1063/UN4.6.4.5.31/PP36/2019] and registered in the Indonesian trial registry [INA-EW74C1N].

Regardless of the trial outcomes, the results on safety, feasibility and bone formation efficacy from the BCP-MFAT combination will be published.

Keywords

Microfragmented fat, calcium phosphate, bone regeneration, regenerative medicine, alveolar bone grafting

Introduction

An alveolar cleft is defined as a bone gap in the primary palate from the nasal sill to the incisive foramen [1]. The defect occurs due to disruption of primary palate development between 4 to 12 weeks of gestational age, specifically in the frontonasal prominence [2]. The treatment protocol varies based on the following factors: timing, surgical procedure, and grafting material. Secondary alveolar bone grafting (SABG) is the most preferred and successful method that is done during the mixed dentition period (6-11 years), which allows support teeth eruption and facial growth [1]. Iliac crest as a bone graft donor for alveolar cleft reconstruction has gained popularity since it was first introduced by Schmid in 1954 [3], and in particular for SABG procedures because it allows harvesting of large amounts of bone for alveolar cleft surgery [4].

Other bone graft sources include the cranium, tibia, and mandibular symphysis [5]. However, several studies have reported risks of general postoperative complications using autografts, such as pain, prolonged hospital stay, and donor site-specific complications such as scarring, cutaneous nerve injury near the iliac crest and hematoma after harvesting the cranial bone [6-9]. Therefore, alternative materials have been studied for alveolar cleft surgery.

Biphasic calcium phosphate (BCP) is a bioceramic that consists of two materials, hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP), mixed in different ratios [10]. It is a biocompatible, easy-to-handle, safe, and osteoinductively proven material with similar mineral compositions to human bone tissue [10]. BCP has been mixed in vivo and in vitro with autografts, inducing factors, and/or cells to improve osteoinductivity [11,12]. Studies have reported its applications in the field of dentistry and maxillofacial surgery [13-15]. Although calcium phosphate ceramic is not yet considered the standard of care, it has been used for alveolar cleft reconstruction in human models with satisfactory bone results acquired [16] and even enabled to support teeth eruption [17].

Adipose tissue is one of the mesenchymal stem cells (MSC) sources, and adipose stem cells (ASCs) can be collected with minimum risk and discomfort from the buccal fat pad (BFP) [18]. The BFP is surrounded by the buccinators muscle and other superficial muscles such as the masseter, the zygomaticus major, and the zygomaticus minor [19]. Moreover, multiple studies have shown that the cell yield of

ASCs per volume is at least 100-500 times higher than that of MSCs in bone marrow aspirates [18,20]. Commonly, ASCs are prepared using enzymatic (collagenase) digestion which, however, is considered as “more than minimally manipulation” of the cells by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [21].

An alternative method that takes considerably less time is processing the adipose tissue mechanically into MFAT [22]. MFAT is reported to have similar or even higher secretory activity of regenerative growth factors, cytokines, and pericyte content compared to an enzymatically derived stromal vascular fraction (SVF) [23]. In addition, the MFAT procedure can be used in regular hospitals because its harvesting and processing do not require major invasive surgery, expensive disposables, or Good Manufacturing Practice (GMP)-compliant cell culture expansion. The autologous application of MFAT has been utilized successfully for maxillofacial reconstructions [24].

Therefore, using BCP mixed with MFAT for alveolar cleft reconstruction, we describe the first-in-human clinical safety trial protocol. Since the osteoconductive BCP is supplemented by the regenerative capacity of the MFAT, we hypothesize that the combination will be a safe, efficient, and effective alternative to conventional autograft.

Materials and Methods

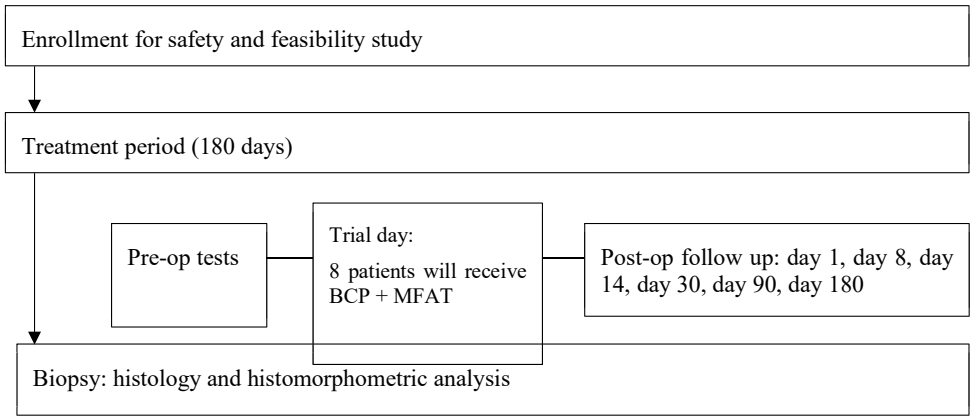
Study Design

According to the IDEAL framework, this first-in-human surgical study can be classified as a “stage 1” study [25]. It is a single-center prospective clinical trial comprising eight patients, assessing the safety of a combination of MFAT and biphasic calcium phosphate (BCP; BoneCeramic™, Straumann®, Switzerland)) as bone graft material for alveolar cleft reconstruction. The BCP is a synthetic bone graft with the following characteristics: contains 60 % hydroxyapatite (HA) and 40 % β-tricalcium phosphate (β-TCP); a porosity of 90%; and a 100–500 µm interconnected pore size. The BCP will be combined in a 1g:1cc ratio with MFAT prepared from the patient’s buccal fat pad (BFP) processed with Tulip Gen II Nanofat™ Kit single-use sizing transfer 1.2 mm (Tulip Medical, California, United States).

The primary endpoint will be set at six months. AEs and/or SAEs will be documented at each follow-up visit, and clinical assessments will be performed at time points specified in the Intervention section (below). After these six months, a bone biopsy will be taken using a hollow drill during dental implant preparation and processed for histological/histomorphometric analysis (see below). Finally, a report on safety and proof of concept with regard to bone formation will be made and published.

Fig. 1 Simplified diagram of the study protocol.

Figure 1. Simplified diagram of the study protocol (adapted from SPIRIT figure).



Inclusion and exclusion criteria

Patients will be included based on the following criteria: [26]

1. Healthy male or female, age ≥ 15 years old
2. Have unilateral alveolar cleft without any history of grafting procedure(s) previously
3. Categorized as ASA1 for anesthetic risk and having a normal blood count

Patients will be excluded based on the following criteria:[26]

1. Have poor oral hygiene with mouth plaque
2. Have systemic disease
3. Have a systemic or local infection
4. Have received chemotherapy, radiotherapy, immunosuppressives, or anticoagulants that may interfere with the healing process
5. Have received bone growth-inducing factors, malnutrition, or active influenza
6. Pregnancy

Interventions

Under general anesthesia and infiltration with lidocaine (1%) with 1:100,000 epinephrine, the surgeon will identify the Stensen's duct with a lacrimal probe and make an incision 2-3 cm below the duct [27]. A dissection penetrating the muscles and

the superficial fascial will allow spontaneous herniation of the fat pad [27]. This procedure will be done bilaterally on both cheeks to obtain approximately 3cc fat. After vasoconstrictor infiltration with adrenaline 1:100,000, an incision will be made to create palatal flaps at the cleft margins. These flaps will then be elevated from the palate. After exposure of the full alveolar cleft, the nasal floor is reconstructed, and the palatal flaps are sutured to create a soft-tissue pocket [4].

At the same time, the harvested fat will be chopped into small pieces with a scissor and soaked in normal saline for 15 minutes. The normal saline will be drained, and the chopped fat will be processed into MFAT using two syringes (size 10cc) connected with the Tulip Gen II Nanofat™ Kit single-use sizing transfer 1.2 mm (Tulip Medical, California, United States) according to its protocol. MFAT will be mixed with BCP (Straumann Bone Ceramic, Villeret, Switzerland) in a ratio of 1cc:1g respectively until it reaches a homogenous consistency. The mixture will be placed in the alveolar cleft defect as a graft material. If the defect is large and requires additional bone grafts, another mixture with the same mixing ratio will be created. A membrane will be used to cover the grafted defect if necessary. Finally, the defect will be closed with absorbable 3/0 vicryl sutures for the mucosa and 4/0 vicryl sutures for nasal reconstruction. All postoperative patients will be prescribed antibiotics and painkillers.

Table 1. Patients' assessment table

	Consent form	OPT	CBCT or CT	Physical examination	CBC	Thermometer	Biopsy
Pre-operative	x	x	x	x	x	x	
Operative day				x		x	
Post-op day 1		x		x	x	x	
Post-op day 8		x	x	x	x	x	
Post-op day 14				x		x	
Post-op day 30				x	x	x	
Post-op day 90		x		x		x	
Post-op day 180		x	x	x	x	x	x

OPT: Orthopantomogram; CBCT: Cone-beam computed tomography; CT: Computed tomography; CBC: Complete blood count; Post-op, post-operative

Adverse Events Assessment

Any change in subjects' health will be documented in their medical history, and required medical care will be given. Any unexpected physical and laboratory change, symptom, or disease in a treated patient who has been administered the graft will be documented as an adverse event (AE). An adverse event will be graded according to World Health Organisation (WHO) Classification [28] as serious or non-serious based on intensity. The Clavien-Dindo Classification of Surgical Complications will also be used in case of any incidence [29]. In the case of a serious adverse event (SAE), a report will be made to the sponsor within 24 hours and to the ethical committee within three days from the onset date. The trial will be terminated immediately if the SAE concerns severe toxicity or infection associated with graft products.

Sample size

This is a first-in-human phase I clinical trial aimed at obtaining insight into the safety and proof of concept of the treatment with the BCP-MFAT combination. We assume that no SAEs or AEs will occur based on clinical experience with other applications of MFAT and the well-proven safety of BCP. Upon consultation with a statistician, an n = 8 is expected to be sufficient for this trial.

Recruitment

Patients will be recruited from an existing database maintained by the Hasanuddin University Dental Hospital. After thoroughly explaining the procedure, candidates and their parents or legal guardians willing to participate in the trial will provide informed consent. Then, the candidates' inclusion and exclusion criteria will be determined. Before the clinical trial, the ethical and surgical teams will conduct a comprehensive assessment and training regarding the safety measures at the Hasanuddin University Dental Hospital research site.

Randomization and blinding

Since this trial comprises only one type of treatment, no randomization or blinding to the treatment is possible.

Data collection and access

The research team will be informed about the rules and their responsibilities. All research team members who will collect the data according to the evaluation table (Table 1) will receive training on how this collection should be performed. The data manager will document the data in a patient-coded manner (i.e., each patient will get a study-specific code under which the data will be stored to conceal the patients' identity), which will subsequently be handed over to the clinical evaluators and investigators. Each patient will be followed up for up to 6 months.

Post-trial care

After the participants exit the trial, they will be followed up for an additional three years to ensure their safety and to record whether any delayed side effects will occur due to the BCP-MFAT treatment [26].

Monitoring

Internal monitors of the Ethics and Research Committee of the Faculty of Medicine, Hasanuddin University, will evaluate whether data collection is done accurately. A data safety monitoring board is not installed since a negligible risk for the patient is expected as both materials (MFAT and BCP) have been tested in other clinical trials [16,17,24]. A safety report will be submitted annually to the Medical Research Ethics Committee of the Faculty of Medicine, Hasanuddin University. No interim analysis is deemed necessary.

Amendments

Amendments to the current protocol will be submitted to the ethical committee and competent authority if necessary. They should be approved before implementation to ensure the safety and integrity of participants and the scientific value of the trial.

Evaluation methods

Safety assessment based on physical examination and laboratory measurements

When an SAE occurs, it will be concluded that the combination of MFAT and BCP is not (yet) safe in the current setting. If AEs do not occur at a higher frequency than in patients treated with standard care (autologous bone) and/or can be resolved by non-invasive conventional methods (e.g., analgesics, antibiotics), the combination of MFAT and BCP will be considered safe. In all other cases, the combination of MFAT and BCP will not be considered safe (yet).

Radiographic analysis

The Chelsea scale will be utilized to assess the success rate of the bone graft. The scale grades the position of the cleft's bone rim in relation to the adjacent teeth. To begin the evaluation, an imaginary midline is drawn between the teeth on either side of the cleft location. Each of these teeth (mesial and distal roots) will be divided into four sections beginning at the cemento-enamel junction and ending at the root apex. When there is no bone up to the midline, the score is 0; when a bone does not reach the midline, the score is 0.5; and when the bone extends from the root surface to the midline, the score is 1 [30].

Histological and histomorphometric analysis

According to previously published procedures, the histological and histomorphometric analysis will be performed in at least 3 patients who received dental implants after alveolar cleft reconstruction [31]. The implant preparation site will be made using a trephine burr (⌀ 2.0 mm × 10.0 mm in length) that allows biopsy collection from the implant site without interfering with the regular procedure. The biopsies will be fixed in 4% phosphate-buffered formaldehyde, dehydrated in ascending series of ethanol, and embedded in 80% methylmethacrylate (BDH Chemicals) supplemented with 20% dibuthylphtalate (Merck), 8 g/L lucidol CH-50 L (Akzo Nobel) and 22 µL/10 mL N, N-dimethyl-p-toluidine (Merck).

The biopsies will be cut into 5-micrometer thick sections, and two different stainings (Goldner's trichrome and Tartrate Resistant Acid Phosphatase (TRAP)) will be performed. Several histomorphometric parameters will also be determined for quantitative analysis [31]. Two trained examiners will do the histologic and histomorphometric analysis. The biopsies will be re-analyzed in case of dispute to reach a consensus.

Statistical analysis

Since this is a single-arm safety study, statistical analyses will not be performed.

Discussion

There has been a growing interest in using adipose tissue to reconstruct cleft lip and palate [32]. Its applicability is largely dependent on the amount of tissue, the ease of surgical harvesting, and the type of surgical reconstruction in which the tissue is used, such as correction of cleft lip volume asymmetry [33,34], improvement of velopharyngeal insufficiency after cleft lip and palate repair [35,36], or as an additional flap in cleft palate repair [37-39]. In this study, the BFP will be used to reconstruct bone. BFP is a specialized adipose tissue with a high vascular supply that is easy to harvest via the oral cavity with minimal morbidity and discomfort [40].

A phase I clinical trial by Khojasteh et al. [41] and an animal study employing adipose-derived stem cells for alveolar cleft repair [42] are the only reports on the use of adipose tissue as a regenerative compound for bony cleft reconstruction to date. Both studies

obtained adipose stem cells for personalized cleft reconstructions by digesting the tissue with collagenase and expanding them in culture. An alternative is the SVF derived from adipose tissue by collagenase digestion, which requires less time and may yield stem-cell-like quantities, allowing intra-operative applications [43,44].

An earlier clinical study by Prins et al. [43] demonstrated that adding SVF to calcium phosphate ceramics intra-operatively enhanced bone formation, indicating that SVF can impart osteoinductivity when combined with calcium phosphate. However, regulatory concerns and relatively costly SVF production procedures prevent its widespread application [22,23]. Mechanically-processed fat, or MFAT, has emerged as a fast-processing alternative to SVF; it is regarded as minimally-manipulated and, therefore, less regulated [22,23].

This is the first study to evaluate MFAT and biphasic BCP as regenerative grafts for alveolar cleft reconstruction in humans [45,46]. BCP is a ceramic scaffold with a balanced ratio of the less soluble HA to the more soluble TCP, resulting in mechanical and biological properties that support the production of bone and cartilage tissue [47]. It is adequate for reconstructing bone in non-load-bearing applications. It is currently the standard of care for certain maxillofacial reconstructions [48].

Recently, calcium phosphate has been used in alveolar cleft operations [16,17]. In this study, patients were treated between the ages of 9 and 10, which falls within the optimal age range for SABG [1]. However, because we did not wish to enroll children in a safety study involving this novel concept in clinical practice, we decided to include only adolescents and adults capable of making their own decisions. This study will be conducted in Indonesia because it is difficult to find non-operated patients in this age group in Europe.

Declarations

Ethics approval and consent to participate

The clinical trial was approved by the Ethics and Research Committee of Hassanudin University-Makassar, Indonesia, with code number 1063/UN4.6.4.5.31/PP36/2019.

The trial was also registered in the Indonesian Trial registry [INA-EW74C1N]. All participants shall be asked an informed consent to participate.

Consent for publication

All participants shall be asked an informed consent for personal data publication.

Availability of data and materials

Not applicable.

Conflict of interest

None declared.

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Trial Status

Recruitment started in November 2019 and is planned to end in May 2022, with 8 patients randomized. The current protocol is version 1.0, dated 15 September 2019.

Supporting information

S1 Fig. Simplified diagram of the study protocol.

List of abbreviations

Abbreviation	Explanation
MFAT	Microfragmented fat
BCP	Biphasic calcium phosphate
MSCs	Mesenchymal stem cells
SABG	Secondary alveolar bone grafting
HA	Hydroxyapatite
β-TCP	β-tricalcium phosphate
ASCs	Adipose stem cells

FDA	United States Food and Drug Administration
EMA	European Medicines Agency
SVF	Stromal vascular fraction
GMP	Good manufacturing practices
BFP	Buccal fat pad
OPT	Orthopantomogram
CBCT	Cone-beam computed tomography
CT	Computed tomography
CBC	Complete blood count
Post-op	Post-operative
WHO	World Health Organisation
AE	Adverse event
SAE	Serious adverse event
TRAP	Tartrate Resistant Acid Phosphatase

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Chapter 9

General Discussion

General Discussion

Bone tissue engineering (or bone regeneration) using growth factors, scaffolds, and mesenchymal stem cells (MSCs) is a promising technique for inducing new bone formation. Regenerative materials may enhance the activity of differentiated bone cells. These regenerative materials can serve multiple purposes, including promoting cellular recruitment to the scaffold, differentiation of other cells essential for bone regeneration, and inhibition of antagonistic activity (such as osteoclasts).[1]

In addition to reviewing the currently tested regenerative graft materials for maxillary sinus augmentation and alveolar cleft augmentation, the purpose of this thesis was to assess the safety and feasibility of a novel regenerative graft material, Ca-Polyphosphate, in a human alveolar cleft defect model.

Analysis of the state of the art

Although autogenous bone graft is still considered the gold standard for treating alveolar clefts, studies have shown that it can be safely and effectively performed with tissue engineering methods as well. Several studies have shown that this procedure is less invasive than bone grafting. The systematic review papers in this thesis in craniofacial bone models (maxillary sinus floor augmentation (MSFA) and alveolar cleft augmentation (ACA)) were categorized according to the various components of tissue engineering (growth factors, cell therapy and a combination of both).

Today, few studies use regenerative grafts in the ACA model. Therefore, we decided to focus on the MSFA model first. MSFA is a powerful and often used model to test novel graft materials. It allows researchers to take biopsies before dental implant placement and evaluate bone formation using high-resolution μ-CT and histomorphometric analysis. Our review was conducted to evaluate the state-of-the-art in the use of regenerative grafts and to provide an overview of the various types of regenerative graft materials that showed adequate bone formation.

In MSFA, the regenerative materials used were platelet-rich fibrin (PRF), platelet-rich plasma (PRP), recombinant human Growth and Differentiation factor 5 (rhGDF-5), Platelet-derived growth factor (rhPDGF), the bone morphogenetic proteins 2 and 7 (rhBMP-2, rhBMP-7), bone marrow aspirate concentrate (BMAC), and MSCs. Among the wide range of osteo-inducing growth factors, rhBMP-2 has often been used. The impact of these factors is significantly affected by the utilized dose. For example, Froum et al. (2013) showed in their studies that low-dose rhBMP-2 did induce bone formation. However, treatment with higher doses of rhBMP-2 showed better results.[2] On the other side of the spectrum, rhBMP-7 showed a dual negative effect, i.e. slow development of new bone formation and concomitant resorption of new bone was observed.[3] Our results in MSFA showed no significant difference between control grafts and regenerative grafts except in residual bone graft analysis. From our review, it can be deduced that regenerative materials such as PDGF, PRF, and higher concentrations of rhBMP-2 could be beneficial as regenerative agents. In contrast, we feel that the use of rhBMP-7 should be discouraged due to its negative effects on bone formation.

The regenerative graft materials used in alveolar cleft augmentation were the bone morphogenetic proteins 2 (rhBMP-2, platelet-rich fibrin (PRF), platelet-rich plasma (PRP), buccal fat stem cells (BFSCs), bone marrow aspirate concentrate (BMAC), plasma rich growth factor (PRGF) and MSCs. Within these studies, the various regenerative materials, such as PRP, BFSCs and PRF, were mixed with the autogenous bone graft. However, the ACA model did not provide definitive evidence of their additive effects compared to the application of autograft alone. Again, the most studied regenerative graft material was rhBMP-2. In multiple studies, the results of the application of rhBMP-2 were positive or at least similar to autologous bone grafts.

However, rhBMP-2 is costly, overdosing can result in resorption, and underdosing may have no effect. In addition, the conclusions of both reviews should be interpreted with caution because the included studies were frequently improperly conducted and exhibited a high risk of bias, thus preventing the development of sound conclusions regarding the efficacy of the examined regenerative grafts.

New Treatment Modalities

Müller et al. (2018) demonstrated that a novel bioactive compound, polyphosphate (polyP), has strong regenerative capacities and, by providing extracellular energy, may activate repair processes including osteogenic healing and strong angiogenic activity.[4]

PolyP, an inorganic polymer present in the body in a free state in platelets, is a source of metabolic energy by forming ATP as a result of the enzymatic cleavage of the high-energy phosphoanhydride bonds of this polymer. Based on these promising preclinical studies, we designed and conducted a clinical trial employing a human alveolar cleft model.

The protocol was designed to treat adolescent and adult patients legally capable of making decisions regarding their study participation. It excluded children to avoid potential risks for them. The protocol additionally included the possibility of histomorphometric analysis of biopsies taken with a hollow drill prior to the placement of dental implants. The trial was planned to be conducted as a randomized study to determine the optimal mode of treatment for patients with polyP, i.e., either with Ca-polyP-microparticles (Ca-polyP-MPs) alone or in combination with biphasic calcium phosphate (BCP) scaffold. Treatment with osteoconductive calcium phosphate (CaP) scaffold had already been shown to be feasible and suitable for alveolar cleft reconstruction.[5] We aimed to improve efficacy by adding bioactivity to the CaP scaffold.

Due to the complexity of handling the Ca-polyP-MP graft material which we experienced in our trial, we concluded that the treatment with Ca-polyP microparticles only was not feasible in its current form, and we abandoned this treatment arm after two patients. Six patients had reconstructions with the BCP- Ca-polyP-MP mixture. No adverse reactions were observed in both treatment arms. Despite the small sample size and the absence of some data points because of the COVID-19 pandemic, we can conclude that using Ca-polyP MP and BCP composites as graft material for alveolar cleft repair was safe. However, we suggest combining microparticles with stable graft material to allow defect augmentation effectively. Moreover, we should ensure that the BCP scaffold can retain the polyP at the target site, which should be tested in in vitro experiments.

Recently, another proof-of-concept clinical study was reported, i.e., the application of polyP-soaked collagen mats for wound dressing. It was found that the engineered polyP and collagen mats significantly improved the re-epithelialization rate and reduced the wound area. We do not fully understand the discrepancy between their

positive findings and our not-yet-clear efficacy in the alveolar cleft model. However, we speculate that the effect of the mat on the wound dressing was helping the stability of dressing and polyP concentration on the wound site and may also have sequestered the polyP better than our BCP did in our study, as also addressed above.

Conclusion and Perspective:

Although we believe we have demonstrated that the use of Ca-polyP MP alone and in combination with BCP for the repair of alveolar clefts is safe, we must acknowledge that further optimizations are necessary to achieve efficacy. Despite this, we continue to believe that polyP may be an innovative and intriguing technique for (bone) tissue engineering. Numerous preclinical studies on tissue engineering have demonstrated its efficacy. For example, PLGA-polyP combination particles are effective in preclinical studies (Müller et al., 2018) for bone reconstructions, or a combination of polyP with collagen mats has been recently applied to a skin wound healing model (Müller et al., 2018). The latter would resemble the current application of rhBMP-2 in bone TE, which is a collagen membrane. In addition, studies on polyP dosing, sequestration within scaffolds, and release profiling may be necessary to achieve maximum efficacy. These studies can be conducted in part in vitro and in part in bioreactors.

Another possibility would be to first test the efficacy of polyP for bone tissue engineering in a less demanding and more well-established model, such as the maxillary sinus augmentation model, which could provide a better enclosure for the polyP graft and lead to a better understanding of the efficacy of polyP in terms of bone regeneration. Moreover, MSFA provides the opportunity to obtain bone biopsies during the dental implant procedure.

Finally, based on the positive results with the buccal fad flap of chapter 7 and recent literature,[6,7] it appears likely that the BFP may have healing effects and that MSCs preparations derived from the BFP may be used as a bioactive means to stimulate bone regeneration as well. It was previously shown that BFP contains similar amounts of MSCs with multi-differentiation potential and activities as abdominal fat (Farré-Guash et al., 2010).[8] We devised a protocol for the intra-operative and cost-effective use of microfragmented fat (MFAT) for alveolar cleft reconstruction. In a parallel project, this concept will be further explored. We expect that this alternative approach

may also be promising and provide not one but two new intra-operative and cost-effective procedures upon optimization of the MFAT and PolyP regenerative protocols.

We can conclude that rhBMP2 currently seems to be the best studied and best performing tissue engineering inductive compound, but that optimizations as discussed above may improve the polyP and/or MFAT applicability. It may provide potent and cost-effective new alternatives for future bone reconstruction strategies.

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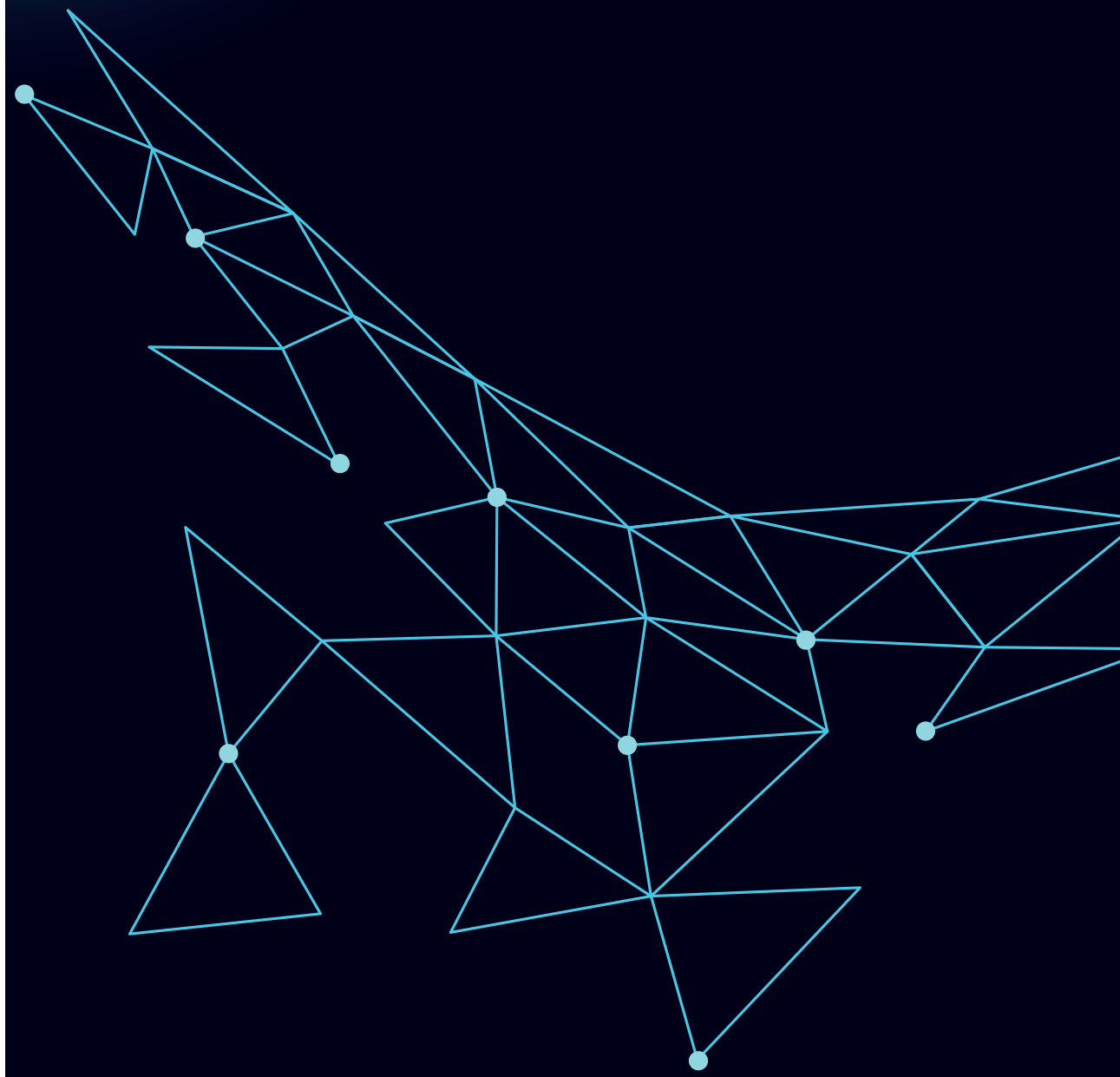
Chapter 10

Summary



Summary

The main objective of this thesis was, firstly, to review the regenerative graft materials tested in maxillary sinus lift augmentation and alveolar cleft grafting, and secondly, to evaluate the safety and feasibility of a novel regenerative graft material, Ca-Polyphosphate, in a human alveolar cleft defect model.

In Chapter 2, we have performed a systematic review of the clinical trials that used regenerative graft materials in the sinus lift augmentation procedure since it is the most common surgical model being used to test regenerative graft materials in the maxillofacial area. As is well known that randomized clinical trials are the gold standard in the evaluation of medical intervention, we performed a meta-analysis and a risk of bias assessment as well. Of the thirty-two studies included in this review, the only significant difference was seen in the residual bone graft volume, whereas no significant differences were found between regenerative grafts and non-regenerative grafts in terms of new bone formation, augmented bone height, soft tissue area, total bone volume, and bone density. Noteworthy, it is important to point out that most of the trials displayed a high risk of bias.

In chapter 3, a systematic review was performed on stem-based tissue engineering treatments in animal cleft models. The objective was to analyze the effectiveness of stem-based tissue engineering in alveolar and palatal clefts in animals. Out of twenty-five trials, 21 were carried out using animal alveolar cleft model. The majority of these studies have used bone marrow mesenchymal stem cells in combination with ceramic grafts. Although the results were not significant between stem cell tissue engineering versus scaffolding or empty grafts, the result was in favor of tissue engineering grafts. In conclusion, adding osteogenic cells to biomaterials may enhance and improve alveolar reconstruction.

In chapter 4, a further systematic review focused on the human alveolar cleft model was conducted. This review intended to review all control clinical trials using regenerative graft materials in alveolar cleft reconstructions. Meta-analysis and risk of bias were also considered in this review. Fifteen studies could be retrieved from the literature until October 2020. Of these, zero % reported being double blinded, 73,3% did not explain the path of randomization, and 20% did not randomize the trail pathways. According to Jaded and Delphi scores, the risk of bias was relatively high in these clinical trials. Although the meta-analyses did not show any significant difference between the regenerative graft and the iliac crest graft in terms of new bone formation, it could be deduced that regenerative grafting has promising clinical prognosis compared to iliac crest grafting. Furthermore, upcoming clinical trials should consider improving quality in term of risk of bias.

Chapter 5 presents a protocol of a prospective non-blinded first-in-man clinical pilot study. The objective of this protocol was to evaluate the safety and feasibility of Ca-PolyP (Polyphosphate) as a regenerative graft material in alveolar cleft patients. Polyphosphate is a physiological polymer found in the bloodstream specifically in platelets. One of the main advantages of polyP is that it plays a role in the deposition of bone calcium and simultaneously provides the necessary energy for the regeneration process. Under this protocol, young adolescents aged 13 years of old or older were planned for recruitment in this trial. The sample size was set at eight patients with two treatment groups, one receiving Ca-polyP and the other receiving a combination of Ca-polyP combined with biphasic calcium phosphate (BCP). The monitoring of most important outcome parameters, safety as well as radiographically and histologically/histomorphometrically determined bone volume, was set at 6 months after intervention. The radiologist and histologist will be kept blinded to the treatment during the evaluation. In conclusion, with this protocol we aimed to evaluate the safety and feasibility of Ca-polyP MP as a new graft material.

Chapter 6 describes in detail the first-in-human clinical trial using Ca-PolyP as graft material in the reconstruction of the alveolar cleft. The age ranged between 13 to 34 years with a mean age of 18.1. The study was intended to consist of two equal groups of four patients, half of them receiving Ca-polyP alone and the other half receiving Ca-polyP + BCP. However, an amendment was necessary for surgical reasons, ending up with only 2 patients receiving Ca-polyP and 6 patients receiving Ca-polyP + BCP. Outcome parameters were safety evaluations including routine blood tests, regular health checks and physical examinations, and radiographic imaging. None of the patients experienced any local or systemic allergic reactions, or other side effects. According to the Bergland scale radiographic evaluation, the combination of Ca-polyP + BCP graft showed more stability compared to Ca-polyP alone in alveolar cleft reconstruction. Based on these findings, we could conclude that both grafts were safe to use. Future clinical trials with a larger sample size are recommended.

In chapter 7 we evaluated the influence of patient-related factors on intraoperative

blood loss during double opposing Z-plasty Furlow palatoplasty using the buccal fat pad. It was a prospective study involving 109 patients with cleft palate for three months at Hasanuddin University as well as during charity trips to the rural areas of Eastern Indonesia. The blood loss was calculated intraoperatively by measuring the weight differences before and after the surgery of the gauze swabs that were used to control the surgical bleeding. A complete blood count was performed three days post operatively. The results show that both a high weight of the patient and a long duration of the operation led to significant effects on the increase in blood loss. It was shown that the DOZ Furlow palatoplasty combined with buccal fat pad graft is relatively safe procedure with promising results. Furthermore, surgery in younger patients was recommended not only to reduce the blood loss but also to start a better life, since it is still difficult to provide medical care at the adequate (prepuberal) time in Indonesia. In chapter 8, we present the first-in-human prospective clinical trial protocol on the use of microfragmented buccal fat pad (MFAT) combined with BCP to repair alveolar cleft. BCP graft material is an osteo-conductive graft which serves as a substitute for the autologous graft of the iliac crest. MFAT prepared from the buccal fat pad aims to add osteoinductivity to the BCP after mixing the two components. In the protocol, the design of a clinical trial aimed at evaluating the feasibility and safety of BCP-MFAT combination is described. The sample size of this protocol will be eight alveolar cleft patients. All patients will receive BCP-MFAT graft. Subsequently, a blood test, physical examinations, radiographic evaluations and, after 6 months, a histological/ histomorphometric analysis will be performed on biopsies obtained by trephine drills during dental implantation.

AUTHORS' CONTRIBUTIONS

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Chapter 2 was published as:

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Authors:

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Authors:

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Microfragmented fat (MFAT) and BCP for alveolar cleft repair: A prospective clinical trial protocol

Authors:

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LIST OF PUBLICATIONS

Published

1. S. A. Alkaabi; G. A. Alsabri; D. S. Natsir Kalla; S. A. Alavi; T. Forouzanfar; R. Nurrahma; M. N. Helder. Regenerative graft materials for maxillary sinus elevation in randomized clinical trials: A meta-analysis. Advances in Oral and Maxillofacial Surgery, ISSN: 2667-1476, Vol: 8, Page: 100350. DOI10.1016/j.adoms.2022.100350.

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14. Alavi SA1, Imanian MA3, Alkaabi SA1,2, Alsabri GA1, Forouzanfar T1,3, Helder MN1. Socket preservation using regenerative graft materials in randomized clinical trial. systematic review and meta-analysis.

Curriculum vitae

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Since 2018, he has been pursuing a Ph.D. in the field of Oral and Maxillofacial Surgery/Oral Pathology at the Department of Oral and Maxillofacial Surgery, VU University Medical Center/Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, The Netherlands, under the supervision of Professor Tymour Forouzanfar, MD., DDS., PhD., and Dr. Marco Helder PhD as his supervisors. His research and clinical interests revolve around stem cells/tissue engineering and orthognathic surgeries. Additionally, he has also been a member of the Scientific and Training Committee of the Arab Board in Oral & Maxillofacial Surgery since 2018. Furthermore, he serves as a reviewer for several highly regarded journals in the field of oral & maxillofacial surgery.

“Here, the journey knows no end “

Salem A. Alkaabi, born in 1985, in Al Fujairah, United Arab Emirates (UAE), currently holds the position of a Consultant in Oral and Maxillofacial Surgery at Al Fujairah and Al Kuwait Hospital Dubai, affiliated with Emirates Health Services. He completed his Bachelor's degree in Dental Surgery from the Faculty of Dentistry at Ajman University of Science and Technology in 2008. In 2012, he pursued specialized training and obtained a Master's degree in Oral and Maxillofacial Surgery from Jordan University of Science and Technology. He has been registered as an Oral and Maxillofacial Surgeon by the Ministry of Health in the UAE since 2012.

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