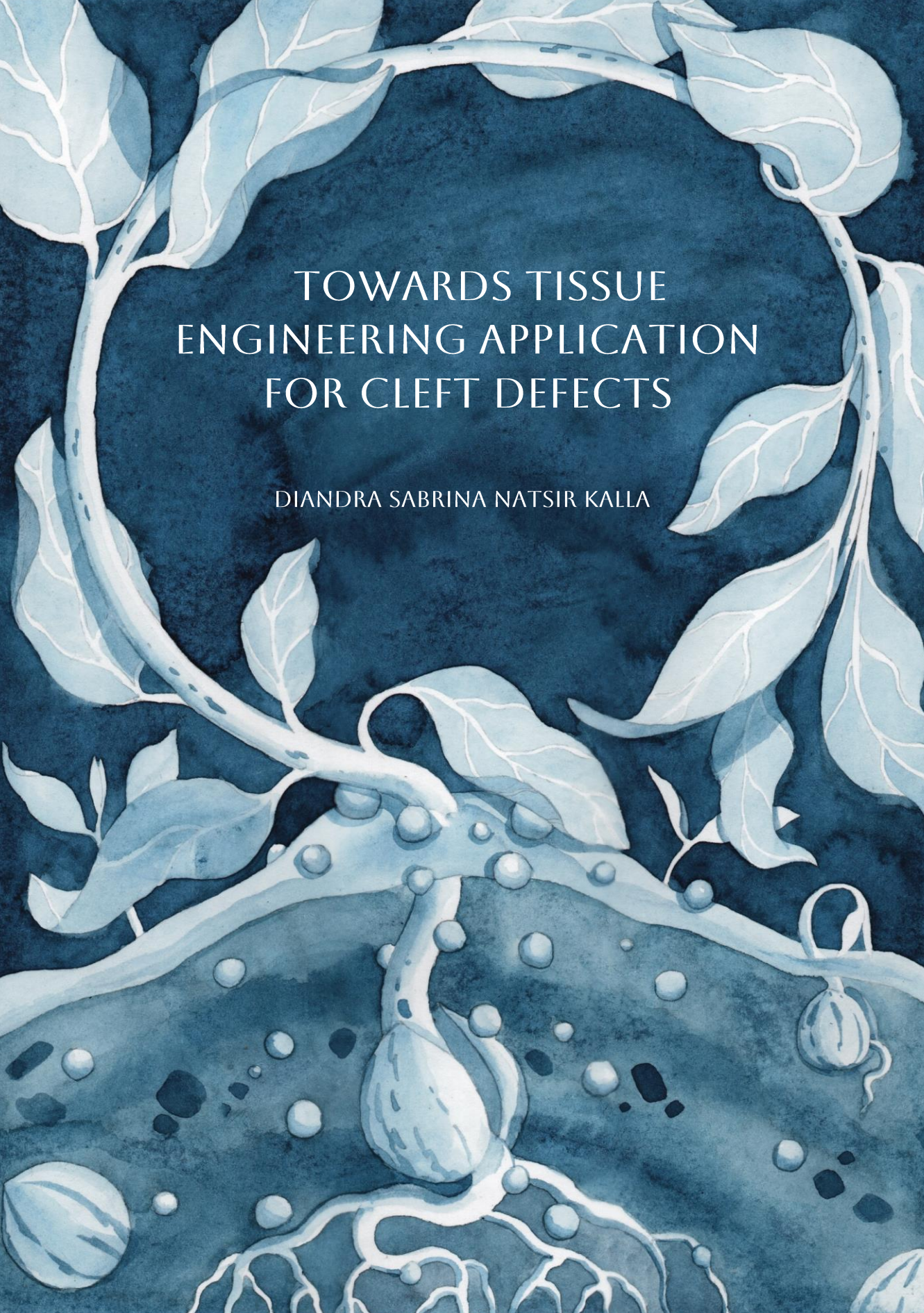

TOWARDS TISSUE ENGINEERING APPLICATION FOR CLEFT DEFECTS

DIANDRA SABRINA NATSIR KALLA

Towards tissue engineering application for cleft defects

Diandra Sabrina Natsir Kalla

The studies presented in this thesis were conducted at the Department of Oral and Maxillofacial Surgery, Amsterdam University Medical Center, location VU University Medical Center, Academic Centre for Dentistry Amsterdam (ACTA), and Amsterdam Movement Sciences, Amsterdam, the Netherlands.

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For my family

“Whoever follows a path to seek knowledge, Allah will make the path to Jannah (Paradise) easy for them.”

(HR. Muslim)

CONTENTS

CHAPTER 1

General Introduction

GENERAL INTRODUCTION

Background

Oral clefts, which include cleft lip, cleft lip and palate, and cleft palate alone, are a variety of conditions affecting the lips and oral cavity [1]. The overall prevalence of orofacial clefts is estimated to be 1 in 700 live births, with a range of 3,4 to 22,9 per 10.000 births for cleft lip with or without cleft palate and 1,3 to 25,3 per 10.000 births for isolated cleft palate [1]. Across all ethnic groups, males are more likely to have cleft lip with or without cleft palate (CL/P), while females are more likely to have isolated cleft palate (CP) [1]. The sex ratio varies depending on the severity of the cleft, the presence of other malformations, the number of affected siblings in a family, the ethnic origin, and possibly the paternal age [1]. Cleft lip with or without cleft palate and isolated cleft palate is usually linked to other serious congenital abnormalities. Studies have found a wide range of proportions, but in general, additional problems appear more common in individuals with isolated cleft palate than in those with cleft lip with or without cleft palate [1]. Causes of oral clefts are suggested to be lifestyle and environmental risk factors (maternal exposure to tobacco smoke, alcohol, poor nutrition, viral infection, medicinal drugs, and teratogens in the workplace and at home in early pregnancy have all been investigated), genetic factors (including regions on chromosomes 1, 2, 4, 6, 14, 17, and 19 (MTHFR, TGFA, D4S175, F13A1, TGFB3, D17S250, and APOC2), with putative loci suggested at 2q32–q35 and 9q21–q33), or gene-environment interactions [1].

Oral clefts impose a large psychosocial and economic burden on affected families and society. They associated with several health problems and complications early in life, such as problems with speech, hearing, appearance, and cognition. This can eventually lead to long-lasting adverse outcomes for health and social integration, and may even increase mortality and morbidity, especially in less developed settings, where early systematic pediatric care may not be commonly accessible [1–3].

The traditional classification of oral clefts is based on phenotypes, which can range in severity from microform to complete clefting and may involve the alveolar ridge and palate (Figure 1) [3]. Specific genetic linkage patterns have been

associated with certain phenotypes, according to evidence [3]. A clinical spectrum of cleft lip with or without an accompanying cleft palate is referred to as CL/P or CP, respectively [3]. CL/P and CP are embryologically different processes interrupted at diverse embryonic stages and have separate epidemiologic and genetic features [3]. Despite potential epidemiologic variations, involvement of the palate often denotes a similar but more severe congenital anomaly [3]. Lip clefting can be either complete (covering the entire vertical height of the lip) or incomplete [3]. Complete cleft lips are frequently accompanied by an alveolar cleft, a gap in the dental arch between the maxillary segments anterior to the incisive foramen, and are caused by malformation of the frontonasal prominence [3–5]. The alveolar cleft typically occurs between the lateral incisor and the canine [5].

Affected children need multidisciplinary care from birth until adulthood. Care for children born with oral clefts ideally includes many disciplines: nursing, plastic surgery, maxillofacial surgery, otolaryngology, speech therapy, audiology, counseling, psychology, genetics, orthodontics, and dentistry [1]. Cleft lip repair is influenced by the deformity of multiple anatomical structures, which can occur with varying severity [3]. Surgical techniques of cleft lip repair include creating an intact and appropriately sized upper lip especially on the cleft side, repairing the underlying muscular tissue for normal oral competence and function, and primary repair of nasal deformity [3]. As for palatoplasty, the various surgical methods include a tension-free multilayered closure with repositioning of the velar muscle sling [6].

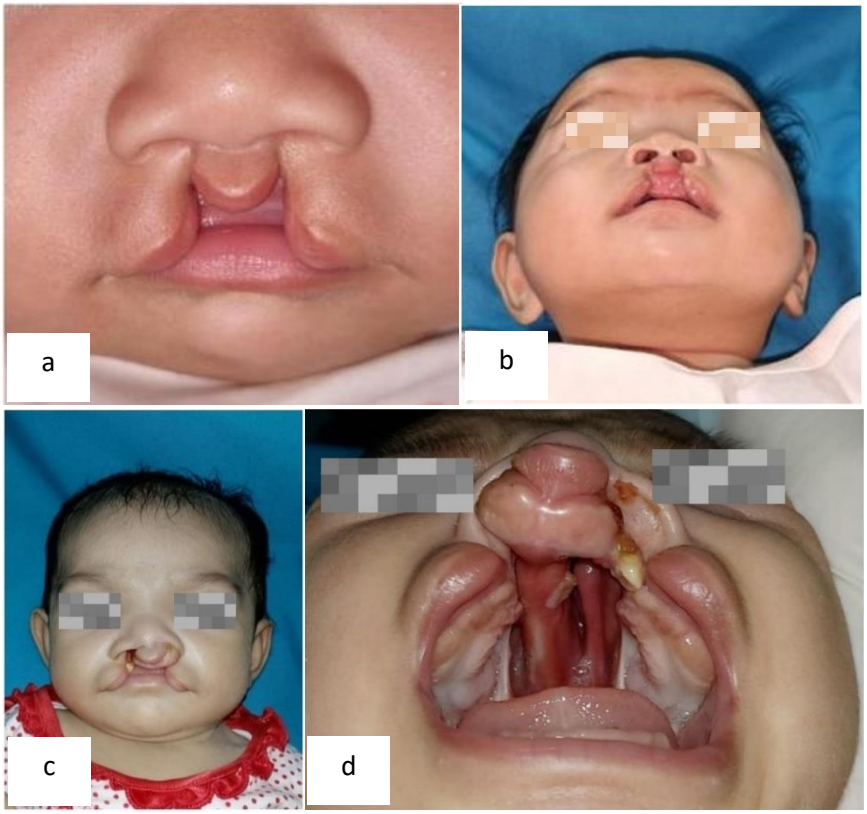

Figure 1. Oral Clefts. (a and b) bilateral cleft lip, (c and d) bilateral cleft lip and palate.

Specific strategies to manage the alveolar cleft must be incorporated into the complete treatment of oral clefts [4,6]. The goals of alveolar cleft repair include both functional goals (closure of the nasolabial fistula, creation of a stable and continuous maxillary dental arch, improved support of teeth adjacent to the cleft site, allowance for the eruption of teeth into the cleft site, provision of unrestricted orthodontic movement, facilitation of oral hygiene, and speech improvement) and aesthetic reconstruction [5]. Alveolar cleft repair should be done at the right timing in order to provide proper attention to other accompanying orofacial conditions and to prepare bony scaffold for adult dentition [7]. As a result, it stands to reason that any treatment for an alveolar cleft should be carried out before the eruption of permanent teeth or ‘‘secondary’’ bone grafting [7].

Although alternatives are gaining recognition, the currently accepted strategy for the repair of an alveolar cleft remains autologous bone grafting [7]. The autogenous graft is usually harvested from the anterior iliac crest [6]. Autogenous bone has various advantages over other choices, including osteogenic activity and osteoinductive potential with the availability of healthy cells and growth factors while not inducing an immune response [6]. However, employing autogenous bone has drawbacks such as morbidity at the donor site and lengthy recovery times [6]. Thus, improving the quality of the already available graft materials and searching for new and more potent materials are crucial for creating a material that is therapeutically suitable.

There has been a lot of research done to identify the best donor site and materials to use for alveolar cleft repair. In order to reduce donor site morbidity, provide a higher quality outcome, shorten operating times, and reduce hospital stays, recent advancements have moved toward using tissue-engineered bone graft materials. The tissue engineering process might be a choice for creating bone grafts that can promote the osseous repair of craniofacial defects. The utilization of these new procedures in treating individuals with cleft lip and cleft palate is undoubtedly of interest, given the advancements in regenerative medicine and tissue engineering.

Tissue Engineering for Oral Cleft Defects

Tissue engineering construction using biomaterials, cells, and growth factor(s) has established its value as an alternative method to regenerate bone [8]. They are currently also being investigated for the regeneration of oral clefts, particularly alveolar cleft defects [9,10]. Particular, the addition of stem cells to the alveolar gap is an appealing tissue engineering strategy [11–13]. Stem cells are, among others, found in bone marrow and adipose tissue, the latter present in large quantities [14]. These cells can be differentiated into bone cells. Another approach is the use of inducing factors to recruit endogenous stem cells to improve healing and promote tissue regeneration [15]. The use of cells and inducing factors, however, need a scaffold as a carrier that will help bone formation by providing attachment and movement support to the invading cells, may facilitate the

establishment of the required reconstruction shape, and that will aid in providing sufficient strength to withstand external stresses [16].

Although the tissue engineering field for oral clefts regeneration is emerging, the golden formula regarding the optimal combination of cells, inducing factors, and scaffolds has still not been found. Underlying this problem might be the many differences between conducted experiments and different evaluation techniques [9]. Moreover, in research involving animal models and clinical trials, there are often many more variables than just the intended regenerative strategy. This will hamper the ability to draw scientifically sound conclusions required for the proper development of tissue engineering strategies. Hence, before effective tissue engineering strategies for the regeneration of oral clefts can be accomplished, novel, relevant, repeatable, and more standardized combinations of tissue engineering building blocks need to be devised and validated. The process for tissue engineering is intended to be used in the future, particularly in developing countries where inadequate antenatal care led to poorly thought out therapy of oral clefts [17].

Several tissue engineering approaches have been proposed to reconstruct other anatomical defects of craniofacial area, most commonly in the calvarial model [18–22] and maxillary sinus floor augmentation (MSFA) models [23]. All of these techniques mainly involved the use of autogenous bone graft and/or bone substitute materials [21,23], and often in combination with cells and/or growth factors[18–20,22]. These accessible craniofacial constructions offer biologists, bioengineers, and clinicians a convenient way to test tissue-engineered prototypes [24].

Müller and colleagues discovered a novel polyphosphate (polyP)-based bone graft, which is a physiological polymer made up of orthophosphate units joined together by high energy phosphate bonds similar to ATP and abundantly present in platelets [25]. This makes PolyP, which acts as an energy source for bone tissue regeneration, represent a novel class of inducing factors and is now thought to be a safe material for human applications [25]. In Chapter 7, this novel bone graft will be explored in terms of safety and feasibility for craniofacial bone tissue regeneration. In addition, adipose stem cells (ASC) have potential implications in tissue engineering for craniofacial structures [22]. One of the sources of ASCs is the

intraoral buccal fat pad (BFP) that is said to contain stromal vascular fraction (SVF) with a subpopulation of ASC with osteogenic differentiation potential just like other ASC depots, show little donor size change, and be independent of body weight and fat distribution [26]. The pro-angiogenic and bone formation-enhancing actions of SVF have been described in a phase I investigation by Prins et al., and this offers significant promise for therapeutic bone tissue engineering for MSFA [27]. However, recent US and European recommendations that seek to categorize enzymatically digested SVF as "more-than-minimally manipulated" cells could hinder the anticipated rise in the therapeutic usage of SVF [26,28]. Thus, mechanical dissociation methods have been proposed as an alternative to conventional enzymatic isolation of SVF. These techniques apply physical pressure to compress fat particles into an injectable form of cell aggregation called micro- or nanofat [29], which can be applied for bone tissue regeneration. This may offer a possibility to optimize or test as an easily applicable and cheaper alternative for the enzymatically processed SVF described by Prins et al. This topic will be covered in Chapter 9.

Aims and Outline of the Thesis

One of the major objectives of this thesis is to examine current clinical practice and difficulties associated with the treatment of cleft lip and palate (Chapter 2 and 3). These analyses ought to be beneficial for the planning and developing of alternative tissue engineering methods for the reconstruction of oral clefts. Other objectives are to investigate current tissue engineering strategies for oral cleft reconstruction (Chapter 4 and 5) and assess the safety and feasibility of novel tissue engineering approaches for oral cleft reconstruction (Chapter 6, 7, 8, and 9).

In **Chapter 2** the results are reported of a retrospective comparative cohort study, with regard to postoperative daycare after Secondary Alveolar Bone Grafting (SABG) procedure are evaluated.

In **Chapter 3** the findings of a prospective human clinical investigation to ascertain the impact of patient-related variables on intraoperative blood loss during double opposing Z-plasty and buccal fat pad coverage are presented.

In **Chapter 4** a systematic review and meta-analysis of the literature on evaluation of the efficacy of stem cell-based tissue engineering for cleft palate and alveolar cleft defects in pre-clinical models is given.

In **Chapter 5** a systematic review and meta-analysis of the literature on regenerative products used in clinical trials to treat alveolar clefts is provided.

In **Chapter 6** the protocol is reported of a prospective control clinical trial to investigate the safety and feasibility of two grafting materials, polyphosphate (PolyP) or combination of PolyP and biphasic calcium phosphate (BCP), with regard to alveolar cleft surgery.

In **Chapter 7** the results of a prospective control clinical trial, with regard to safety and feasibility evaluation in alveolar cleft repair with polyphosphate (PolyP) or combination of PolyP and biphasic calcium phosphate (BCP).

In **Chapter 8** the protocol is reported of a prospective control clinical trial to investigate the safety and feasibility of a grafting material, combination of microfragmented fat (MFAT) and biphasic calcium phosphate (BCP), with regard to alveolar cleft surgery.

In **Chapter 9** a prospective control clinical trial study is described, which evaluated the safety and feasibility of combination of microfragmented fat (MFAT) and biphasic calcium phosphate (BCP) in alveolar cleft repair.

Finally, **Chapter 10** and **Chapter 11** comprise a general discussion and a summary of the results reported in this thesis.

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CHAPTER 2

Postoperative daycare as a safe and cost-effective option for Secondary Alveolar Bone Graft (SABG) surgery: A retrospective comparative cohort study

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ABSTRACT

Objective: To evaluate the outcomes of Secondary Alveolar Bone Grafting (SABG) in patients treated either in daycare or with multiple day hospitalization (MDH) in relation to costs and complication rates.

Design: Retrospective comparative cohort study.

Setting: The data was collected from two settings: Postoperative daycare or MDH after oral cleft surgery in an Academic Medical Center in The Netherlands.

Patients: Data of 137 patients with unilateral Cleft lip, alveolus, and palate (CLAP) treated between 2006-2018 were evaluated. Registered clinical variables: age, gender, cleft subtype, bone donor site, type of hospitalization, length of stay, additional surgery, complications, surgeons, and costs.

Interventions: Closure of the alveolar cleft with/without closure of the anterior palate.

Main outcome measures: Univariate analyses.

Results: Of the 137 patients, 46.7% were treated in MDH, and 53.3% in daycare. Total costs for daycare were significantly lower ($p<0.001$). All patients treated in daycare received mandibular symphysis bone, whereas in MDH, 46.9% received iliac crest bone instead. Bone donor site was associated with postoperative care type. Complication rates were slightly but not significantly higher in daycare (26%) vs. MDH (14.1%) ($p=0.09$). Most were Grade I (minor) according to Clavien Dindo classification.

Conclusions: Daycare after alveolar cleft surgery is about as safe as MDH, but significantly cheaper.

Keywords: Alveolar cleft, surgery, bone grafting, postoperative care, cost, complication.

INTRODUCTION

Oral clefts, representing diverse malformations affecting the lip and oral cavity, are among the most frequent congenital facial defects in children, with a prevalence of about 1 in 700 livebirths [1]. In The Netherlands, a time trend analysis over the years 1997-2006 estimated the prevalence of oral cleft as 16.8 per 10.000 livebirths [2]. The etiology of oral clefts is heterogenous and mostly caused by lifestyle and environmental factors, genetic factors, or interaction of these factors [1].

Many different therapeutic programs exist for patients with oral cleft [1]. In our academic center, patients are followed from birth to 22 years old by a multidisciplinary Cleft Team comprised of among others a plastic surgeon, an ear nose throat (ENT) specialist, a speech therapist, a pediatric dentist, an orthodontist, a maxillofacial surgeon, a clinical genetic scientist, a pediatric nurse, and a pediatric specialist. One of the strategies to provide an efficient and effective treatment for oral clefts is reducing the treatment costs by minimizing the length of postoperative stay for patients [3]. Unlike conventional care, whereby children come the day before surgery, have surgery performed and are discharged after multiple day hospitalization (MDH) within 48 to 72 hours, postoperative daycare allows children to come on the day of the surgery and be discharged on the same day [4,5]. This type of hospitalization will be beneficial not only for the patients and their family but also for health care centers [3]. The child can be returned to his/her own home sooner and the parents can give the remaining care without having to neglect the other family members [4,5].

Cleft lip and palate surgery followed by postoperative daycare has been shown to be safe and cost effective in properly selected patients [6]. Postoperative daycare following alveolar cleft surgery, in contrast, has been reported in limited data [5] or focused on financial aspects associated with the procedure without emphasizing postoperative outcomes [3,7].

This retrospective study aimed to examine patients with unilateral cleft lip and alveolus, with or without cleft palate, in 137 patients who had surgical treatment (closure of the alveolar cleft and palate) followed by postoperative daycare or MDH. Surgical outcomes in both types of postoperative care, and the

associations of postoperative care type with costs and complications were evaluated.

PATIENTS AND METHODS

Data

A data registry of patients with clefts treated at our academic center between 2006 and 2018 was used. Selection criteria were: unilateral cleft lip and alveolar cleft with or without cleft palate, age younger than 18 years, and received surgical treatment by the oral and maxillofacial surgeon between 2006 and 2018. Syndromic patients were excluded. The following data were collected from the registry: age (at surgery), gender, cleft subtype, bone donor site, type of hospitalization, length of stay, additional surgery, complications (classified using the Clavien-Dindo classification [8]), surgeons, and costs. The study was not subjected to the Medical Research involving human subjects Act, as confirmed by the ethical committee of our center.

Surgical methods

According to our Cleft Protocol, closure of the hard palate and closure of the alveolar cleft using bone graft is performed between ages 7 and 10 years old. This procedure of alveolar cleft repair is also known as secondary alveolar bone grafting (SABG) during the mixed dentition period [9,10]. At what time SABG is performed is related to the eruption of lateral incisors or permanent canines [11,12]. At our academic center, the timing of SABG is decided around the age of 6 years old based on a standard radiological documentation of the eruption path of the teeth. All of the included cases were treated consecutively. Iliac crest bone is the most common donor site for the SABG procedure [9,10]. However, in this study group, the mandibular symphysis was the preferred donor site if enough bone was available in this area. The size of the alveolar cleft and the amount of available bone were assessed on a panoramic radiograph. Monocortical harvesting of mandibular symphysis bone was preferred because of the easy access and the reduction of postoperative discomfort for the patient. Especially in older patients (over 9 years old), the amount of available bone in the mandibular symphysis is larger due to further eruption of the permanent canines. In contrast, for younger

patients sometimes the iliac crest was chosen as the donor site due to insufficient bone being available in the mandibular symphysis region.

During the surgery, which is performed under general anesthesia, the palatal and buccal fistula are circumcised and mucoperiosteal flaps are raised. The nasal mucosa is closed from posterior to anterior with interrupted sutures. Then the oral mucosa of the hard palate is closed using a continuous suture. After harvesting of the autologous bone (either from the mandibular symphysis area or from the iliac crest) the bone is grinded using a bone mill and the particles are positioned at the alveolar cleft site. Finally, the oral mucosa and release incisions are closed with interrupted sutures. If indicated, additional procedures such as the removal of deciduous or supernumerary teeth, or secondary correction of the lip scar were performed concomitant with the SABG procedure.

Peri-operative care and follow up

All patients were seen by the Cleft Team at ages 1, 6, 9, 12, 15, 18 and 22 years old for regular follow up. At age 6 a panoramic radiograph was made for the first time to determine the eruption path of the lateral incisor and canine at the cleft side. When the lateral incisor would erupt into the cleft, we performed an early closure of the alveolar cleft (8-9 years old). If the canine would erupt into the cleft, we planned the closure later (9-11 years old). If necessary, pre-surgery orthodontics were started to better align the dentition in order to facilitate the closure.

All patients were admitted to the hospital on the day of their surgery. No surgeries were delayed, so the hospitalization time is a good measure of the time patients needed to recover at the hospital after the surgery. Standardized peri-operative and post-operative antibiotics were given to all patients (Amoxicillin 50mg/kg/day for one week starting on the day of the surgery). Parents and child received elaborate oral and written information about the surgery and post-operative care instructions, both pre-surgery and post-surgery before release from the hospital. All patients were seen by the surgeon 4 weeks after the surgery, at which time further follow ups were scheduled. Patients who received iliac crest bone came back after 7-10 days for sutures removal at the donor site. If

subsequent orthodontic treatment was deemed necessary, the patients were also seen by the orthodontist after 4-6 weeks to plan the post-surgery orthodontics.

Costs

The costs are calculated based on standard costs for hospital stay at our center and multiplied by number of hospitalization days. For daycare, the standard cost is 275.89 euros and for MDH the standard cost is 371.52 euros/day (based on the Netherlands national guideline valid at 12 July 2019). The costs of anesthesia and surgery were excluded because this was the same for both groups. Because the majority of the complications were minor according to the Clavien Dindo classification and did not lead to additional costs, a calculation of the costs per complication was not performed.

Statistical methods

Frequency, median, minimum, and maximum values were calculated in this study using SPSS version 26 for Windows. The following clinical variables were registered: age, gender, cleft subtype, bone donor site, type of hospitalization, length of stay, additional surgery, complications, surgeons, and costs. We did not conduct a power analysis because this was a series of consecutive cases. The X^2 test was used to evaluate all sets of comparisons between categorical variables, while comparisons of continuous data were analyzed using Mann-Whitney U test. Logistic regression was used to analyze the association between the potential factors and development of complications. All statistical tests were considered significant at $p < 0.05$.

RESULTS

Description of selected patients and surgeons in charge

One hundred thirty-seven patients with unilateral cleft lip and alveolus with or without cleft palate were included in the study. There were two types of care for patients, i.e. multiple day hospitalization (MDH) or daycare. Seventy-three patients belonged to the daycare group with 44 male patients (60.3%) and 29 female patients (39.7%), while 64 patients belonged to the MDH group with 40 male patients (62.5%) and 24 female patients (37.5%) (Table 1). Thus, the sex

distribution was similar in both care types. Children were slightly, though not significantly ($p = 0.18$), younger in daycare than in MDH as can be deduced from Table 1. The age at which most of the surgeries were performed was 9 years for both groups (61 patients out of total 137 patients (44.5%)).

The prevalence of unilateral cleft lip and alveolus was higher in patients in the daycare (27.4%) compared to the MDH group (7.8%) ($p = 0.003$) (Table 1). In contrast, the prevalence of the diagnosis unilateral cleft lip, alveolus, and palate was higher in the MDH group (92.2% vs. 72.6% in the daycare group; $p = 0.003$) (Table 1). There was no difference for alveolar cleft side in both care types (left side: n=46 and n=47 for daycare and MDH, respectively, and right side: n= 27 for both care types) (Table 1).

Patients who needed bone graft from the iliac crest automatically got MDH postoperatively because of the healing period of the donor site location. This is in line with the study of Swan and Goodacre [13] that showed that bone donor site was significantly associated with the type of postoperative care. All patients in the daycare group received mandibular symphysis bone graft during the surgery. As for the MDH group, 34 patients (53.1%) received mandibular symphysis bone graft and 30 patients (46.9%) received iliac crest bone graft (Table 1).

The vast majority of the patients (134 of the 137) in this study group were treated by one of two experienced cleft surgeons. During the time period 2006-2018 the main cleft surgeon (JA Baart) retired and his successor (MG) was trained to take over the practice and ensure a safe transfer of care. Thirty seven of the 137 patients were treated by both surgeons together during this period. As can be deduced from the data presented in Table 2, we could rule out that the surgeon performing the procedure attributed to the complication rate, in contrast to what was reported in other studies [14,15].

Table 1. Characteristics of Alveolar Cleft Patients

Variable	Daycare		MDH		p-Value
	N	Result	N	Result	
Gender (N, %)					0.79
Male	44	60.3	40	62.5	
Female	29	39.7	24	37.5	
Diagnosis status (N, %)					**0.003**
CLA	20	27.4	5	7.8	
CLAP	53	72.6	59	92.2	
Alveolar cleft side (N, %)					0.534
Left	46	63	37	57.8	
Right	27	37	27	42.2	
Donor site (N, %)					**< 0.001**
chin	73	100	34	53.1	
Iliac crest	0	0	30	46.9	
Closure of the palate (N, %)					**0.004**
No	23	31.5	7	10.9	
Yes	50	68.5	57	89.1	
Additional surgery (N, %)					**0.006**
No	24	32.9	36	56.3	
Yes	49	67.1	28	43.8	
Complications (N, %)					0.083
No	54	73.9	55	85.9	
Yes	19	26.1	9	14.1	
Surgeon (N, %)					**0.004**
1 & 2	31	42.5%	6	9.4%	
1	33	45.2%	29	45.3%	
2	9	12.3%	26	40.6%	
Other	0	0%	3	4.7%	
Age, years (N median (min - max))	73	9 (6 - 15)	64	9 (7 - 16	0.18
Care time,hour (N median (min - max))	73	8 (4.8 - 13.3)	64	30 (22.6 - 94)	**< 0.001**
Cost euro (N median (min - max))	73	275.89	64	743.04 (743.04 - 1857)	**< 0.001**

Variable	Daycare		MDH		p-Value
	N	Result	N	Result	
Gender (N, %)					0.867
Male	66	61.7	18	60	
Female	41	38.3	12	40	
Diagnosis status (N, %)					**0.003**
CLA	25	23.4	0	0	
CLAP	82	76.6	30	100	
Alveolar cleft side (N, %)					0.619
Left	66	61.7	17	56.7	
Right	41	48.3	13	43.3	
Postoperative care (N, %)					**< 0.001**
Daycare	73	68.2	0	0	
MDH	34	31.8	30	100	
Closure of the palate (N, %)					**0.004**
No	23	31.5	7	10.9	
Yes	50	68.5	57	89.1	
Additional surgery (N, %)					**0.001**
No	28	26.2	21	70	
Yes	79	63.6	9	30	
Complications (N, %)					0.656
No	86	80.4	23	76.7	
Yes	21	19.6	7	23.3	
Surgeon (N, %)					**< 0.001**
1 & 2	35	32.7%	2	6.7%	
1	43	40.2%	19	63.3%	
2	29	27.1%	6	20%	
Other	0	0	3	10%	

Abbreviations: CLA, cleft lip and alveolus; CLAP, Cleft lip, alveolus, and palate; MDH, multiple day hospitalization

[a]Results based on X2, Fisher's Exact Test, or Mann-Whitney U test. The statistically significant results are given in bold.

Closure of the palate and additional surgery

The prevalence of patients who required palate closure at the time of alveolar cleft surgery in the MDH group was higher than in the daycare group (89.1% vs 68.5%, p = 0.004, respectively) (Table 1). In contrast, the percentage of patients who had an additional surgery was lower in the MDH group than in the daycare group (43.8% vs 67.1%, p = 0.006, respectively). Additional surgery mainly

consisted of small procedures such as removal of deciduous teeth in the cleft area, removal of hypoplastic or supernumerary teeth, ligation of teeth, naevus or fibroma removal, placement of eardrum tubes and secondary corrections of the lip scar.

Table 2. Univariate logistic regression analysis for alveolar cleft patients' characteristics associated with complications

Factors	No complications (N = 109)	Complications (N =28)	Univariate Analysis	
			OR [95% CI]	p-Value
	n (%)			
Age	9 (7-16)[a]	9 (6-11)[a]	0.822 [0.579-1.166]	0.272
Gender				0.612
Male	68 (62.4%)	16 (57.1%)	Reference	
Female	41 (37.6%)	12 (42.9%)	0.804 [0.346-1.867]	
Diagnosis status				0.626
CLA	19 (17.4%)	6 (21.4%)	Reference	
CLAP	90 (82.6%)	22 (78.6%)	1.292 [0.461-3.617]	
Alveolar cleft side				0.085
Left	62 (56.9%	21 (75%)	Reference	
Right	47 (43.1%)	7 (25%)	2.274 [0.892-5.796]	
Donor site				0.657
Chin	86 (78.9%)	21 (75%)	Reference	
Iliac crest	23 (21.1%)	7 (25%)	0.802 [0.304-2.119]	
Closure of the palate				0.341
No	22 (20.2%)	8 (28.6%)	Reference	
Yes	87 (79.8%)	20 (71.4%)	1.582 [0.615-4.065]	
Additional surgery				0.911
No	48 (44%)	12 (42.9%)	Reference	
Yes	61 (56%)	16 (57.1%)	0.953 [0.412-2.205]	
Daycare				0.087
No	55 (50.5%)	9 (32.1%)	Reference	
Yes	54 (49.5%)	19 (67.9%)	0.465 [0.193-1.118]	

Surgeon				
1&2	30 (27.5%)	7 (25%)	Reference	0.381
1	46 (42.2%)	16 (57.1%)	0.467 [0.037-5.903]	0.556
2	31 (28.4%)	4 (14.3%)	0.696 [0.059-8.199]	0.773
Other	2 (1.8%)	1 (3.6%)	0.258 [0.019-3.533]	0.310
Care time (in hour)	22.6 (5.3-94)[a]	8.6 (4.8-76.3)[a]	0.996 [0.979-1.014]	0.654
Cost	743.04 (275.89-1857.60)[a]	275.89 (275.89-1486.08)[a]	1.000 [0.999-1.001]	0.457

Abbreviations: CLA, cleft lip and alveolus; CLAP, Cleft lip, alveolus, and palate
[a] Median, minimum, and maximum.
The statistically significant results are given in bold.

Type of complications

One hundred and nine patients (79,6%) were uneventful after the surgery, while 28 patients (20.4%) experienced complications after the surgery (Table 3). Minimal discomfort after the surgery was considered as no complications. For daycare, 54 patients (73.9%) showed no complications, while 19 patients (26.1%) had (Table 1). For MDH, these numbers were 55 patients (85.9%) and 9 patients (14.1%), respectively (Table 1). The overall complication types were wound healing disturbance which also comprised infections (8.8%), small fistula (3.7%), fistula needing additional surgery (0.7%), bone loss needing additional surgery (2.9%), minor bone loss (2.2%), foreign body in the transplant (0.7%), postoperative bleeding (0.7%), and nausea requiring readmission to the hospital (0.7%) (Table 3). These complications were also categorized based on the Clavien Dindo classification [8]. Most of the complications were of Grade I and can therefore be considered as minor (Table 3).

Odds ratios were calculated between patients' characteristics and the presence of postoperative complications. None of the characteristics of patients with alveolar cleft were associated as potential risk factors for complications. However, although with a really wide confidence interval, there appears to be a trend towards the group of MDH patients having less complications after the surgery [odds ratio (OR), 0.465; 95% CI, 0.193-1.118; p = 0.087] (Table 2).

Table 3. Distribution of complication types or no complication based on postoperative care types

Complication Types or No Complication	Grade[a]	Daycare N (%)	MDH N (%)	Total N (%)
No complication	n.a	54 (73.9%)	55 (85.9%)	109 (79.6)%
Wound healing disturbance	Grade I	9 (12.3%)	3 (4.7%)	12 (8.8%)
Small fistula, no need for treatment	Grade I	3 (4.1%)	2 (3.1%)	5 (3.7%)
Fistula in need of additional surgery	Grade IIIb	-	1 (1.6%)	1 (0.7%)
Bone loss in need of additional surgery	Grade IIIb	1 (1.4%)	3 (4.7%)	4 (2.9%)
Minor bone loss	Grade I	3 (4.1%)	-	3 (2.2%)
Foreign body in the transplant	Grade I	1 (1.4%)	-	1 (0.7%)
Postoperative bleeding	Grade I	1 (1.4%)	-	1 (0.7%)
Nausea readmitted to hospital	Grade I	1 (1.4%)	-	1 (0.7%)

[a]Grading based on Clavien Dindo Classification (Dindo et al.[8])

Hospitalization, donor site, and costs

As can be expected, the postoperative care time was shorter in the daycare group compared to the MDH group ($p < 0.001$) (Table 1). For daycare, hospitalization time ranged from 4.8 – 13.3 hours, whereas in the MDH group this ranged from 22.6 hours – 94 hours (Table 1). The cost for all patients in the daycare group (275.89 euros) was significantly lower ($p < 0.001$) than the costs for patients in the MDH group (median (min-max) 743.04 (743.04-1857.6) euros).

DISCUSSION

The aim of this study was to assess patients with unilateral cleft lip and alveolar cleft with or without cleft palate in our center who had surgical treatment in daycare or in multiple day hospitalization (MDH). The evaluated factors were outcomes of the surgery and associated costs and complications. Previous studies already compared cleft lip and palate surgery in daycare versus MDH and recommended postoperative daycare as an alternative to MDH[3–7,16,17] in the US, Australia, and Nigeria. To the best of our knowledge, this is the first study that

examined postoperative care type associated with oral cleft surgery in the Netherlands.

One hundred thirty-seven unilateral alveolar cleft patients with or without cleft palate were included in the study. The postoperative care types were categorized into daycare or MDH. The patient characteristics were similar in both care types, but prevalence of unilateral cleft lip and alveolus with cleft palate was higher in the MDH group compared to daycare (92.2% vs 72.6% respectively $p = 0.003$) (Table 1). The postoperative care type for the patients was really dependent on bone donor site ($p < 0.001$) (Table 1). This was because patients who received iliac crest bone were automatically assigned to postoperative MDH because of the risk of donor site morbidity [13], while patients who received mandibular symphysis bone were assigned to either daycare or MDH. One fifth (20.4%) of total patients experienced (minor) complications in the current study. Costs for daycare were significantly lower than costs for MD care in our study ($p < 0.001$) (Table 1).

The surgeries in this study were conducted in the period of 2006 – 2018 in our center meaning that the earliest data are from over 15 years ago. Thus, certain aspects now considered relevant in current standard practice were unfortunately not recorded in this study. We chose to report the costs based on the standard costs in 2019, both because it is more applicable to today's practice and because the relative cost differences will be similar and independent of the time period.

In literature, bone donor sites for alveolar bone grafting include calvarium, tibia, mandible, iliac crest, and rib [7]. Iliac crest is the most common donor site because it can provide a large volume of bone [7]. In our patient group, the choice for either iliac crest bone or mandibular symphysis bone as donor graft was made based on the size of the cleft and the available amount of bone that could be harvested from the mandibular symphysis region. For example, when a child has a relatively wide cleft and only little available bone in the chin area due to the position of the developing lower permanent canines or young age, iliac crest will be chosen as the donor site. Vice versa, when the cleft is smaller or when the permanent canines have erupted further and more bone is available in the mandibular symphysis region, this will be chosen as the donor site. In our study group the mandibular symphysis was the preferred donor site if possible (according to cleft size and amount of bone available).

The choice of bone donor site determines the postoperative care. Patients who received iliac crest as bone graft would stay more than 24 hours postoperatively for donor site recovery [18] (Table 1). The type of bone graft seemed correlated with postoperative complications. All patients in the daycare group received bone from the mandibular symphysis region and 26% had complications. In the MDH group, only 9 (14.1%) patients had complications, 2 patients received mandibular symphysis bone and 7 patients received iliac crest bone (Table 1). Surprisingly, the complication rates of patients who received mandibular symphysis bone in the MDH group were slightly lower than patients who received mandibular symphysis bone in daycare. The most common complication in daycare was wound healing disturbance, which included postoperative infections (12.3%) and may be consistent with the study by Kantar et al [6]. They showed that patients who received postoperative daycare after oral cleft surgery, in particular primary cleft palate repair, are almost twice as likely to develop wound dehiscence than patients who were hospitalized. One contributing factor might be the limited knowledge of parents about wound care so they were unprepared to take care and protect the child's postoperative wound at home in less than 24 hour after the surgery [6].

Our center is an academic hospital with an experienced Cleft Team, which for this study period only involved two experienced main maxillofacial surgeons. The number of patients in the daycare group was higher in our study than the MDH group. In a study by Nguyen et al. [17], it is stated that if the length of stay is prolonged to more than two days, the complication rates such as iatrogenic risks like nosocomial infections increase four-fold, and that hospital stays should therefore preferably be kept to less than 2 days. Since both our daycare and MDH groups have short hospital stays (8 and 30 hours on average, respectively; Table 1) we cannot confirm or decline their observations in our study. In addition, it has to be kept in mind that our study was smaller compared to their study [17] which involved 2 US national databases from 1997-2006 (resulting in the inclusion of 11792 patients with cleft palate), and that their study also only involved palate repair which is a more straightforward procedure than alveolar cleft closure. Moreover, hospital conditions in the US and The Netherlands are not in all aspects comparable; in the US hospitals, Methicillin-resistant Staphylococcus aureus

(MRSA) is frequently occurring [19], whereas due to the strict antibiotics policy in The Netherlands health system [20] the proportion of MRSA is below 5% according to EARS-Net annual report 2019 [21]. This also holds true for some other European countries such as Denmark, Norway, Sweden, Finland, and Estonia [21]. Data from antimicrobial resistance surveillance in Europe 2022 showed that the Dutch, along with the Nordic European countries, have the lowest MRSA prevalence in the world [22]. In the Netherlands, the estimated nasal colonization rate is 0.03-0.17%, compared to 0.9-1.5% in the United States [20,23]. Thus, the iatrogenic risks to patients mentioned by Nguyen et al. [17] are likely not a major risk factor for complications in The Netherlands.

Even though our study showed that the complication rate in the MDH group was slightly lower than in daycare, we still consider daycare safe after oral cleft surgery if patient selections are carefully conducted. No major complications were found in our study for both care types, and only one of our patients needed a readmission to the hospital after they were discharged. This is in line with Perry et al. [5] who recommended postoperative daycare following alveolar cleft surgery with adequate consideration on pain control, anesthetic recovery, sufficient oral intake, and comfortable postoperative setting provided by reliable and motivated caregivers. A study by Rosen et al. also recommended postoperative daycare after cleft palate surgery with an expected hospital stay of no longer than 23 hours [4].

This study has several limitations. One is that our study has a relatively small sample size which may affect accuracy of complication rate estimates. Also, since all treatments were standardized care, a case-control study design was not relevant in our study design. This study did not consider other factors associated with length of stay such as comorbid factors, type of hospital, and hospital volume [4,17]. According to Rosen et al., by documenting comorbid factors of patients, precautions can be made so length of stay can be reduced [4]. Information regarding patient satisfaction was also not presented in our study. This implies that during the regular consultations, no major complaints were reported by either parents or patients. Besides, patient related outcome measures (PROMS) were only introduced within the last 10 years [24], and were therefore not recorded in our study. The patient satisfaction aspect, however, is something that would enrich future studies.

CONCLUSION

In this study, the complications were slightly lower in the MDH group, but overall, there were no major complications for both daycare and MDH. Donor site selection was based on the amount of bone needed for reconstruction as deduced from radiographic evaluation of the defect and donor sites prior to surgery, and thereby the major determinant for the postoperative care type. In conclusion, if symphysis bone is present in sufficient quantity for reconstruction, postoperative daycare can be considered a safe and more cost-effective option following alveolar cleft surgery.

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CHAPTER 3

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. The aim of this study was to investigate the intraoperative and early postoperative blood loss using the buccal fat pad (BFP) during cleft lip and/or cleft palate (CL/P) surgery.

Material and methods: This prospective study included a total of 109 patients with cleft palate (CP) during a three-month period of treatment at Hasanuddin University Dental Hospital (permanent center) and charity trips in rural parts of Eastern Indonesia. All patients were treated with DOZ Furlow technique combined with BFP graft. Before and after surgery, the total amount of intraoperative blood loss was calculated by measuring the weight differences of the gauze swabs that were 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 ($P<0.05$). Overall, 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 is among the countries with a high number of CL/P cases in which some of them left untreated specifically those who lived in the rural area [1,2]. In the developing and low-middle income countries (LMC), a common model to provide cleft treatment in remote sites is through charity events [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, lack of associated specialists and anesthesia providers who can undertake the surgical treatments [4]. Consequently, these various shortages cause the surgical capacity in the remote area to be generally inadequate [3].

In the course of CL/P repair, the surgery is usually carried out when the patients is 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 in the treatment delay [2] .

While the topic of blood loss during palatoplasty is much discussed in the literature, there is not much consensus between these articles [5,7]. What is missing in the literature is a study that discusses the relationship between intraoperative blood loss and patient-related factors. Therefore, this study aims to identify which factors affect the amount of blood loss during palatoplasty using the DOZ Furlow technique combined with BFP as graft materials.

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 guardian who were willing to join the study after being fully educated about the procedure. Patient data were collected during a three-month period (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

We firstly collected the number of patients in charity group and the number of patients in the hospital group were adjusted accordingly. The reason was because charity surgeries were conducted in limited settings that made it less adjustable than hospital.

Study inclusions and exclusions

Before starting with this study, a number of inclusion and exclusion criteria were set up: patients with cleft palate which needed primary palatoplasty would be included in this study, while patients with syndromic clefts, fistula after palatoplasty which needed reconstruction, cleft after trauma, patients affected with multiple syndromes, and patients with a family history of blood loss conditions were excluded from this study. In addition, procedures using techniques to close palatal clefts other than the DOZ Furlow technique combined with BFP graft were also excluded.

Operation technique

The patients were put under general anesthesia and got prepared for surgery. The patients were injected with a local anesthetic solution of 2-5cc lidocaine 2% with epinephrine 1:100.000 alongside the line of incision [8]. The first incision was made five minutes later and from that moment on, the operative time was measured. The operation procedure of cleft palate closure is depicted in Figure 1. At first, the oral flaps were created by making an incision alongside the margin of the cleft and then continuing into a relaxing incision alongside the processus alveolar and ending posteriorly at the hamular processes of the palatinal bone [9,10] (Figure 1a). With a raspatorium, the mucosa was lifted of 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 midline [9,11]. To prevent scarring and post-operative complications the surgeons transplanted the BFP into the open relaxing incisions [12,13] (Figure 1c).

Data collections

The intraoperative surgical form was completed with following data recorded: width of cleft, type of cleft, technique used, and operative time. Patient's age was categorized in 4 groups according to the previous study with some adjustment: 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].

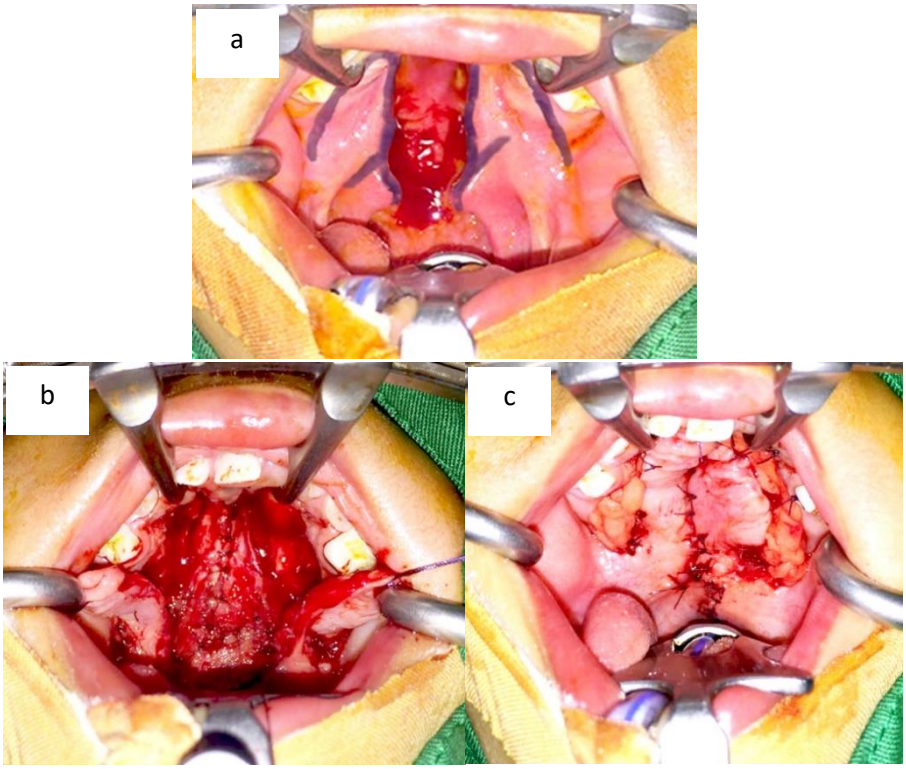

Figure 1. Operation procedure: (a) Flap design; (b) Bisection of mucosal and muscle layers, then suturing the nasal mucosa lining with the z-plasty technique; (c) Suturing the oral mucosal and placing the BFP towards the defect area with the interrupt technique.

Prior to 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 noted on the intraoperative form. The patients got admitted to the hospital postoperatively for three days and 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 of blood loss between charity trips and permanent hospital, nonparametric Mann Whitney test was used. To find the difference between the amount of blood loss from the two groups based on age category and cleft type, nonparametric Kruskal Wallis was done. To evaluate a potential relationship between the amount of blood loss and the numeric and categorical variables, the linear regression with backward method was used. P-values <0.05 were considered statistically significant.

Ethical Consideration

Ethical approval (approval number: UH14060319) was granted by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Because of the general age of the patients, the parents or legal guardian signed the research form if they would consent to 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 7 years), while the mean age of patients in group 2 was 118 months (about 9 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 3 days after surgery.

Table 2 highlights the comparison of the total amount of blood loss between patients treated during charity trips and permanent hospital. It was seen that the measured blood loss differed much in 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, in all surgeries, wound closure was performed with the use of BFP graft. No other techniques or additional relaxing incisions were used.

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. 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

Table 3 shows the results of the Kruskal Wallis test where we are looking at the difference between each age category and cleft type based on the amount of bleeding from the two groups. It was found that the difference between age categories is significant in group 1 (P <0.05). Thus, Pairwise Comparisons were done to investigate the partial difference in this category using superscript code. Based on the analysis, it can be said that there is a significant difference in the amount of blood loss between age categories of patients treated during charity

trips. The category of young child has significantly lower blood loss compared to the child, adolescent, and adult.

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

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

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

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

The aim of this study was 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 age were 90.52 months (±7 years) and 118 months (±9 years) for charity trips group and permanent hospital group respectively. In the previous study conducted by Katzel et al. (2009), palatoplasty is performed in patients aged between 6 and 12 months, resulting in satisfying outcomes [18]. Our different age range is due to the fact that our study was performed in a developing country with a poorly developed health care system causing a late diagnosis on 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 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 to be consistent with the condition in rural areas in Indonesia, thus, patients have to wait for a charity surgery at nearby village/city that further will delay 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, but 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 the rural areas. We have to take into account 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 to patients with CL/P are highly recommended.

Intraoperative time seemed to be correlated with the amount of blood loss in the present study. Longer operative time will result in higher amount of intraoperative blood loss. One contributing factor might be that the surgeries were performed by a team of surgeons and not all of them were experienced in performing palatoplasty using DOZ technique with BFP graft. Nevertheless, no blood transfusions were deemed necessary to any of the patients because pre-operative screening was performed according to the Practice Guidelines for perioperative blood management (2015) as 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 the blood loss by 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. In this study, the amount of blood loss was measured by 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 that weight in mg can be converted into mL [5,17]. This method, however, might still underestimate the amount of blood loss because of technical reasons such as a surgeon accidentally using the suction system, or the inability to measure the amount of blood loss left on the instruments, gloves, and surgical drapes. Postoperative blood loss was also not measured because the present clinical setting prohibited that to be performed. Nevertheless, we still think that the last point was not detrimental to the study because our focus was on the intraoperative blood loss during palatoplasty.

CONCLUSION

Our results suggest that DOZ Furlow palatoplasty combined with BFP graft is a relatively safe procedure. This study found that the procedure resulted in minimal to mild amounts of blood loss, however, higher weight and longer operation time caused significantly more blood loss. The first recommendation from this study is to operate patients at an earlier age. Operating on young children does not only reduce the amount of intraoperative blood loss but also gives the patients a better start at life. Our second recommendation is to shorten the operation time as much as possible since this will significantly decrease the amount of intraoperative blood loss. Furthermore, standardized postnatal holistic planning is recommended in Indonesia for the improvement of cleft care.

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CHAPTER 4

Stem cell-based tissue engineering for cleft defects: Systematic review and meta-analysis

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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 [5]. 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 disturbances [6], 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 conducted 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

Title and abstract screening were conducted by two independent reviewers (DSNK and SAA) to obtain full texts of all eligible articles. Disagreements in determining the eligible articles were resolved by discussion. A third reviewer (FNH) was consulted for statistical analysis and, if necessary, to evaluate the articles. Three authors (DSNK, SAA, and NEN) reviewed the full-text articles and decided on the final eligible studies based on the inclusion and exclusion criteria. A summary of the whole screening process is presented in Figure 1.

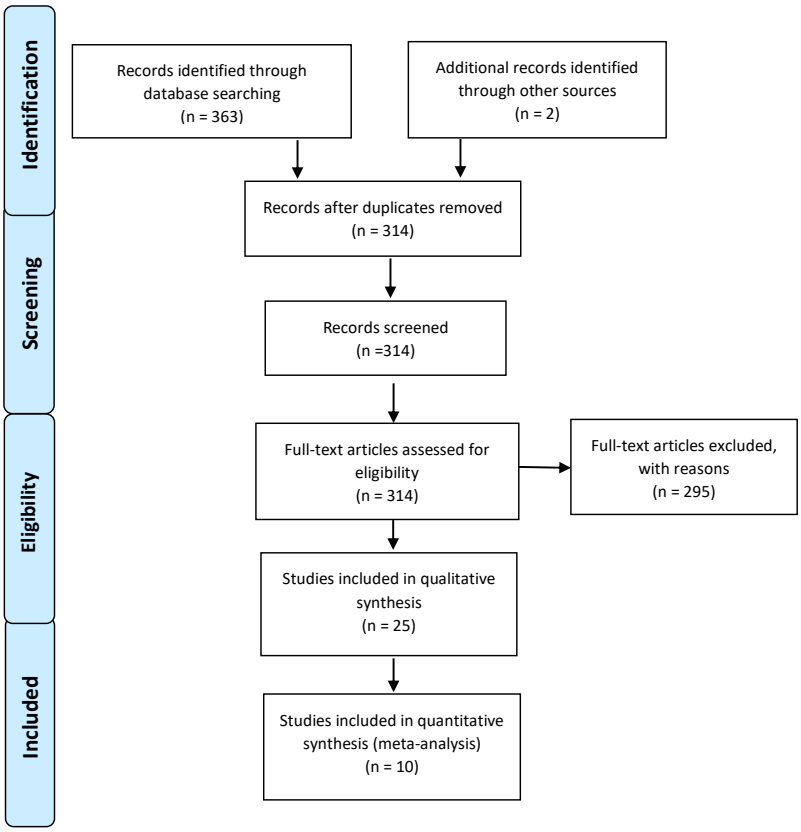


Figure 1. Flowchart of the study selection process

The extracted data from the eligible articles were the following: 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, dose/concentration), control group, observation time, method, and result (histomorphometry, CT, other). Descriptive data of the included studies were stored in tables. Quantitative data of histomorphometric new bone formation (%NBF), radiographic assessment of bone formation via computerized tomography (CT) or micro-CT (%NBF), histomorphometric assessment of remaining defect (RD), and/or radiographic assessment of RD or BMD using CT or micro-CT data were extracted for possible meta-analysis. If data were only expressed graphically, numerical values were requested from the authors, and if no response was received digital ruler software was used to measure graphical data (ImageJ; National Institutes of Health,

Bethesda, MD, USA). When studies reported outcomes at multiple time points, outcomes from the latest time points were extracted. The outcome data from similar time-points of different studies were pooled for meta-analysis by DSNK and NEN. 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 days0029. The characteristics of the included studies were summarized in Tables 1 and 2.

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	Immuno competent Lewis rats (alveolar cleft)	16 (4 dropouts, total 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/Chitosan/ Gelatin + rhBMP-2 3) HA/Chitosan/ Gelatin + hUMSC + BMP-2	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 Pura Matrix™ (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	Immuno competent Sprague-Dawley 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 2: NBF 60.5% ± 6.9% Micro-CT (8 weeks) Group 1a: 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 implant

No	Study	Model	Number	Cell Source	Scaffold	Growth Factor	Experimental group(s)	Control Group	Results
5.	Korn 2020	Immunocompe-tent Lewis rats (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 (60° rotated layers) only 1c) Scaffold B (30° 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.	Shahna-seri 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 (immune status unknown) (alveolar cleft)	24	Xenogenic (hUMSC)	Bone collagen particles (HA and collagen 1) + collagen membrane	n.a	2) Bone collagen particles (Collagen+HA) + hUMSC	1) Empty control 1b) Bone collagen particles (HA and collagen)	Histology (6 months) Group 1a: no NBF Group 1b: Small amount of NBF Group 2: Large portion of NBF Micro-CT (6 months) 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 (Michael Fanning Farms, Howe, IN) (alveolar cleft)	22	Autologous (UC-MSCs)	PLGA	BMP/CS nanoparticles	2) Undiff - MSCs 3) Diff - MSCs	1a) Empty control 1b) Autologous ICG	CT (30 days) Group 1a: NBG 1.94 ± 1.35 mm3/kg Group 1b: NBG 2.58 ± 2.83 mm3/kg Group 2: NBG 7.23 ± 4.99 mm3/kg Group 3: NBG 5.82 ± 4.48 mm3/kg
10.	Korn 2017	Immunocompe-tent Lewis rats (alveolar cleft)	84	Syngenic (BMSC donor adult Lewis rats)	bHA	n.a	2) bHA 3) Undiff -MSCS + bHA 4) Diff – MSCs + bHA	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

No	Study	Model	Number	Cell Source	Scaffold	Growth Factor	Experimental group(s)	Control Group	Results
11.	Wen 2016	Immunocompe-tent 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 condition, 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	Immunocompetent 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.	Yuan-zheng 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	Immunocompe-tent 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

No	Study	Model	Number	Cell Source	Scaffold	Growth Factor	Experimental group(s)	Control Group	Results
16.	Raposo - Amaral 2014	Non-immunosup-pressed Wistar rats (alveolar cleft)	28	Xenogenic (human MSCs from orbicularis oris muscle of cleft patients)	1) Bio-Oss collagen 2) α-TCP-matrix	n.a	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.	Poureb rahim 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	Miniature 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 the group 1, extending from the apical aspect of the defect through the coronal extension 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 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

No	Study	Model	Number	Cell Source	Scaffold	Growth Factor	Experimental group(s)	Control Group	Results
20.	Zhang 2012	Immuno competent Sprague-Dway-ley 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) Autologous graft 3) β-TCP 4) BMSC/ β-TCP	1) Empty control	Histomorphometry (20 weeks after orthodontic treatment is completed) Group 1: no data Group 2: NBF 78.68±4.91% 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(s)	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 vDawley rats (Janvier Labs, Le Genest-Saint-Isle, France) (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	Wistar 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

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

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 cells [44] (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 scaffold [30,33], one article used autologous bone [35], 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 [27,28,41–43,45–47,29–34,36,37].

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].

Cleft Palate

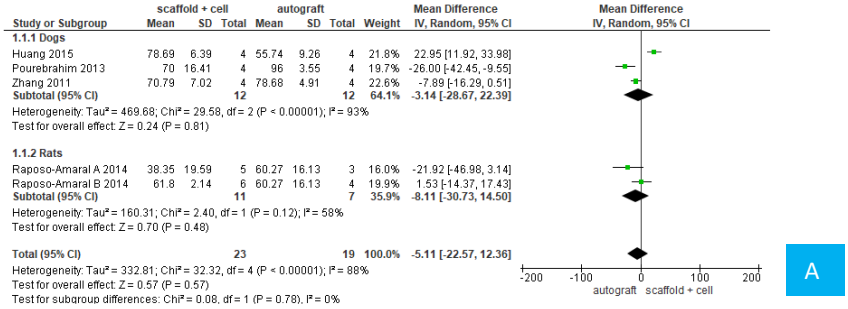
A total of four articles have provided information on cell-based tissue engineering in cleft palate animal models. Three animal groups were used, namely rat [17,48], dog [49], and rabbit [16]. Cell transplantation was comprised of only two types (autologous and allogenic). Bone marrow was the sole source 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.

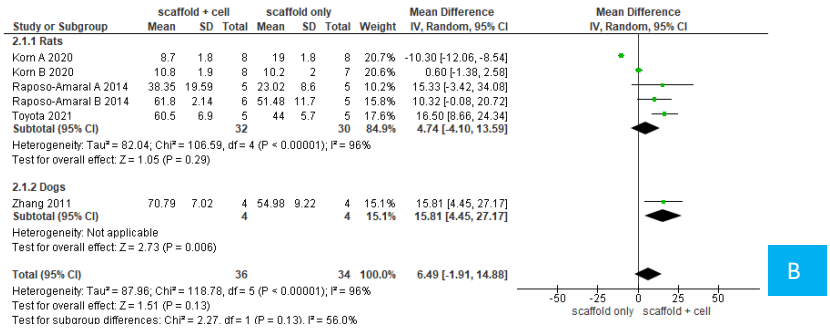
The osteogenic potential was assessed as the primary outcome parameter in all studies. One study expressed more bone formation clinically with cell + scaffold application for cleft palate reconstruction [17]. Three studies described higher bone formation levels for cleft palate reconstruction with cell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

Meta-analysis

Figure 2A 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. In the dogs' group, one study 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.





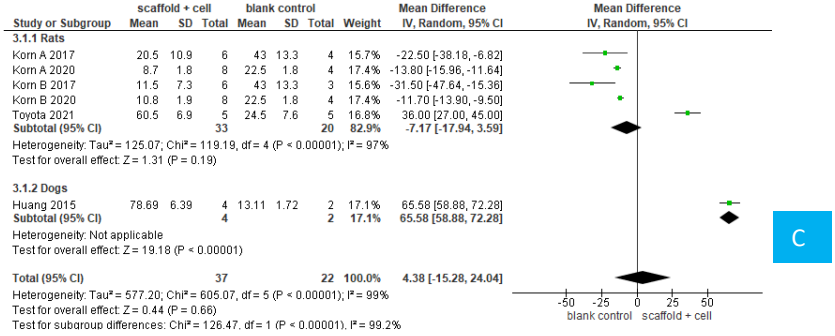


Figure 2. Forest plots for the new bone volume formation (%) histomorphometry analysis alveolar cleft in dog and rat models: (A) autograft vs cells-loaded scaffold group; (B) scaffold-only group vs cells-loaded scaffold group; (C) blank control group vs cells-loaded scaffold group.

Figure 2B 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 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 scaffold + cell group compared to scaffold only group [37] SMD of 15.81 [95%CI (4.45,27.17), P=0.006]. The overall result, although not significantly, favoured scaffold + cell over scaffold-only with a 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.

In figure 2C, 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 higher new bone formation of blank control compared to the scaffold + cell group [28,29]. One study reported higher new bone formation of the scaffold + cell group compared to the blank control group [41]. These studies showed a SMD of -7.17 [95%CI (-17.94,3.59), P=0.19, with heterogeneity I^2=97%]. In the dog's group, one study reported the higher new bone formation of scaffold + cell group compared to the blank control group [34] with a SMD of 65.58 [95%CI (58.88,72.28), P<0.00001].
The overall result, although not significantly, 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 3A 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 a SMD of 0.03 [95%CI (-1.19,1.24), P=0.97, with heterogeneity I^2=58%].

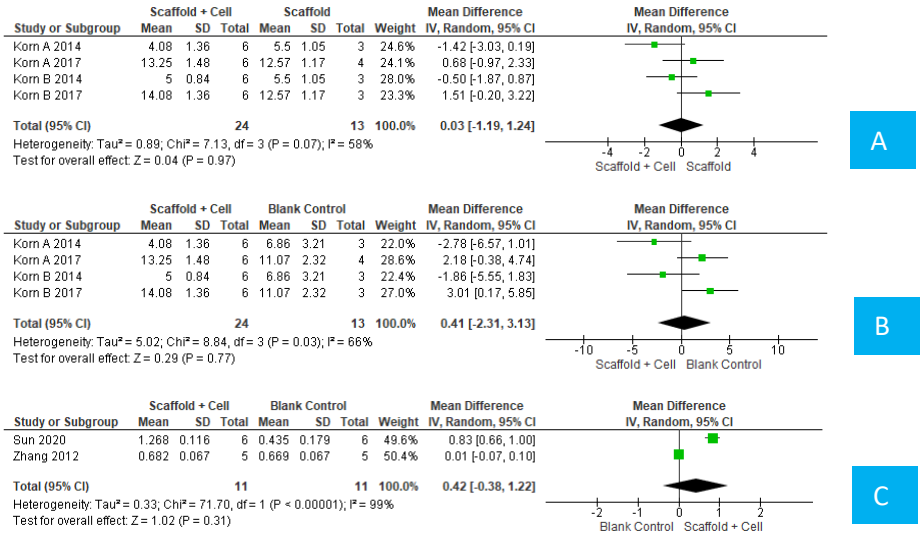
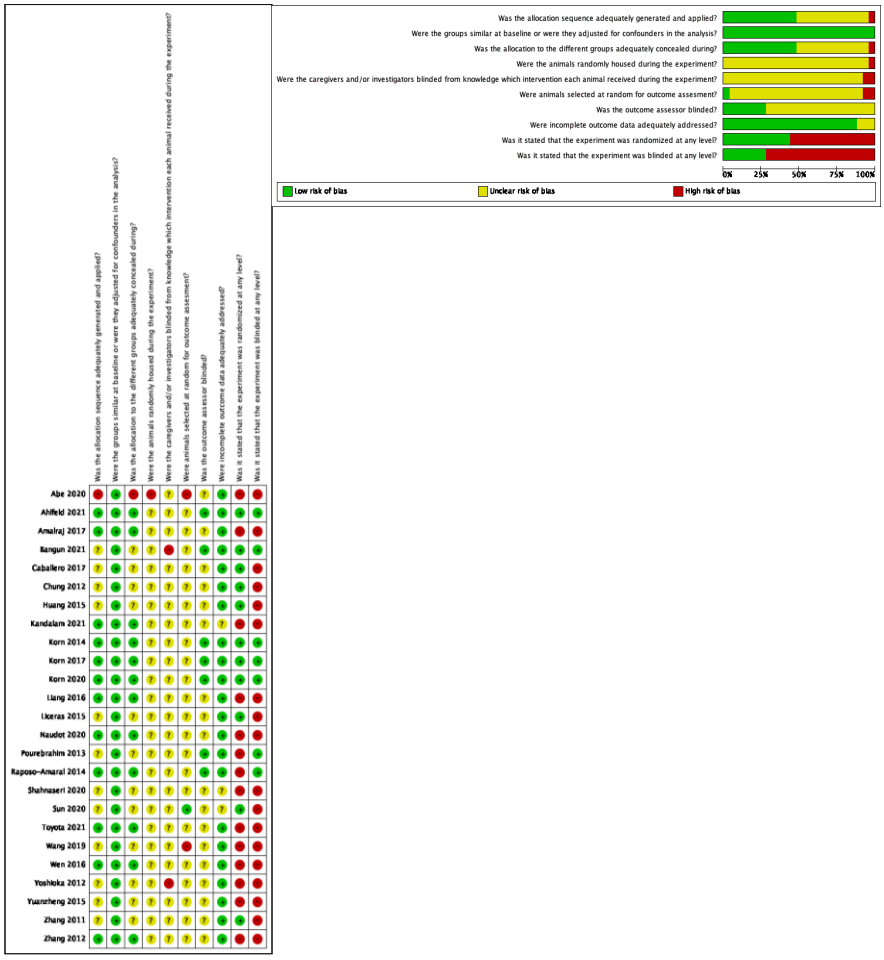


Figure 3. Forest plots for CT scan analysis in the alveolar cleft rat model: (A) the remaining defect volume of scaffold-only vs cells-loaded scaffold group; (B) the remaining defect volume of blank control vs cells-loaded scaffold group; (C) bone mineral density of blank control vs cells-loaded scaffold group.

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 3B. 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 showed a slightly lower remaining defect volume than the scaffold + cell group, with a SMD of 0.41 [95% CI (-2.31,3.13), P=0.77, with heterogeneity I^2=66%].

The meta-analysis evaluating the bone mineral density CT scan analysis of blank control group vs. cells-loaded scaffold group in the alveolar cleft rat model is shown in figure 3C. One study reported higher bone mineral density in the scaffold + cell group compared to the blank control group [42], whereas 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 a SMD of -0.42 [95%CI (-0.38,1.22), P=0.31, with heterogeneity I^2=99%].



Risk of bias within and individual studies

Figure 4. Risk of bias graph & summary: review authors' judgments about each risk of bias item presented as percentages across all included studies and as the item for each included study.

Figure 4 shows the overall results of the risk of bias assessment of the 25 studies included in this systematic review. Regarding 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 and 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.

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 [27,28,37–46,29,47,30–36] 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.

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CHAPTER 5

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 to investigate the efficacy of treatments. Nevertheless, risk of bias (RoB) can still affect the validity of these trials. We aimed to conduct a systemic review of all control trials (CT) CTs using regenerative materials for alveolar cleft reconstructions, to evaluate their RoB, and to perform a meta-analysis of new bone formation.

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

Results: A total of 15 trials met the inclusion criteria, none of which reached a full score. Of these, 20% didn't randomize the trails, 73,33% failed to describe the way of randomization, and none reported double-blinded criteria. Furthermore, allocation concealment (99.9%), intention to treat (100%), and patient awareness (100%) were inadequately described. The meta-analysis found no significant difference between regenerative materials and iliac crest graft.

Conclusion: This review showed high RoB in CTs implying 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 as a result of 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, the 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 kept in mind when choosing a grafting source such as the bone volume available, surgeons 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 the autogenous bone graft [13,14]. Hydroxyapatite (HA) is considered a good biomaterial, which shows high biocompatibility with negligible negative reactions. Hydroxyapatite provides osteoconductivity in bone formation [15]. β-tricalcium phosphate (β-TCP) is another reliable and highly biocompatible biomaterial that also uses the osteoconductive property in bone formation [16].

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

Moreover, stem cell therapy showed a promising alternative method to promote and to accelerate bone regeneration [19,20].

Multiple growth factors have also been used in regenerative alveolar bone graft 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 the differentiation of osteogenic cells to promote bone reconstruction and healing [21-23].

It is difficult to assess the term “quality”; it has been defined in RCTs as “the likelihood of the trial design to generate unbiased results” [24]. The application of proper quality assessment methods in RCTs shall enhance the validity of the trial results.

In order 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 on 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 autogenous bone graft.

MATERIAL AND METHODS

Study design

All studies on controlled CTs referring, in their title or abstract, to the utilization of regenerative materials in the treatment of alveolar bone defects were considered in this study. To be included, all studies must have a control group and intervention groups. Tissue engineering, cell therapy, growth factors, or a combination of these therapies are considered as regenerative medicine. Only human studies up to October 2020 in the English language 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, 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 given a score of 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 is given for either "no" or "do not know" answers. A higher score also indicates a low risk of bias. A score of 4-5 in the Jaded scale and 6-9 in the Delphi list considered as 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

For the meta-analysis of new bone formation by regenerative materials vs. autogenous bone graft, descriptive continuous data i.e., mean, sample size, standard deviation, and weight were used.

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 follow: I^2 < 30% - low heterogeneity, I2 = 30-60% - medium heterogeneity, I^2 > 60% - high heterogeneity [27].

RESULTS

Search Results and Study Characteristics

The overall primary outcome from Medline, Embase, Cochrane, and Google scholar included 112 articles (**Figure 1**). After the screening of the title and abstract, a total of 19 articles were obtained, and upon applying the eligibility, inclusion and exclusion criteria, the result of 15 studies was obtained and fully evaluated (**Table 2**).

Figure 1. Literature Search Strategy

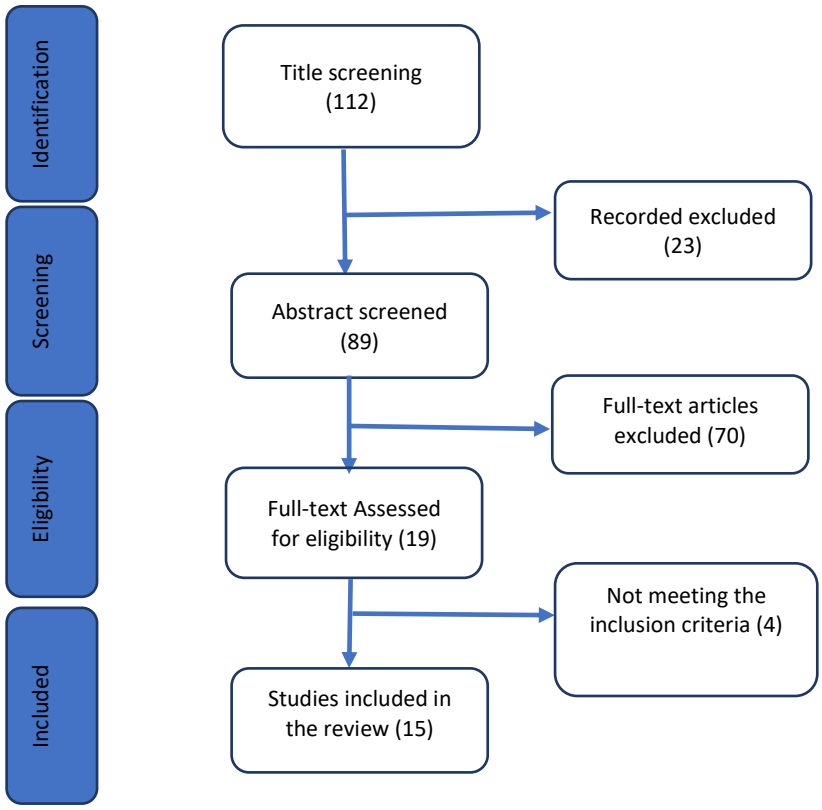


Table 2. List of the studies

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 volume. 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 have 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 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 defect, caution in large defect.
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 in bone volume or bone resorption

Study Id	Author, year	Study type	Number of patients	Control	Graft material/Test/ Intervention	Regeneration type	Result
11.	Khojasteh et al. 2017 [36]	CT	10	AIC bone and a collagen embrane	1. LRCP with BFSCs mounted on a natural bovine bone mineral 2. AIC bone, BFSCs cultured on natural bovine bone mineral	Cell	AIC shows least new bone formation. (AIC or LRCP) +BFSC shows the 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 + allograft +collagen membrane	Ixmyelocel-T (Stem cell) mixed with β -TCP and covered with collagen membrane	Cell	Safe to use stem cell therapy in alveolar cleft. Limitation in large alveolar defect.
13.	Al-Ahmady et al. 2018 [38]	CT	20	ICBG	BMMCs seeded on a collagen sponge in combination with Nanohy-droxyapatite 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 densiy 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 the 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 β-TCP group and the control group (iliac crest bone graft (IC)). They concluded that BMMCs with β-TCP is 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, but only reported that the combination of β-TCP with stem cell is safe to use, however, should be limited to not-too-large defects.

The third and the 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, while the second group underwent grafting using anterior iliac crest (AIC) bone and BFSCs cultured on natural bovine bone mineral. Khojasteh et al. (2017) revealed that no statistically significant differences were found in bone regeneration rates among all groups, although 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, and the study concluded that the combination graft (Chin graft + allogeneic bone + leukocyte + PRF) can 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 & Seifeldin (2016) compared a combination of PRF with anterior iliac crest bone graft (study group) to anterior iliac crest bone graft only (control group). However, in this study the quantity of new bone formation was significantly higher in the study group. The quality of bone, on the other hand, was lower in the study group but without a significant difference.

One of those ten studies (Huang et al. 2018) used a CGF (concentrated growth factors) preparation as a regenerative material combined with ICBG (CGF+ICBG) and compared it to acellular dermal matrix combined with ICBG (ADM+ICBG) in

alveolar grafting. Although there was a significant increase in bone density in the CGF+ICBG group, there was no significant difference between the two groups in terms of bone resorption.

In the fifth study which was conducted by Shirani et al. (2017), plasma rich growth factor (PRGF) was combined either with autogenous graft or allograft in alveolar bone defects, and differences in bone formation were assessed. They concluded that the autogenous bone graft combined with PRGF resulted in a significant increase in bone regeneration compared to the allograft counterpart.

Four out of ten studies used rh-BMP-2 mixed with collagen in comparison to ICBG. Three studies showed no significant difference in term 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.

Only one study (Marukawa et al. 2011) investigated the use of PRP combined with autogenous bone graft in alveolar grafting (interventional group) and compared it to a standard ICBG (control group). They found less bone resorption in the PRP study group. This difference was statically significant.

In our included studies, two reports used a combination of cells and growth factors. In one study, the effect of regenerative combination (ICGB + marrow graft+ PRP) was compared to grafting the alveolar cleft with autogenous bone only. The difference in resulting bone volume between of the two groups was not statistically significant (Sakio et al. 2017). In the other study, the regenerative combination (BMMCs + collagen sponge + Nanohydroxyapatite + autogenous PRF) was compared to iliac crest bone graft (ICBG). The regenerative combination showed better bone regeneration (Al-Ahmady et al. 2018). They stated that using autogenous bone marrow mononuclear cells (BMMCs) in combination with nanohydroxyapatite and PRF is a reliable alternative treatment for alveolar bone defect and showed a complete bone union in 90% compared to only 70% for the control group (ICBG).

Risk of assessment result

In our study we used the Jaded scale and the Delphi list as tools to assess the quality of trials design (**Figure 2,3**). The results of this analysis showed high risk of

bias (RoB) in all the included trials. In general, the mean value of the Jaded score was 1.2 with a SD of 0.909, while the mean value of the Delphi list was 3.13 with a SD of 1.454 (**Table 3**). Overall, the items that showed high RoB were noticed in both the Jadad scale and the Delphi list as following: blindness, intention to treat analysis, concealment of allocation, patient awareness, and provider awareness. The mean (SD) of randomization score was 1.06 (0.679) with a percentage of 80% in 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%) was the care provider blinded to the treatment used, and none of the studies (0%) reported that the patients were blinded to the treatment allocation.

Figure 2. Jadad scale scores

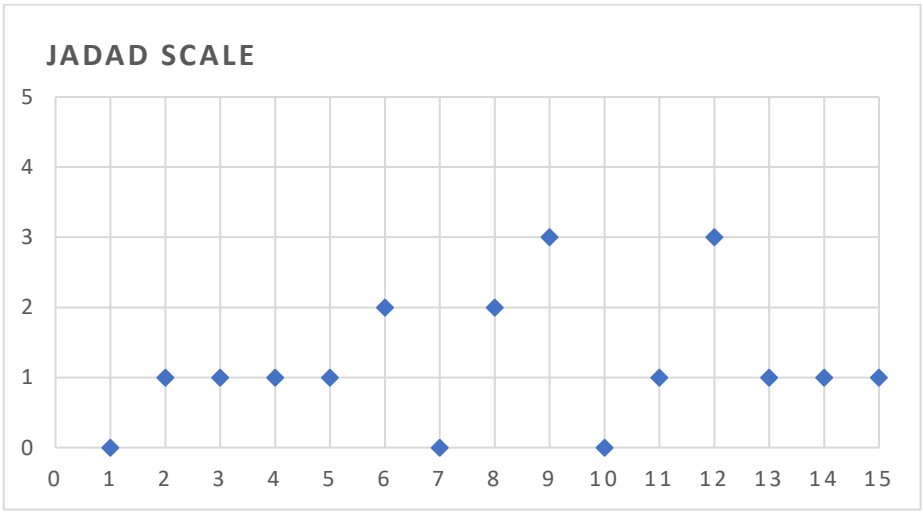


Figure 3. Delphi list scores

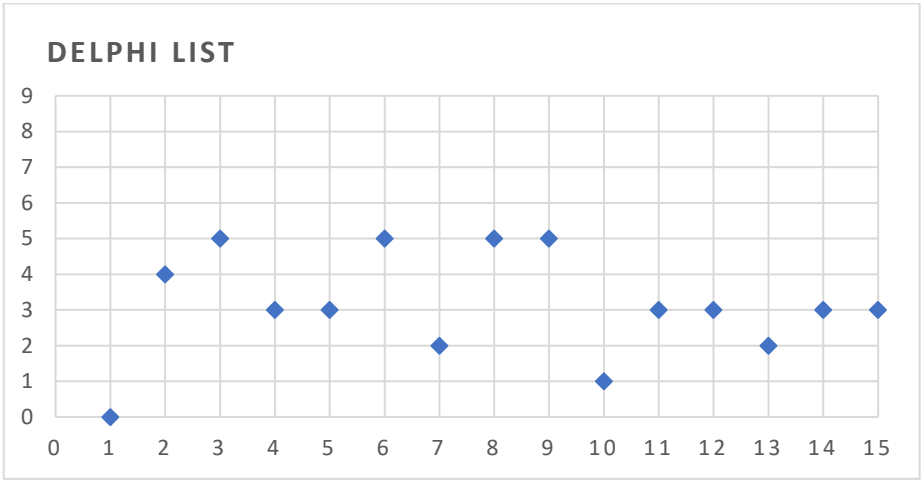


Table 3: Scores/risk of bias (n= 15 in each case)

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)

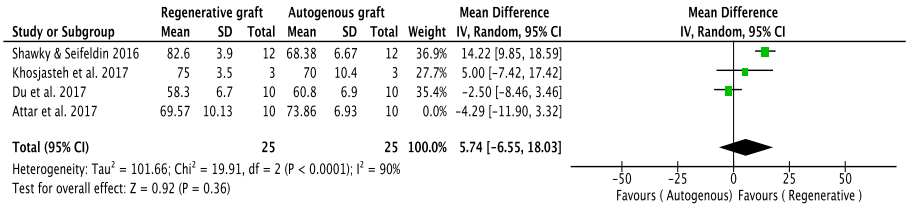
Meta-analysis

Three of the 15 controlled CTs could be included in the meta-analysis. These studies have measured new bone formation using regenerative methods and compared them to autogenous bone. Three studies [Du et al. (2017), Khojasteh et al. (2017) and Shawky & Seifdin (2016)] evaluated new bone formation after 6 months post-operatively. Two studies used the BMMCs and BFSCs stem cells (Du et al. 2017; Khojasteh et al. 2017; respectively), and one study used PRF as growth factors source (Shawky & Seifeldin 2016).

The meta-analysis revealed favorable new bone formation when regenerative tissue engineering methods were used compared to autogenous bone graft, however, the difference was not significant (P value = 0.36) with high heterogeneity I2= 90% (**Figure 4a**). In contrast, another study conducted by Attar et al. (2017) also compared new bone formation rates by regenerative methods and autogenous bone, but this study favored autogenous bone graft over the regenerative approach. Of note, evaluation was only performed after 12 instead of 6 months. However, even when this study was included in the meta-analysis, the overall result revealed bone formation after the use of regenerative methods

was still favorable when compared to autogenous bone. Again, the difference was not significant (P value = 0.55) with high heterogeneity (I2= 89%) (**Figure 4b**). The P-value and the heterogeneity results indicate that the studies are neither comparable nor exploitable.

a:



b:

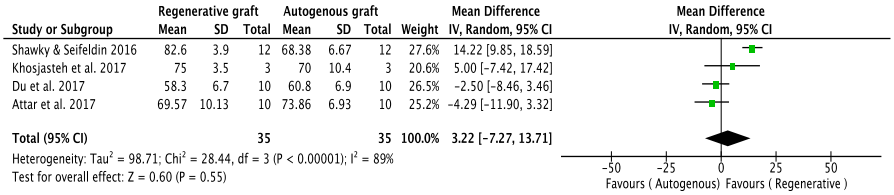


Figure 4 a, b. a: Three studies forest plot for cumulative weighted of the new bone formation rate in regenerative materials compared to control autogenous bone (Iliac crest graft), b: four studies forest plots for cumulative weighted of the new bone formation rate in regenerative materials compared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

DISCUSSION

The overall reason for considering regenerative medicine approaches over autogenous bone, in particular iliac crest bone, is to avoid a second surgical site and thereby the risk of co-morbidity. However, in order to be regarded 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 a myriad of regenerative approaches has been described in literature, not many were performed in a controlled trial design or RCT, even though this type of trials is considered the most optimal format for drawing conclusions based on evidence-based medicine.

Recently, multiple systematic reviews have been conducted in alveolar bone grafting. In 2018, Kamal et al. have published an interesting study in alveolar cleft tissue engineering, on which the study reviewed all the retrospective and

prospective clinical trials [42]. Therefore, we decided to update and confirm the result of that study, and to focus only on the prospective studies to evaluate the risk of bias. Osorio et al. 2020 have also discussed in general about bone substitutes in compare to autogenous bone graft. Although the review stated that rh-BMP-2 has shown a satisfactory result in their conclusion, the study did not use meta-analysis to evaluate the regenerative bone graft material results [43].

Another three systematic reviews were focused only on using rh-BMP-2 but we wanted to address all alternatives to autogenous used in 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].

The aim of our review was to conduct a systemic review of all CTs using regenerative materials for alveolar cleft reconstructions. In this review, the majority of these trials (10 out of 15) used growth factors as the regenerative method. Of these, four studies showed significant difference results toward the 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]. It is important to mention here that in the study of Shirani G et al. 2017, the PRGF used in both intervention (FDBA) and control group (ICBG), hence, a clear conclusion cannot be obtained with regards to the effect of the regenerative factor. Together, these studies show that different kinds of growth factors can be used and have been applied in CTs to repair alveolar cleft defects, of which rh-BMP-2 appears to be preferred and most promising, since it showed comparable efficacies in three studies and in one even superior bone formation in comparison with autogenous bone.

Three out of 15 used cellular therapies only [13, 36, 37]. The setups of these trials were very divergent: Du and colleagues found similar effects of the cellular therapy compared to autogenous bone, Khojasteh and coworkers analyzed two different bone sources both seeded with the same type of stem cells, and Bajestan's report only described that their cell therapy was safe but without reporting efficacy data. This makes it virtually impossible to draw conclusions.

Only two out of 15 studies used cell-growth factor combinations. The outcomes showed that the regenerative therapies showed 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 in our meta-analysis evaluation: it revealed favorable new bone formation when regenerative tissue engineering methods were used compared to autogenous bone graft, however, the difference was not significant (P value = 0.36) with high heterogeneity (I2= 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 in 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 [41]. At that time, it was concluded that due to insufficient evidence and controlled CTs, the treatment efficacy of tissue engineering in alveolar cleft bone defects could not be determined, and that well-designed controlled studies were needed so that detailed outcomes could be properly compared. Unfortunately, the current study reveals that up till now, no substantial improvement has been accomplished, and that also the studies performed since then suffered of a high RoB and insufficient design quality to draw evidence-based conclusions. Thus, there is still a strong advocacy for markedly improved trials in this field.

CONCLUSION

Alveolar cleft grafting CTs using the regenerative materials have a high risk of bias according to our review. 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 in term of avoiding risk of bias.

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CHAPTER 6

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 sides 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 6 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 feasibilty 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 a written approval by the ethical committee of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia with code number 1063/UN4.6.4.5.31/PP36/2019. On completion of the trial, the results on safety, feasibility, and bone formation with polyP as graft material will be published.

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

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 [20-22]. While other reports such as de Lange et al. showed that BCP has osteoconductive properties facilitating the bone formation and re- modeling in a maxillary sinus lift model [23].

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 the first-in-human.

Primary objective

To assess the safety of amorphous Ca-polyP MPs as graft material in the human alveolar cleft reconstruction model.

Secondary objective

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 combination with BCP granulate, will accelerate the quantity and quality of bone formation in a timely manner. Further, 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.

Eligibility criteria

Inclusion and exclusion criteria

After written informed consent will be obtained by research team member, the participant will be screened further for eligibility. Patients should be ≥ 15 years old, healthy male or female patients with an alveolar cleft bone defect, non-smoker, with no history of previous grafting procedure(s), 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, and have local infection, 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, 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 approach 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 1g: 1.5 ml and 1g:2g:3-5 ml respectively. A homogenous mixture should be reached before placing the graft material into the cleft defect. A good adaptation of bone graft material should be considered while placing it in cleft defect. No membrane will be used. A different graft quantity will be considered for larger defects, however, with the same mixing ratios. Absorbable sutures with 3/0 vicryl for mucosa, and 4/0 vicryl for nasal reconstruction will be used for closure. Post-operative, suitable antibiotic 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 Classification. Any change in health which occurs between screening examination and first administration of amorphous Ca-polyP microparticles or related procedures will be recorded as part of the subject's medical history, and full medical care will be given to all participants. In the case of a SAE, the sponsor will be reported within 24 hours 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 2x4 patients, with the primary 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.

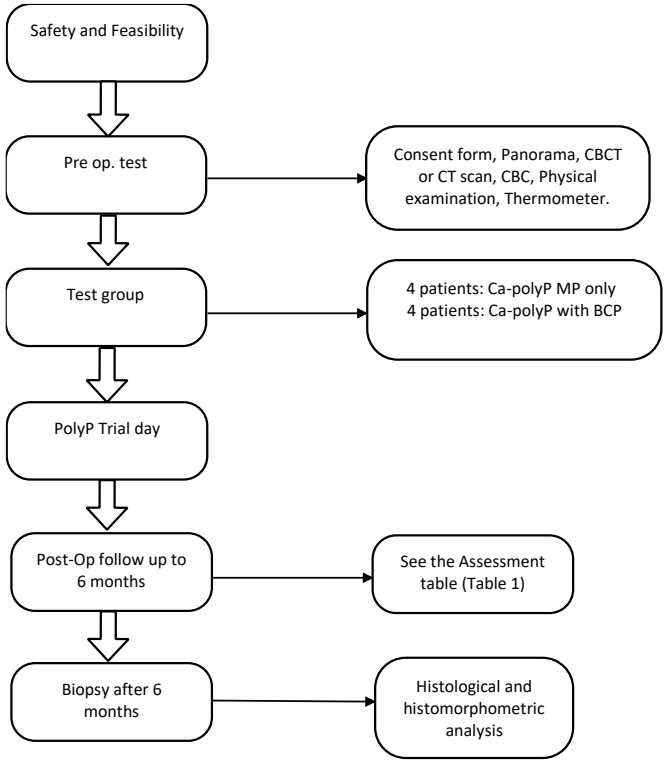
Randomization and treatment allocation

Because of this is a first in human study, it is not possible to keep all personnel blinded to assignment group. After written informed consent will be obtained by the main surgeon, randomization will be performed with regard 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 (eg, 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 [24].

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 evaluation of hard tissue formation. After sectioning, different stainings (Goldner's trichrome, Toluidin blue, Tartrate-resistand 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 disagreement between observers, the specimen will be re-evaluated to reach 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. 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 three years to ensure their safety and to record any delay side effect 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 piv- otal 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 [25] and to increase progenitor cell differentiation into osteoblasts [15, 26]. 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 [27, 28]. 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 posi- tive 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 [29–34]. Some reports describe that BCP may also have osteoinductive properties [35], which implies that BCP may add to the osteoinductivity as well. Moreover, a recent clinical study applying microstructured β-TCP for alveolar cleft repair demonstrated that calcium phosphate could be used safely and effectively for this purpose as well [36]. 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 demon- strated before in other bone regeneration studies per- formed by our group [30, 31, 37, 38].

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 alveolar cleft model.

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CHAPTER 7

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 present in blood, primarily in platelets. PolyP plays a role as phosphate source in bone calcium phosphate deposition. Moreover, the cleavage of high-energy bonds to release phosphates provides local energy necessary for regenerative processes. In this study, polyP is complexed with calcium to form Calcium polyP microparticles (Ca-polyP MPs), which were shown to have osteoinductive properties in preclinical studies. The aim of this study was to evaluate the feasibility, safety and osteoinductivity of Ca-polyP MPs, alone or in combination with BCP, in a first-in-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 residual alveolar bone cleft. Randomization in two groups (four receiving Ca-polyP MPs only, four a combination of Ca-polyP MPs and biphasic calcium phosphate (BCP)) was performed. 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 not possible due to COVID restrictions.

Results: Due to surgical and feasibility reasons, eventually only 2 patients received Ca-polyP MPs, and the others the combination graft. All patients were assessed up to day 90. Four out of eight were able to continue with the final assessment day (day180). Three out eight were unable to reach the hospital due to Covid-19 restrictions. One patient decided not to continue with the study.

None of the patients showed any allergic reactions, or any remarkable local or systematic side effect. 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 alveolar cleft repair.

Trial registration: Indonesian Trial Registry under number INA-EW74C1N by the ethical committee of 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 craniofacial region and considered as the second most common congenital deformity after the clubfoot [1]. An alveolar cleft is seen in 75% of the CLP patients [2,3]. Alveolar bone grafting (ABG) is an essential functional and esthetic procedure to reconstruct the bony defect in the maxilla as well as the nasal floor [4]. ABG not only plays an important rule to facilitate teeth eruption, but also to fill the bony defect by closing the oronasal fistula that routinely occurs in alveolar cleft patients.

The alveolar bone grafting can be performed either by using autogenous bone, allograft bone, or bone substitutes. Autogenous bone graft is still considered as 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 raise from harvesting bone at the donor site [6-8]. Risks such as gait disturbance, hematoma, donor site morbidity and other concerns that are associated with the growth (through harvesting from the rib or the iliac crest), could be avoided if having a good allograft or bone substitute material [9].

Polyphosphate (polyP) is a molecule that is naturally present in platelets in the blood stream. 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 anhydrides linkages as well as the metabolic process in the cells. Such signals could accelerate the cell growth and differentiation [15].

On the other hand, Biphasic calcium phosphate (BCP) is another type of graft that contain a phosphate molecule mixed with Hydroxyapatite (HA) in different ratios. Ambivalent outcomes have been reported to the BCP as graft material; some stated that the BCP has osteoconductive characteristic [16,17], while others concluded that it also can be osteinductive in nature [18,19].

Objective

This first-in-human study aims to evaluate the safety, feasibility and osteoinductivity of Ca-polyP MPs, alone or in combination 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 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

This study enrolled eight patients with residual alveolar bone cleft. The inclusion criteria were non-syndromic, nonsmoker, age of ≥ 13, no history of previous grafting procedure(s) and ASA1 regarding anesthetic risks. The exclusion criteria were systemic diseases, syndromic patients, localized infection, active influenza, obvious malnutrition or patient under any active medical treatment. Randomly using closed envelopes, four out of eight patients were selected to receive the Ca-polyP MPs alone, while the other four patients were to receive a mixture of Ca-polyP MPs and BCP as a graft material. However (see the “Results” section), eventually, two patients only received Ca-polyP MPs alone, while six received the mixture. The surgeon and the patients were revealed to the graft type, nevertheless, the assessor was kept completely blinded from the patient grouping. The time schedule of the surgical procedure and follow-up moments is presented 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 in order to minimize the risk of the graft exposure in case of any adverse effect. The current trial sample were limited to only 2x4 patients, with the primary goal to gain a first insight on the feasibility and safety of the treatment with polyP.

Randomization and treatment allocation

After written informed consent, randomization was performed with regard to the treatment group, but 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

Doctors, nurses, and rest of the research team were provided with a list of rules and responsibilities. The doctors and nurses collected the data according to the assessment Table 1. All research team members received training on how to collect data at all study visits. Each patient has been followed up to 6 months. Patient confidentiality was protected by the data manager.

Polyp and BCP preparation

PolyP graft comes in a form of Ca-polyP MPs powder produced by NanotecMARIN GmbH (Mainz, Germany), while 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. Full mucoperiosteal flap was reflected from 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 oro-nasal fistula (Figure 2a). A Ca-polyP MPs preparation or the Ca-polyP MPs and BCP mixture was applied into the alveolar cleft defect (Figure 2b). Tension free closure was realized in all wounds.

a b

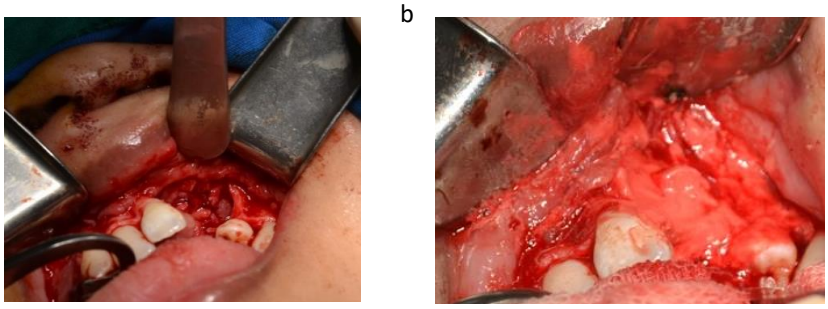

Figure 2. a: nasal floor reconstruction and exposing the bony edges, b; ca-polyP graft placed in the defect

Post-operative care

Oral hygiene instructions were given to all patients including mouth rinsing with 0.12% Chlorhexidine. Antibiotics (Amoxicillin / Clavulanic acid) and pain killers were prescribed for 7 days according to the standard of care. During 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 then subsequently after 8, 30, 90 and 180 days. The OPGs were used to assess the vertical graft formation employing the Bergland scale, which is 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 a normal height, grade II: a bone height at least 75% of 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, and 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 were able to comply with the study requirements up to assessment day 90. Unfortunately, four out of eight patients were unable to continue with the final assessment (day 180). One patient decided not to continue with the study, while the other three patients were unable to approach the hospital due to the Covid-19 lockdown at their towns/villages (Table 2).

All eight patients underwent bone grafting surgery by the same surgeon. There were no reported postoperative complications, local or systematic, in both study groups. All patients were in close follow-up from day 1 until they were discharged from hospital (day 3). Thereafter, the patients were followed up according to Table 2. Although not included in the initial trial design, all patients were contacted with video or telephone calls up for a 1-year follow-up. No adverse events were reported, and all patients reported that they were content with the treatment.

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

Feasibility

Two different application modes of Ca-polyP-MP should have been tested in a randomised manner, but as a consequence of the difficulty to handle Ca-polyP

microparticles when not complexed with BCP, we had to abandon the randomization of graft type and applied the BCP-polyP graft type only. Thus, feasibility appeared valid for the combination graft, but not (in the current setting) for the application of Ca-polyP MPs only.

Safety

Adverse events

The main goal of this study was to evaluate the safety of the Ca-polyP MPs, alone or in combination with BCP, in terms of adverse events (local or systematic) using clinical assessment, radiographic, and laboratory investigations (a.o. white blood cells, neutrophil, lymphocyte, and if needed C-reactive protein) (Table 3). All patients were kept hospitalized postoperatively for 72 hours to maintain close follow-up. In the case of a SAE concerns severe toxicity or infection associated with the graft site, the trial would be terminated immediately.

Osteoinductivity

Since acquirement of biopsies was not possible 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	Within normal limits	Within normal limits	Within normal limits	Within normal limits	Within normal limits	Within normal limits	Within normal limits	Within normal limits

Ca-polyP MPs: Calcium polyphosphate microparticles, BCP; Biphasic calcium phosphate, ND; nothing detected

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.

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

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

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 at both day 8 and day 180. Likewise in the Ca-polyP MPs-BCP group, the CT scans 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 be coinciding 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

There were no complications reported intra- and/or post-operatively in both study groups.

DISCUSSION

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

The optimum age for the alveolar bone grafting is considered to between 9 - 11 years old [20,23]. Since we did not want to enroll children in a safety study with this novel material in clinical practice, we chose to only include older adolescent and adult patients, being capable themselves to be involved in decision making. We performed this study in Indonesia, because non-operated patients in this age group are difficult to find in Europe.

In the Ca-polyP MPs group, the main challenge was in the handling and application of the material in the alveolar defect. The characteristics of the Ca-polyP MPs can be determined by 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 resulting Ca-polyP MPs graft material was easily lost from the surgical sites once it got saturated with blood, which made maintaining a space-occupying scaffold within the alveolar defect virtually impossible. We therefore had to conclude that the physical characteristics of the Ca-polyP MPs used as a stand-alone scaffold material were insufficient and unfeasible. As a consequence, we had to reduce the Ca-polyP MPs only group to 2 patients instead of 4 patients as planned originally in the study protocol. Retrospectively, the reason that the microparticles were previously shown to be effective in bone formation in preclinical studies may be due to the location used: it was implanted in a subcutaneous pocket instead of a not well contained, large void such as the alveolar cleft [24,25].

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 in general have been used as a graft material several times in craniofacial surgery before. For example, Levitt et al. had already used calcium phosphate in 1969 for this purpose, and calcium phosphates were subsequently used in dental implant, 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 [26]. Based on our results, we recommend that to achieve 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 sequestration of the polyP on or within the carrier, of which we could not be sure in the current study.

Our study was limited by several aspects, the most severe being the COVID-19 pandemic allowing only 4 patients to be evaluated after 180 days of follow-up and thereby resulting in a rather short postoperative follow-up period. Another limitation was the rather radiolucent characteristic of the Ca-polyP MPs, which hampered visualization of the graft in radiographic images considerably and making evaluation with the Bergland scale virtually impossible. We also tried the Chelsea scale [27], which analyzes the bone position in relation to the adjacent teeth on the grafting site radiographically. However, this did not result in other outcomes as the Bergland scale, so we omitted these results. We can therefore not be completely sure whether defect filling was sufficient and if some initial bone regeneration events occurred, but at least no solid bone formation was demonstrated after 3 months, and also not in the one patient evaluated after 6 months. Last but not least, it may be that the choice to include only adolescent and adult people in our study and to exclude prepuberal children may have affected the efficacy of the treatment. Bone formation activity usually has its highest peak during puberty, and our post-puberal patient population may therefore have more restricted bone formation capacity per se. In addition, the cleft defects in our patients were mostly rather large, thus reducing the likeliness of effective bone regeneration as well.

To our knowledge, this study is the first clinical trial to investigate the safety and feasibility of polyP, either as Ca-polyP MPs alone or in combination with BCP in humans. A histological examination of the bone at six months was not performed due to the COVID 19 restrictions in Indonesia, which hampered osteoinductivity assessment considerably. We could now only evaluate this aspect based on the radiographic results.

Despite this limitation, since we have now performed video/phone calls at 1 year post-operative, and all patients did report that they had no adverse events and that they were content with the treatment, this indicates that the treatment with polyP-containing grafts may be safe and in combination with BCP appears feasible for alveolar cleft repair. Nevertheless, new studies with a larger group of patients, biopsy evaluations, and suitable polyP formulations encompassing appropriate carriers such as BCPs or polymeric scaffold materials are required for sound conclusions about their regenerative capacities. Eruption of the teeth through the site, periodontal and health of the root surface of the adjacent teeth, orthodontic movement of adjacent teeth to the grafted site need to be taken into account as well.

CONCLUSION

Despite the small sample group size and some missing data points due to the COVID-19 pandemic, we were able to conclude that Ca-polyP MPs and the Ca-polyP MP/BCP composites appear to be safe graft materials, however, Ca-polyP MPs alone may not be sufficiently stable defect-filling scaffolds to be used in alveolar cleft repair.

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CHAPTER 8

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

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ABSTRACT

Background

Biphasic calcium phosphates (BCP) may serve as off-the-shelf alternatives for iliac crest-derived autologous bone in alveolar cleft reconstructions. To add osteoinductivity to the osteoconductive BCPs in order to reach similar regenerative capacity as autologous bone, locally harvested buccal fat pad will be mechanically fractionated to generate microfragmented fat (MFAT), which was shown to have high regenerative capacity due to high pericyte and MSC content and a preserved perivascular niche.

Objective

Primary objectives will be feasibility and safety of the BCP-MFAT combination. The secondary objective will be efficacy, which will be evaluated using radiographic imaging and histological and histomorphometrical evaluation of biopsies taken 6 months postoperatively, concomitant with dental implant placement.

Methods

Eight alveolar cleft patients (≥ 15 years) will be included in this prospective non-blinded first-in-human clinical study. MFAT will be prepared intraoperatively from the patient's own buccal fat pad. Regular blood tests and physical examinations will be conducted, and any (serious) adverse events ((S)AEs) will be meticulously recorded. Radiographic imaging will be performed prior to surgery, and at regular intervals after reconstruction of the alveolar cleft with the BCP-MFAT combination. Biopsies obtained after 6 months with a trephine drill used to prepare the implantation site will be assessed with histological and histomorphometric analyses after methylmethacrylate embedding and sectioning.

Results

The primary outcome parameter will be safety after 6 months follow-up, as monitored closely using possible occurrences of (serious) adverse events based on radiographic imaging, the blood tests, and the physical examinations. For efficacy, radiographic imaging will be used for clinical grading of the bone construct using the Bergland scale. In addition, bone parameters such as bone volume, osteoid

volume, graft volume, and number of osteoclasts will be histomorphometrically quantified.

Discussion/Conclusions

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

When an SAE occurs, it will be concluded that combination of MFAT and BCP 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 (eg. analgesics, antibiotics), the combination of MFAT and BCP will be considered safe. In all other cases, combination of MFAT and BCP will not be considered safe (yet).

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].

The results of this study will be published regardless of the trial outcomes.

Keywords

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

INTRODUCTION

Alveolar cleft is defined as a bone gap in the primary palate from the nasal sill to the incisive foramen [1]. The defect occurs as a result of 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 usually done during the mixed dentition period (6-11 years), which allows to support teeth eruption and facial growth [1]. Iliac crest as 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 the mandibular symphysis [5]. However, several studies have reported risks of general postoperative complications using autograft 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 are being evaluated 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 material with a mineral composition comparable to human bone tissue [10]. BCP has been mixed in vivo and in vitro with autografts, inducing factors, and/or cells to improve its osteoinductivity [11,12], also in the field of dentistry and maxillofacial surgery[13–15]. Although calcium phosphate ceramic is not yet considered as standard of care , it has been used for alveolar cleft reconstruction with satisfactory results [16] provided support for teeth eruption [17].

Adipose tissue is one of the mesenchymal stem cell (MSC) sources, and adipose stem cells (ASCs) can be collected with minimum risk and discomfort from the buccal fat pad (BFP) [18]. BFP surrounds 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 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, which also takes considerably less time, is by processing the adipose tissue mechanically into MFAT [22]. MFAT is reported to have similar or even higher secretory activity of regenerative growth factors and cytokines and pericyte content compared to enzymatically derived stromal vascular fraction (SVF) [23]. In addition, the MFAT procedure can be applied even in regular hospitals because its harvesting and processing does not require a major invasive surgery, specialized equipment or expensive disposables, or good manufacturing practices (GMP)-qualified cell culture expansion. Autologous application of MFAT has among others been used with success for clinical reconstructions in the maxillofacial area [24].

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

MATERIAL AND METHODS

Study design

This first in human surgical study can be classified as a “stage 1” study according to the IDEAL framework [25]. It is a single-center prospective clinical trial comprising 8 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 containing 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 patients’ own buccal fat pad (BFP) which is 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 6 months. At each follow-up visit, adverse events (AEs) and/or serious AEs (SAEs) will be documented, 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 subsequently 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.

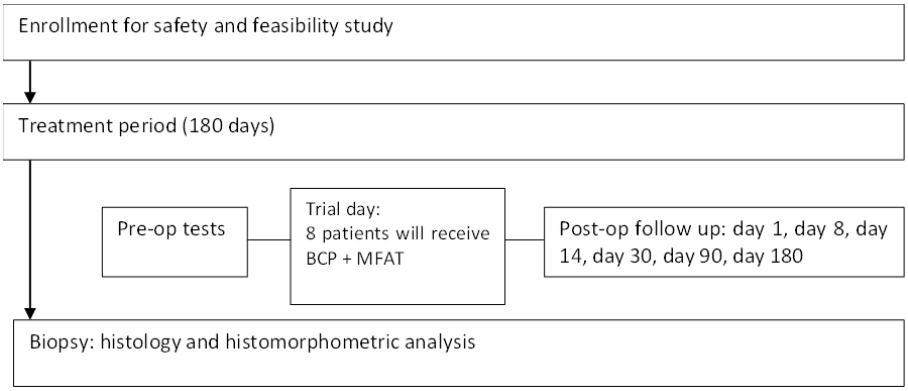
Ethical Considerations

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].

Participants will be recruited from general practices of Hasanuddin Dental Hospital and in the area around Makassar. Since we did not want to enrol children in a safety study with this novel concept in clinical practice, we chose to only include older adolescent and adult patients, being capable themselves in decision making. Within Indonesia this age group is more readily found, due to cultural and religious backgrounds causing abstinence from cleft surgeries.

The trial will be conducted in Hasanuddin Dental Hospital. All participants shall be asked to sign an informed consent after risks and possible complications of the procedure (e.g., bleeding, infection, cheek asymmetry, parotid duct injury, possibility of facial nerve branches injury, and (although not likely) non-closure) were appropriately communicated with the patient. Data will be handled and stored in coded i.e., de-identified format, so that data cannot be traced back to the patient without a decoding key, which is kept in a locked place and only accessible to the study PI. Implants will be offered free of charge. This study complies with the principles of the Declaration of Helsinki.

Figure 1. Simplified diagram of the study protocol



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 normal blood count

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

1. Having poor oral hygiene with mouth plaque
2. Having systemic disease
3. Having systemic or local infection
4. Having received chemotherapy, radiotherapy, immunosuppressives, or anticoagulants that may interfere with the healing process
5. Having 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 in order to obtain approximately 3cc fat. After vasoconstrictor infiltration with adrenaline 1:100,000,

a full mucoperiosteal flap spanning the first molar to the central incisor is lifted. After exposure of the full alveolar cleft and to separate the nasal layer from the oral mucosa, the tissue was meticulously dissected. Following the reflection of a palatal mucoperiosteal flap from either side of the cleft, the palatal tissues were elevated. The oro-nasal fistula was repaired cranially by elevating and suturing the nasal mucosa [4], thereby creating a pocket for BCP-MFAT deposition.

In parallel with the defect surgery, the harvested fat will be chopped into small pieces with a scissor and soaked in normal saline for 10-15 minutes. The normal saline then will be drained and the chopped fat will be processed into MFAT using 2 syringes (size 10cc) connected with the Tulip Gen II Nanofat^{TM} Kit single use sizing transfer 1.2 mm (Tulip Medical, California, United States) according to manufacturer's protocol. MFAT will be mixed with BCP (Straumann Bone Ceramic, Villeret, Switzerland) in a ratio of 1g per cc until it reaches a homogenous consistency. The BCP-MFAT mixture will be placed as a graft material into the alveolar cleft defect. If the defect is large and requires more bone graft, another mixture will be made with the same mixing ratio. If necessary, a membrane will be used to cover the grafted defect. Finally, the defect will be closed by suturing the palatal mucoperiosteal flaps using absorbable sutures with 3/0 vicryl for mucosa and 4/0 vicryl for nasal reconstruction. All patients will be prescribed with antibiotics and painkillers postoperatively.

Table 1. Patients' assessments

	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 the health of subjects will be documented in their medical history, and required medical care will be given. Any unexpected physical and/or laboratory change, symptom, or disease which occurs 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 either serious or non-serious based on its 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 3 days from the date of onset. If the SAE concerns severe toxicity or infection associated with graft products, the trial will be terminated immediately.

Sample size

This is a first-in-human phase I clinical trial, aimed to obtain insight on the safety and feasibility 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 of the Hasanuddin University Dental Hospital. Informed consent will be obtained from candidates and/or their parents or legal guardian who are willing to join the trial after being fully educated about the trial procedure. Thereafter, it will be checked if the candidates fulfill the study inclusion and exclusion criteria. A thorough assessment and training regarding the safety measurements at the research site in the Hasanuddin University Dental Hospital will be performed prior to the trial by the ethical and surgical teams.

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 members of the research team which 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 in order to conceal the patients' identity), which will subsequently be handed over to the clinical evaluators and investigators. The primary endpoint is set at 6 months.

Post-trial care

After the primary endpoint assessment, they will be followed up for an additional period of 3 years to ensure their safety and to record whether any delayed side effect will occur as a result of the BCP-MFAT treatment, like previously done in a similar study [26].

Monitoring

Internal monitors of the Ethics and Research Committee of Faculty of Medicine, Hasanuddin University will evaluate whether the collection of data is

accurately done. 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], no data safety monitoring board will be installed. A safety report will be submitted every year to the Medical Research Ethics Committee of the Faculty of Medicine, Hasanuddin University. No interim analysis is deemed necessary.

Amendments

If deemed necessary, amendments to the current protocol will be submitted to the ethical committee and competent authority, and should be approved prior to implementation to ensure the safety and integrity of participants as well as the scientific value of the trial.

Evaluation methods

Safety assessment based on physical examination and laboratory measurements

When a SAE occurs, it will be concluded that combination of MFAT and BCP 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 combination of MFAT and BCP will be considered safe. In all other cases, combination of MFAT and BCP will not be considered safe (yet).

Radiographic analysis

To evaluate the success rate of the bone graft, the Bergland scale will be employed[30]. This scale will evaluate the integrity and height of the alveolar bone graft, and will classify the bone height into four grades: grade I, bone height is almost a normal height; grade II, a bone height at least 75% of the interalveolar septum; grade III, the bone height is less than 75%; and grade IV, no evidence of bone integration [31].

Histological and histomorphometric analysis

The histological and histomorphometric analysis will be performed in at least 3 patients who received dental implants after alveolar cleft reconstruction, according to previously published procedures [32] . Briefly, 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 (bone volume, osteoid volume, graft volume, and number of osteoclasts) will also be measured for quantitative analysis [32]. Two trained examiners will perform the histologic and histomorphometric analysis. In case of dispute, the biopsies will be re-analyzed to reach consensus.

Statistical analysis

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

RESULTS

The primary outcome parameter will be safety after 6 months follow-up, assessed by closely monitoring possible occurrences of (serious) adverse events, radiographic imaging, the blood tests, and the physical examinations. For efficacy, radiographic imaging will be used for clinical grading of the bone construct using the Bergland scale. In addition, bone parameters such as bone volume, osteoid volume, graft volume, and number of osteoclasts will be histomorphometrically quantified. We expect that the feasibility and safety of the procedure will be shown, as well as initial efficacy.

DISCUSSION

In recent years there has been increasing interest in the use of adipose tissue for cleft lip and palate reconstruction [33]. Its applicability mostly relies on the quantity of the tissue, the ease of surgical harvesting and the type of surgical reconstruction in which the tissue is used, for example correction of cleft lip volume asymmetry [34,35], improvement of velopharyngeal insufficiency after

cleft lip and palate repair [36,37], or as an extra flap in cleft palate repair [38–41]. In this study, we will make use of the BFP for bone reconstruction. BFP is a specialized adipose tissue rich in vascular supply that is easy to harvest via the oral cavity during an intraoral surgery with minimal morbidity and discomfort [42].

Up till now, there are only few reports on the use of adipose tissue as a regenerative compound for the bony cleft reconstruction: a phase I clinical trial by Khojasteh et al. [24] and an animal study applying adipose derived stem cells for alveolar cleft repair [43]. Both studies used collagenase digestion of the tissue and culture expansion to obtain adipose stem cells for personalized cleft reconstructions. An alternative is the SVF derived from adipose tissue via collagenase digestion, which requires a shorter time frame and may yield similar stem-cell like quantities, allowing intra-operative applications [44,45]. In a previous clinical study by Prins et al. [44] it was shown that addition of SVF in an intra-operative setting to calcium phosphate ceramics had an additive value on bone formation, implying that SVF can provide osteoinductivity when combined with calcium phosphate. However, so far regulatory issues and relative expensive SVF production procedures prohibit its wide applicability [22,23]. Mechanically processed fat or MFAT has emerged as a rapidly processing alternative to SVF, is being considered minimally manipulated, and thereby less regulation-restricted [22,23].

This is the first in human study evaluating a combination of MFAT and biphasic BCP as a regenerative graft for alveolar cleft reconstruction [46,47]. BCP is a ceramic scaffold with a balanced ratio between the less-soluble HA and the more-soluble TCP that results in mechanical and biological properties to support bone and cartilage tissue production [48]. It is sufficient for bone reconstruction in none-load-bearing applications and already accepted as standard of care for certain maxillofacial reconstructions [49].

Recently calcium phosphate has been applied for alveolar cleft surgeries as well [16,17]. Patients within that study were treated at ages between 9 -10 years, which is within the range of optimum age for SABG [1]. However, since we did not want to enroll children in a safety study with this novel concept in clinical practice. Therefore, although we realize that performing surgeries at a later age will (1) not make optimal use of the growth spurt; and (2) may result in cases having larger or

even critical size defects (which will not heal unless supplemented with grafts), we chose to only include older adolescent and adult patients, being capable themselves to be involved in decision making. We will perform this study in Indonesia, because non-operated patients in this age group are difficult to find in Europe.

This is primarily a safety study, so the main conclusions of the study will be based on safety parameters, in particular on the occurrence of (serious) adverse events

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CHAPTER 9

A prospective control clinical trial using microfragmented fat (MFAT) and BCP for the treatment of alveolar clefts

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ABSTRACT

Background: Bone substitutes such as biphasic calcium phosphates (BCP) are alternatives or adjuncts to autologous bone grafting in alveolar cleft (AC) reconstructions. Microfragmented fat (MFAT) as obtained by mechanical fractionation is reported to have high regenerative potency. The aim of this study was to evaluate the feasibility and safety of a BCP-MFAT combination for AC reconstruction in adult patients.

Methods: This prospective non-blinded first-in-man clinical pilot study comprised 8 patients. Reconstruction of the AC was performed with BCP mixed with MFAT prepared from the intraoperatively harvested Buccal Fat Pad. Patient follow-up was six months. Outcome parameters included safety parameters and possible adverse effects assessed by radiographic imaging, regular blood tests, and physical examinations. Osteoinductivity evaluation using histomorphometric analysis of biopsies was not possible due to COVID-19 restrictions.

Results: Mean age of the 8 patients was 22.13 (range 17-32) years. Assessments were up to 90 days for 6/8 patients, while 2/8 missed this follow up due to Covid-19 restrictions. Final assessment (day 180) was only possible for 4/8 patients, of which two were unable to reach the hospital due to Covid-19. Two patients discontinued their study participation. No allergic reactions or local or systemic side effects were noticed. Radiographic assessments (Bergland scale) ranged from I-III.

Conclusion: Our results indicate that the BCP-MFAT grafts appear to be safe and feasible for alveolar cleft reconstruction.

Clinical Trial Registration: The clinical trial protocol was approved by the ethical committee of Hassanudin University-Makassar, Indonesia [protocol 1063/UN4.6.4.5.31/PP36/2019] and registered in the Indonesian Trial Registry https://www.ina-registry.org [INA-EW74C1N].

Keywords: Alveolar cleft grafting, biphasic calcium phosphate, buccal fat pad, microfragmented fat.

INTRODUCTION

Alveolar cleft is defined as a defect in the primary palate and is the result of a disruption in the fusion of the primary palate [1,2]. Management of alveolar cleft often requires secondary alveolar bone grafting (SABG) during the mixed dentition period (6-11 years) in order to close the defect, restore maxillary arch continuity, facilitate tooth eruptions, and to support the nasal base [1]. The gold standard uses autogenous bone graft usually harvested from the iliac crest [1]. Additionally, bone graft sources include the cranium, tibia, and the mandibular symphysis [3,4]. The most common complications using autograft include pain, prolonged hospital stays, and donor site morbidities, for example, scarring and nerve injury [5–8]. Therefore, alternative materials have been studied for alveolar cleft surgery.

Biphasic calcium phosphate (BCP) is a bone graft that consists of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) in variable amounts[9]. The combination made BCP an alternative bone graft material that has a good biocompatibility with osteoinductive properties [9]. Combination of BCP with autografts, inducing factors, and/or cells has been demonstrated to improve osteoinductivity in vitro and in vivo [10–13]. Studies have reported its applications in the field of dentistry and maxillofacial surgery[10,11,14] specifically for alveolar reconstruction where it helped to acquire satisfactory bone reconstruction result [12,13].

Adipose tissue contained multipotential stem/progenitor cells commonly referred as mesenchymal stem cell (MSC) that can be harvested from the buccal fat pad (BFP) [15,16]. The BFP is an encapsulated fat mass between the buccinators muscle and other superficial muscles such as the masseter, the zygomaticus major, and the zygomaticus minor [17]. Autologous application of BFP derived cells has been used successfully for clinical reconstructions in the maxillofacial area [18]. Moreover, multiple studies have shown that the cell yield of ASCs is larger than of MSCs in bone marrow aspirates [15,16,19]. Enzymatic preparation of ASC is still the most used method which in most countries is still considered as “more than minimally manipulation”[20]. An alternative method is by disaggregating the adipose tissue mechanically into small fat particles, so called microfragmented fat (MFAT) [21]. Intact microarchitecture of MFAT preserves similar or even higher number of regenerative cells to enzymatically derived

stromal vascular fraction (SVF) [22]. In addition, the MFAT can be harvested and used directly in the operating room, thus avoiding any enzymatic process or laborious cell culture expansion. Autologous application of MFAT has initially been used for treatment of bone degenerative diseases [23].

In the present study, our aim was to evaluate the safety and feasibility of a BCP-MFAT combination as a graft material for alveolar cleft reconstruction.

Table 1. Timeline of surgical and follow-up procedure of the patients.

	Consent form	OPG	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

OPG, orthopantomogram; CT, computed tomography; CBCT, cone beam CT.

Enrollment and eligibility

Following approval by ethical committee of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (1063/UN4.6.4.5.31/PP36/2019). This prospective control clinical trial was registered in the Indonesian Trial Registry (INA-EW74C1N). The clinical protocol complies with the principles of the Helsinki declaration. All patients and if applicable legal guardians of the patients signed an informed consent after risks and possible complications of the procedure (e.g., parotid duct injury, bleeding, infection, cheek asymmetry, possibility of facial nerve branches injury, and non-closure due to critical size defect) were appropriately communicated with them.

Patient selection and randomization

This study was conducted in eight adult patients with residual alveolar bone cleft with the inclusion criteria as follows; healthy male or female patient with age ≥ 15 years old, having unilateral alveolar cleft without any history of grafting procedure previously, and being categorized as ASA1 for anesthetic risk and having normal blood count. The exclusion criteria were patients having poor oral hygiene, having systemic disease or local infection, having received chemotherapy, radiotherapy, immunosuppressives, or anticoagulants that may interfere the healing process, and having received bone growth inducing factors, malnutrition, or active influenza. All of eight patients received the BCP-MFAT combination as a graft material.

This is a first-in-human phase I clinical trial, aimed to obtain insight on the safety and proof of concept of the treatment with the BCP-MFAT combination. We postulated that the probability that SAEs or AEs will occur would be low, based on clinical experience with other applications of MFAT and the well-documented safety profile of BCP. Based on discussion with a statistician, an n = 8 was considered to be sufficient for this trial. Since this was a one-arm study, no blinding or randomization to the treatment was applicable.

BCP-MFAT graft preparation

We defined BCP-MFAT as a 1g:1cc mixture of BCP and MFAT derived from the BFP. The buccal fad pad isolation protocol was performed as previously published [17,25]. Under general anesthesia, the surgeon identified the Stensen's duct with a lacrimal probe and made an incision 2-3 cm below the duct, then a dissection penetrating the muscles and the superficial fascial was made to allow spontaneous herniation of the fat pad [17] (Fig. 1).

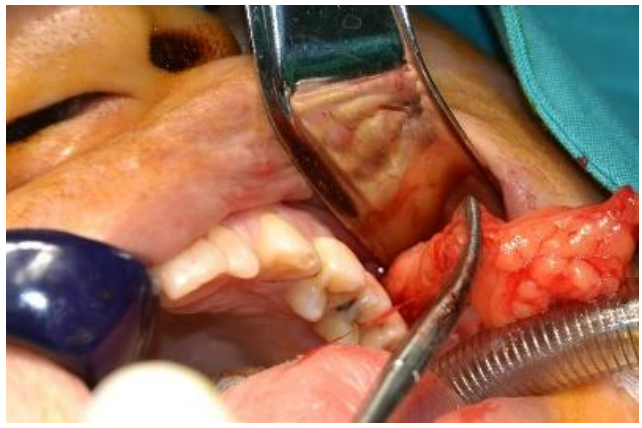

Figure 1. Buccal fat pad harvest

This procedure was done bilaterally on both cheeks in order to obtain approximately 3cc fat. Thereafter, the surgeon continued with the cleft surgery (see below), while in parallel the harvested fat was chopped into small pieces with a scissor and soaked in normal saline for 15 minutes. The normal saline was drained and the chopped fat was processed into MFAT using 2 syringes (size 10cc) connected with the Tulip Gen II Nanofat^{TM} Kit single use sizing transfer 1.2 mm (Tulip Medical, California, United States) according to protocol (Fig. 2A). MFAT was subsequently mixed with 1g BCP per cc MFAT until it reached a homogenous consistency (Fig. 2B). The mixture was placed as a graft material into the alveolar cleft defect (Fig. 2C).

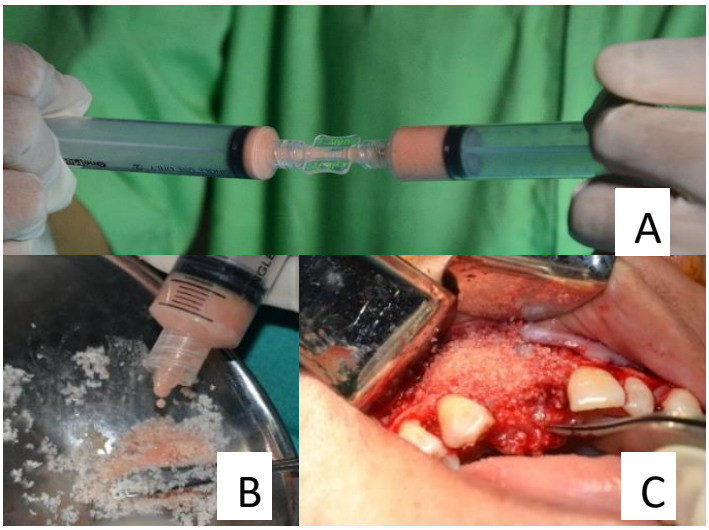

Figure 2. A) Manual microfragmentation of buccal fat pad using Tulip Kit 1.2 mm. B) Microfragmented buccal fat pad being mixed with Biphasic Calcium Phosphate. C) Reconstruction of alveolar cleft using BCP MFAT mixture

Cleft Surgical procedure

Before starting the cleft surgical procedure (Fig. 3), the mucoperiosteal flap was marked on both side of the alveolar cleft. Then local anesthesia of lidocaine 1:100,000 epinephrine was infiltrated in the gingiva, and an incision was made in the mucoperiosteal flap with a no. 15 blade. Afterwards, the reconstruction of the mucosa of the nasal floor was carried out, followed by insertion of the BCP-MFAT graft material in the created alveolar cleft pocket. If necessary, a membrane will be used to cover the grafted defect. Finally, the defect will be closed by suturing the palatal mucoperiosteal flaps using absorbable sutures with 3/0 vicryl for mucosa and 4/0 vicryl for nasal reconstruction (Fig. 4). All patients will receive antibiotics and painkillers postoperatively.

Outcome parameters

Cleft surgery aims to improve speech, and to minimize orofacial growth disturbances and/or dentoalveolar malformities. The main end point regarding this study was to investigate the safety and feasibility of BFP admixed with BCP used in the augmentation of alveolar cleft defects. A secondary outcome of this study was to investigate the volume of bone formation and therefore the continuity of

alveolar bone, which is regenerated after surgery, as assessed by clinical examination, blood tests, and radiographic examination (including orthopantomogram (OPG) and cone-beam computed tomography (CBCT) or computed tomography (CT)).

The OPG pictures were taken pre-operatively and on post-operation-day (POD) 1, 8, 90 and 180. For the assessment of the OPG results, the Bergland scale was used. This scale uses a 4-point scale which is used to assess the percentage of vertical post-graft resorption and therefore the height of the ossified bone on POD180[26].

CT- or CBCT-scans were taken preoperatively and at postoperative days 8 and 180 to verify OPG results in 3D reconstruction of the defect area. For CT-scan assessment, Digital Imaging and Communications in Medicine (DICOM) files were processed by the OsiriX image processing application.

In addition, any unexpected physical and/or laboratory change, symptom, or disease which occurs 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 [27] as either serious or non-serious based on its intensity. The Clavien-Dindo Classification of Surgical Complications will also be used in case of any incidence[28].

Data Collection

The study team, that consisted of doctors, nurses and other researchers were thoroughly trained in the rules and responsibilities of the study, as well as on which, when, and how data should be collected. The assessments are specified in Table 1. Each patient was followed up for 6 months for the primary endpoint, which was safety. Follow-up will end after 3 years. Data were stored in a coded, de-identified manner by the data manager, thereby guaranteeing patient privacy.

RESULTS

All the patients received BCP and MFAT combination. Six out of eight patients were assessed up to day 90, two out of eight patients missed the follow up day 90 due to Covid-19 restrictions. Four out of eight patients were able to continue with the final assessment day (day 180). Two out of eight were unable to reach the hospital for follow up day 180 due to Covid-19 restrictions. Two patients decided not to continue with the study (Table 2).

All patients received alveolar bone grafting by the same surgeon. No complications were reported either local or systemic during surgery and after surgery. Patients were monitored closely from day 1 until they were discharged from the hospital (day 3). Subsequently the patients were followed up according to table 2. Although not included in the initial trial design, all patients were contacted by telephone or video call for a 1-year follow-up. All patient was satisfied with the treatment provided and no adverse effects were documented.

Table 2. Demographic Assessment.

	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 5	Pt. 6	Pt. 7	Pt. 8
Gender	Female	Male	Male	Female	Female	Male	Female	Female
Age	24	17	16	32	19	20	28	21
Affected side	Bilateral: left	Bilateral: left	Bilateral: Left	Bilateral	Right	Left	Right	Left
Assessment day 30	Completed	Completed	Completed	Completed	Completed	Completed	Completed	Completed
Assessment day 90	Completed	Completed	Not assessed due to Covid-19 lockdown	Completed	Completed	Completed	Completed	Not assessed due to Covid-19 lockdown
Assessment day 180	Drop out	Not assessed due to Covid-19 lockdown	Completed	Drop out	Completed	Not assessed due to Covid-19 lockdown	Completed	Completed

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Feasibility

All surgeries went uneventful and according to protocol, thus feasibility of the BCP-MFAT grafting procedure was shown. In none of the surgeries, placement of a membrane was necessary.

Safety

Adverse events

Several examination methods are used to assess adverse effects (local or systemic). All patients experienced minimal to moderate pain levels associated with normal post-operative pain levels, similar to normal post-operative pain scores. Patients remained in the hospital postoperatively for 72 hours. None of the patients had fever, allergic reactions, inflammation or unusual local infections, systemic adverse effects and all laboratory tests showed that all blood parameters were within normal limits.

Osteoinductivity

Due to COVID-19 and since most of the patients are from rural areas, implant surgeries were not possible, and biopsy collection and subsequent evaluation of bone histomorphometric parameters to determine osteoinductivity had to be cancelled as well.

Radiographic evaluation

Bone graft integration in alveolar defects was evaluated with the Bergland scale. This scale was assessed using plain two-dimensional dental radiographs visualizing the alveolar height of the interdental septum. OPG was chosen because it is more patient-friendly (especially postoperatively) and is a good method for assessing the Bergland scale, despite some drawbacks such as image distortion and structural superposition.

Orthopantomogram

The Bergland scale scores after evaluation of OPGs at assessment days POD 1, 8, and 90 bone levels ranged from grade I to III (Table 3). At POD 180 only two patients could be assessed, which had grade III bone levels (Table 3).

Table 3. OPG Evaluation (assessed by Bergland Scale)

	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 5	Pt.6	Pt.7	Pt. 8
POD 1	I	I	I	I	II	II	I	III
POD 8	I	I	I	II	I	I	II	III
POD 90	II	III	ND	III	III	III	III	ND
POD 180	Drop out	ND	ND	Drop out	III	ND	III	ND

ND: No Data

CT scan evaluation

Since performing an occlusal or periapical x-ray on the patient would be uncomfortable, we chose OPG with CT scan as additional support. The latter made it possible to produce a reconstructed 3D image of the patient's area of interest. Scans were performed preoperatively, on POD8 and POD180 (Table 4). When comparing the Bergland scale scores using OPG and CT scans, results were similar (five grade I, two grade II and one grade III vs. four grade I and three grade II and one no data, respectively).

Table 4. CT-Scan Evaluation (assessed by Bergland Scale)

	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 5	Pt.6	Pt.7	Pt. 8
POD 8	I	I	ND	II	I	I	II	II
POD 180	Drop out	ND	ND	Drop out	III	ND	III	ND

ND: No Data

Complications

There were no complications reported intra- and/or post- operatively in this study.

DISCUSSION

The gold standard for alveolar cleft grafting is still autologous bone which, however, is associated to many complications and comorbidities, especially at the bone donor site. New grafting materials with similar bio-efficacy are therefore urgently wanted. We postulated that the combination of an off-the-shelf osteoconductive bone substitute, BCP, with mechanically fractionated buccal fat pad tissue (MFAT) containing high numbers of mesenchymal stem cells would generate a bioactive scaffold that might enhance alveolar cleft repair to a similar level as currently accomplished with autologous bone. MFAT has safely and efficaciously been used in musculoskeletal applications [29–31]. BCP has been extensively applied for bony defect reconstructions, among others in the maxillofacial area [32–34]. BCP has recently also been used for alveolar cleft reconstruction in combination with another bioactive factor, polyp [35].

The aim of this trial was to assess the safety and feasibility of a BCP-MFAT combination as a novel graft material for alveolar cleft reconstruction.

Our findings in the current trial indicated that the BCP-MFAT graft material is a safe, stable graft material and easy to handle, particularly after adding the MFAT into BCP. No adverse reactions such as infection, severe pain, swelling, allergic reaction, or any other local or systemic adverse effects were observed. Bone height reductions were between 25% and 75% (I-III), as measured radiographically and scored using the Bergland scale. We had hoped for a slightly better outcome, but this may be at least partly due to the current study setup. We deliberately chose to only include older adolescent and adult patients in this trial, in order to avoid exposing youngsters to a novel biomaterial in a safety trial and to ensure that the patients could make their own decision to participate in this study. Because of this design, we had to accept suboptimal conditions: (1) we did not have the beneficial effect of the puberal growth spurt, which normally is aimed for by planning alveolar bone grafting at the age of 9-11 years old; and (2) alveolar cleft defects were larger than usual because of this growth spurt being finalized at the time we included the patients. Now that safety of the BCP-MFAT graft was shown, this opens the way to future efficacy studies under more optimal conditions.

Our study was restricted in a number of ways, the worst of which was the COVID-19 pandemic, which only allowed 6 patients to be examined after 90 days, and 4 patients after 180 days of follow-up and led to a comparatively brief postoperative follow-up duration. Moreover, due to the patients not being allowed to travel because of the COVID 19 restrictions, the implant surgeries had to be abandoned, resulting in not being able to take biopsies of the reconstructed defects for micro-CT and histological/histomorphometric analysis.

This study, to our knowledge, is the first clinical trial to look at the feasibility and safety of BCP-MFAT in humans. Although the outcome of the trial allowed confirmation of these two aspects, we could not investigate the bone formation outcome parameters in biopsies, as was the original plan. Thus, additional studies involving a larger and younger patient population, biopsy analyses, and an efficacy comparison with autologous bone are necessary to draw reliable conclusions about their regenerative abilities and autologous bone-replacing potential. In addition, orthodontic factors should also be considered in future studies.

CONCLUSION

Despite the small group size and missing data points due to the COVID-19 pandemic, we were able to conclude that combination of BCP-MFAT appear to be safe graft materials for alveolar cleft repair.

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CHAPTER 10

General Discussion & Future Perspectives

GENERAL DISCUSSION

This thesis provides insight into the current clinical practice of reconstructing oral clefts. It also contributes to systematic reviews of the tissue engineering materials that have been put to the test recently for the preclinical and clinical models of oral clefts reconstruction, with a focus on stem cell-based tissue engineering. In a human alveolar cleft model, it requires a novel approach to investigate the safety and feasibility of the bone graft combination BCP and microfragmented fat (MFAT) or calcium-polyphosphate (Ca-polyP). In the associated publications contained as thesis chapters (2–9), these findings and their implications are presented and discussed; in this section, overall conclusions and future views will be discussed.

Current Clinical Practice of Oral Clefts

Without a question, money is the biggest motivator for people to look for outpatient surgery. Outpatient surgery has increased in the US since the 1980s, with a 300% growth from 1996 to 2006 due to technological advancements as well as a focus on efficiency and cost-cutting [1]. Studies have shown that when choosing patients for outpatient cleft surgery, the surgeon's clinical judgment and assessment of the patient's general state are crucial [1–4]. In chapter 2, we investigated the outcomes of outpatient alveolar cleft repair surgery and related them to the demand for cost-effective alveolar cleft surgery. Depending on the size of the alveolar cleft defect, two different types of bone grafting material were used in this study: iliac crest bone and mandibular symphysis. Individuals who have the mandibular symphysis as the donor site will either receive postoperative daycare or multiple day hospitalization, while individuals who receive the iliac crest are bound for a lengthier hospital stay. According to this study, depending on the defect size, either donor site is feasible and shows similar and few complications after treatment; however, postoperative daycare following alveolar cleft surgery was demonstrated to save treatment costs dramatically. It is crucial to keep in mind that this study was conducted in an academic setting with sufficient funding and a carefully thought-out strategy for treating oral clefts, enabling patients to receive the right care. In chapter 3, we learned that many patients in impoverished nations still have to wait until they reach adulthood

before receiving care because they are reliant on the availability of medical missions that provide free surgery.

Our data confirm developments reported elsewhere: Consideration of costs and the introduction of managed care medical insurance were found to result in a decrease in hospitalization and the introduction of methods that promote a shorter hospital stay in developed and some developing countries [2]. Particularly, outpatient procedures for cleft lip and palate repair are rising in frequency. The literature provides a detailed description of outpatient cleft lip and palate surgery [1–4]. Kantar et al. demonstrated that outpatient cleft lip and palate surgery is both safe and cost-effective when patients are carefully selected. In order to evaluate the safety of outpatient cleft lip repair, Rosen et al. published a research in which they retrospectively reviewed the postoperative care of patients receiving the procedure at two urban tertiary pediatric hospitals [4]. The complications requiring presentation to the emergency room or readmission to the hospital were not significantly different between the patients from the two hospitals in this analysis [4]. In Nigeria, when cellular phones were first made available to facilitate communication, outpatient care following cleft lip surgery was first implemented, according to Ugburo et al. [2]. This retrospective analysis revealed a 2.3% complication rate for outpatient cleft lip surgery, making it a safe and affordable choice [2]. In fact, the majority of the included patients were able to afford surgical correction due to outpatient cleft surgery's lower cost [2].

Alveolar cleft surgery performed in an outpatient setting, however, is still controversial today [3]. One of the factors influencing the rarity of outpatient surgery for alveolar cleft repair is complications related to donor site morbidity (such as pain and trouble walking) [5]. This affects how long patients stay in the hospital postoperatively. The average hospital stay following bone grafting varies between 1 and 6 days in different centers, according to publications in the literature [5].

Current Tissue Engineering Applications for Oral Clefts

Tissue engineering has been established in multiple studies to be a safe and successful alternative to autologous bone grafting, the gold standard treatment for alveolar clefts. One benefit of tissue engineering is the elimination of the requirement for a second (donor) site procedure to collect the bone graft needed for alveolar cleft surgery. However, compared to alveolar clefts, the application of tissue engineering for cleft palates has not received as much attention. One of the key contributions of this thesis is the thorough review of stem cell-based tissue engineering's use in preclinical models of alveolar cleft and cleft palate defects. Additionally, we give an overview of clinical investigations on alveolar clefts that use tissue engineering.

In preclinical studies of alveolar cleft defect reconstructions, the type of cells used were bone marrow stem cells (BMSC), human umbilical cord mesenchymal stem cells (hUMSC), animal umbilical cord mesenchymal stem cells (animal UMSC), human differentiated gingiva mesenchymal stem cells (human dGMSC), animal adipose stem cells (animal ASC), and human mesenchymal stem cells from orbicularis oris muscle. Bone marrow mesenchymal stem cells are the most often utilized cells. Mesenchymal stem cells from bone marrow are the primary cell type employed for preclinical trials for both the alveolar cleft and cleft palate models, as demonstrated in the systematic review in this thesis. Adipose tissue is another often-used source of MSCs. Our risk of bias analysis revealed that there was a significant risk of bias in the published results of the animal research we included. Moreover, the preclinical studies have some pivotal limitations: First, determining the critical size defect in experimental animals for alveolar cleft is crucial for this reason in order to reduce the variety of investigations and arrive at a sound conclusion regarding the combination of tissue engineering that can be the most optimal for managing alveolar clefts. Secondly, the use of the chronic model rather than the acute (fresh defect) model, as has been done by Amalraj et al. [6] by inducing a cleft palate defect through pregnant rats by injecting corticosteroids, is another thing that should be taken into consideration to support the development of animal models for testing the tissue engineering approach for oral clefts. This will make the defect more closely resemble the natural process of oral clefts. To

summarize, we have been unable to come to any solid conclusions regarding the applications that can be converted into human models.

The clinical model systematic review is no different in this regard. Growth factors were utilized in the majority of these trials (10 out of 15 in this review), with rh-BMP-2 appearing to be the most popular and effective. However, our risk of bias analysis showed that the controlled trials on alveolar cleft repair in the 15 studies we identified and presented in this review still had some, and in some cases even many, flaws in the trial design or their reporting of the results, making it difficult to draw reliable and sound conclusions.

With these two systematic reviews, we think we provided a thorough overview of the current state-of-the-art of the current tissue engineering initiatives.

New Treatment Modalities

Although rhBMP2 is so far the most studied and potentially effective compound studied, rhBMP-2 is costly, may lead to adverse effects such as bone resorption when dosed too high or be ineffective when concentrations are too low, and sound conclusions cannot be drawn (see above). Therefore, we sought to evaluate as a first-in-man trial the use of a novel class of reparative compounds acting as an energy fuel for the regenerative process, i.e., polyphosphate (polyP). PolyP was found in many preclinical studies including bone repair studies to show high regenerative potential. In our safety trial employing this compound in two compositions, i.e., as Ca-polyP microparticles only or as a combination of Ca-polyP-microparticles mixed with BCP. Even though our trial showed no adverse effects and could be considered safe, our secondary aim of studying bone formation capacity in bone biopsies was unfortunately prohibited because of the COVID-19 pandemic. Interestingly, an independent clinical study conducted in another field of expertise, using collagen mats soaked in polyP as a wound dressing, confirmed the safety of polyP and showed efficacy as measured by marked reduction of wound size and an increased rate of re-epithelialization [7]. If we compare our results with those of the skin study, we guess that the collagen mats may have been more firmly fixed in situ compared to the cleft site and may have provided a more effective sequestration of the polyP at the implant site. Taken together, we

strongly advocate combining polyP with a more durable graft material for bone repair to avoid the currently experienced difficulties in handling the polyP in our trial setting.

Learning from a successful procedure in the maxillofacial region is another approach to discovering the best bone regeneration technique for oral clefts. Prins et al. were the ones who first developed the SVF and calcium phosphate-based combination in a single surgical procedure for the maxillary sinus floor elevation (MSFE) technique [8]. This model enables the analysis of bone samples collected prior to the insertion of dental implants using the gold standard of bone histomorphometry to measure bone growth. Despite the small number of trial subjects, this study demonstrated the efficacy and safety of using SVF in conjunction with calcium phosphate for the MSFE process.

However, there are some limitations associated with the SVF procedure utilized in the Prins study, such as the use of expensive equipment with still a 1.5-2h processing time, collagenase digestion of the adipose tissue, which is currently regarded as “more than minimal manipulation” by the regulatory agencies, and a second donor site, i.e., the abdomen for procuring the adipose tissue. In this thesis, we present several solutions to tackle these issues:

1. Use of the locally harvested buccal fat pad (BFP) as the adipose tissue source. BFP has already been successfully used in cleft palate grafting surgeries, as we have also shown ourselves (Chapter 3). Moreover, this BFP fat was previously already shown to be equally rich or even richer in ASC content compared to abdominal fat [9].
2. Use of mechanical fractionation instead of collagenase processing of adipose tissue, thus maintaining the “minimal manipulation” classification.

   Intra-operative fractionation was performed using a cheap and easy-to-handle disposable dual syringe-based Tulip Gen II Nanofat™ Kit single-use sizing transfer 1.2 mm fat fractionator device, which produced MFAT within 15 min.

The alternatives, benefits, and drawbacks of the main methodologies established as alternatives to enzymatic processing and intended to manipulate adipose tissue as little as possible were thoroughly discussed in a review by Trivisonno et al. [10]. Microfragmented fat (MFAT), consisting of adipose microparticles about 0.5 mm in diameter, has been used in cosmetic and

reconstructive procedures, including the reconstruction of nasolabial clefts [10]. For tissue engineering/regenerative medicine applications, several other disciplines have already shown the clinical safety of microfat, such as [11–13], and more studies are upcoming [14,15]. This supported our confidence that MFAT would be a beneficial and safe product to use, and we subsequently developed and conducted a clinical trial using MFAT prepared from BFP in conjunction with biphasic calcium phosphate (BCP) scaffold for alveolar cleft reconstruction in patients. It had previously been demonstrated that alveolar cleft reconstruction employing an osteoconductive calcium phosphate scaffold was both feasible and successful [16,17], and we hypothesized that MFAT would add regenerative capacity to the BCP. Moreover, the trial design thereby closely resembled that of the MSFE study of Prins et al., including the histomorphometric evaluation of biopsies obtained using a hollow drill prior to the insertion of dental implants after 6 months follow-up.

Despite the small sample size and the absence of some data points because of the COVID-19 pandemic or drop-outs, we could confirm our 8-patient trial that the reconstructions with the BCP-MFAT mixture were feasible and safe, and that no adverse reactions were observed. Unfortunately, we could not evaluate bone formation (efficacy) since patients were not allowed to travel during the pandemic, so placing implants and concomitantly taking biopsies was prohibited. Moreover, since we did not want to expose children to first-in-man safety trials and therefore included only adolescents and adult patients, the reconstructions were done with alveolar cleft defects of larger sizes, and after the puberty growth spurt had already occurred, which usually facilitates and enhances bone repair. Thus, our results may underestimate the potency of both biomaterial constructs because of these suboptimal conditions.

Conclusion and Future Perspectives

As we have evaluated in this thesis, regenerative medicine approaches for alveolar cleft/palate reconstructions are still in their infancy and have not shown significant benefits over current reconstruction methods as yet. Extrapolation of results from preclinical studies to clinical application has risks since virtually all animal models make use of freshly created defects, while human alveolar cleft

defects are congenital in nature. In this thesis, we investigated two novel regenerative biomaterial constructs, i.e., BCP scaffolds supplemented with either polyP or MFAT. Although severely hampered by the COVID-19 restrictions, which caused several missing data points and patient drop-outs and precluded efficacy evaluation using histomorphometric analysis of biopsies, we could conclude that no adverse effects were observed and that both biomaterial constructs could be considered safe. This confirmed previous safety reports for both compounds in other clinical disciplines. When comparing both new treatment modalities for alveolar cleft restoration, we think the BCP-MFAT combination offers more promising results than the BCP-polyP combination, at least in its current composition. It may be better to consider other carrier materials to be combined with polyP, for example, Poly Lactic-co-Glycolic Acid (PLGA), which has been proven to be effectively applicable combined with polyP in preclinical studies [18], or collagen, which has been tested for skin repair [7]. Nevertheless, this has to be confirmed in additional studies including more participants in which also efficacy assessment by histomorphometric evaluation should be performed. Moreover, now that safety seems not to be an issue, we could consider including pre-puberty patients in order to make optimal use of the growth spurt to enhance the repair process.

Another interesting option is to add easily and rapidly intra-operatively produced autologous preparations of growth factors such as platelet rich fibrin (PRF). Several studies, also reviewed recently by members of our research group (Al-Sabri et al. for MSFE, submitted; Alavi et al. for socket preservation, submitted) have shown that several variants of PRF (e.g., L-PRF, A-PRF) have high regenerative and stem cell-activating potential, and may therefore boost the bone regeneration process even stronger than MFAT from BFP alone. The combination of MFAT and PRF should certainly be pursued in novel clinical trials in craniofacial models, including the alveolar cleft as well.

In conclusion, cells that have undergone minimum manipulation become excellent candidates for bone tissue engineering applications. However, the above-discussed optimizations for these regenerative materials, particularly MFAT, may offer novel, powerful, and cost-effective alternatives for future bone repair techniques.

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CHAPTER 11

Summary

SUMMARY

The main objective of this thesis was to investigate the current clinical practice and associated difficulties in treating cleft lip and palate, the state-of-the-art tissue engineering techniques for the reconstruction of oral clefts, and to assess the safety and efficacy of novel tissue engineering approaches for alveolar cleft defects.

In Chapter 2, we compared the costs and complication rates of Secondary Alveolar Bone Grafting (SABG) outcomes in alveolar cleft patients treated either in daycare or with multiple day hospitalization (MDH). It was a retrospective comparative cohort study that included data from 137 individuals with unilateral cleft lip, alveolar, and palate (CLAP) treated between 2006 and 2018 in two settings: postoperative daycare or MDH following oral cleft surgery in the VUMC academic hospital in Amsterdam, The Netherlands. Age, gender, cleft subtype, bone donor site, hospitalization type, length of stay, additional surgery, complications, surgeons, and costs were the registered clinical variables. According to the findings, 46.7% of the 137 patients were treated at MDH, and 53.3% were in daycare. All patients treated in daycare received mandibular symphysis bone, but in MDH, 46.9% received iliac crest bone. The bone donor site was related to the postoperative care type. Daycare had marginally, but not substantially, higher rates of complications, the majority of which were classified by Clavien Dindo as Grade I (mild). According to the study, daycare after alveolar cleft surgery is about as safe as MDH, although much less expensive.

In Chapter 3, we investigated intraoperative and early postoperative blood loss using the buccal fat pad (BFP) during cleft lip and/or cleft palate (CL/P) surgery. This prospective study involved 109 cleft palate (CP) patients throughout the course of a three-month treatment period at the Hasanuddin University Dental Hospital (permanent center) and humanitarian missions to remote areas of eastern Indonesia. Treatment for all patients used the DOZ Furlow method and a BFP transplant. The total amount of intraoperative blood loss was determined by weighing the gauze swabs that were used to control the surgical bleeding before and after surgery and then doing a full blood count three days later. According to the study, weight and the procedure length can cause more blood loss during

palatoplasty, which suggests that younger patients will have better results from their procedures.

In Chapter 4, we conducted a systematic review and meta-analysis to assess the effectiveness of stem cell-based tissue engineering for the treatment of alveolar cleft (AC) and cleft palate (CP) deformities in animal models. Preclinical studies were included in which animal models of AC and CP reconstruction were carried out using stem cell-based tissue engineering. Bone mineral density (BMD) and/or new bone formation (NBF) were recorded outcome parameters. With an unclear-to-high risk of bias, thirteen large and twelve small animal studies on the AC (21) and CP (4) reconstructions were considered. The most common cell source employed was bone marrow mesenchymal stem cells. Although not significant, meta-analysis for AC favored stem cell-based bone tissue engineering over scaffold alone or blank control. For the CP group, meta-analysis was not possible. In conclusion, adding osteogenic cells to biomaterials improves AC and CP reconstructions.

In Chapter 5, a second systematic review and meta-analysis of controlled clinical trials utilizing regeneration materials for alveolar cleft repairs was carried out. The review also took the risk of bias (RoB) into account. Up until October 2020, a total of 15 trials had been completed; however, none had achieved a perfect score using the Jadad and Delphi list quality assessment scales. Of these, 20% failed to randomize the trials, 73,33% failed to describe the randomization method, and none reported double-blinded criteria. According to the meta-analysis, the regenerative materials and iliac crest grafts did not differ significantly. Additionally, this review's findings indicated that control trials' high RoB indicated the need for quality improvement in control trials.

In Chapter 6, we presented a clinical trial protocol to assess the feasibility and safety of a novel calcium-polyphosphate-complexed bone-inducing graft material for alveolar cleft reconstructions known as Ca-polyP microparticles (Ca-polyP MPs). Ca-polyP MPs have been shown in preclinical studies to have osteoinductive properties. Eight patients with alveolar clefts 13 years or older were planned to participate in this prospective, non-blinded, first-in-man clinical pilot trial to assess the feasibility and safety of Ca-PolyP MPs as a bone-inducing graft material. Patients will either receive Ca-polyP as the only graft material or Ca-polyP

combined with biphasic calcium phosphate (BCP) as a carrier for the bone substitute. Radiographic imaging, routine blood tests, and physical examinations will be used to monitor the study participants for safety-related factors closely. A hollow drill will be utilized to prepare the implantation site for a biopsy after 6 months. The biopsy will be processed for histological/histomorphometric examination of bone formation, while the radiographic imaging will be used for clinical evaluation. Following the trial's conclusion, the findings regarding safety, feasibility, and bone formation using polyP as a graft material will be published.

In Chapter 7, we described in great detail the outcomes of a single-blinded, parallel, prospective clinical pilot research using Ca-polyP MPs for alveolar cleft repairs with 8 adolescent patients (ages 13 to 34), of which the protocol was described in **Chapter 6**. Two groups were randomly assigned, with 4 receiving only Ca-polyP MPs and 4 receiving both Ca-polyP MPs and biphasic calcium phosphate (BCP). However, a change was required for surgical reasons, resulting in administering a Ca-polyP to 2 patients and a Ca-polyP + BCP to 6 patients. Safety criteria and rigorous monitoring of potential side effects utilizing radiographic imaging, routine blood tests, and physical examinations were among the outcome parameters.

None of the individuals had any adverse effects or localized or generalized allergic reactions. Our research showed that both transplants may be used safely. However, compared to Ca-polyP alone, the combination of Ca-polyP + BCP graft demonstrated more excellent stability in alveolar cleft reconstruction, as measured by the Bergland scale. It is advised that future clinical trials include a bigger sample size.

In Chapter 8, we presented a clinical trial design to assess the viability and safety of combining biphasic calcium phosphate (BCP) with microfragmented fat (MFAT) for alveolar cleft repairs. Since iliac crest-derived autograft bone is linked to chronic pain and donor site morbidity, BCP may be an alternative. The locally collected buccal fat pad will be mechanically fractionated to create MFAT, which has excellent regeneration capacity due to high pericyte and MSC content and a conserved perivascular niche. This prospective, non-blinded, first-in-human clinical research will include eight patients with alveolar clefts. The patient's buccal fat pad will be used to prepare MFAT during surgery. Before surgery and after the BCP-

MFAT combination has been implanted in the alveolar cleft, radiographic imaging will be done. A regular physical exam and blood test will also be performed. The clinical evaluation will be conducted using radiographic imaging, and histological and histomorphometric analyses will be performed on biopsies taken six months after the implantation site had been prepared using a trephine drill. Regardless of the trial's findings, the safety, feasibility, and efficacy of the BCP-MFAT combination in promoting bone formation will be disclosed.

In Chapter 9, we described in detail the results of a first-in-man prospective non-blind clinical pilot study of alveolar cleft reconstructions using a BCP-MFAT combination, which included 8 adult patients. For six months, the patient was followed up. Physical examinations, routine blood tests, and radiographic imaging were performed to evaluate safety characteristics and potential adverse effects. It was impossible to measure osteoinductivity using histomorphometric analysis of biopsy specimens because of COVID-19 constraints. The eight patients ranged in age from 17 to 32, with an average age of 22.13. 2/8 patients missed this follow-up due to Covid-19 limitations, but 6/8 patients received examinations up to 90 days later. Only 4/8 patients were able to receive a final evaluation (day 180), and two of those patients were unable to travel to the hospital due to Covid-19. Two study participants willingly withdrew from it. No local or systemic side effects, allergic reactions, or other adverse events were noticed. The Bergland scale had radiographic examinations from I through III. In summary, the BCP-MFAT grafts seem to be secure and practical for alveolar cleft reconstruction.

AUTHORS' CONTRIBUTIONS

Chapter 2 was published as:

Postoperative daycare as a safe and cost-effective option for Secondary Alveolar Bone Graft (SABG) surgery: A retrospective comparative cohort study

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Authors' contributions:

DSNK: data collection, data analysis and interpretation, and drafting the manuscript. MR: conception and design of the study and revising the manuscript critically. IHAA: analysis and interpretation of data especially in statistic and drafting the manuscript. MNH: conception and design of the study, analysis and interpretation of data, and revising the manuscript critically. TF: conception and design of the study and revising the manuscript critically. MG: conception and design of the study, analysis and interpretation of data, and revising the manuscript critically. All authors confirm that the manuscript has been read and approved to be published.

Chapter 3 was published as:

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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DSNK: data analysis and interpretation, and drafting the manuscript. MR: data collection, conception and design of the study and revising the manuscript critically. SAA: conception and design of the study and revising the manuscript critically. ASHY: data analysis and interpretation, and drafting the manuscript. AT: data collection, conception and design of the study and revising the manuscript

critically. TF: conception and design of the study and revising the manuscript critically. S & HK: analysis and interpretation of data especially in statistic and drafting the manuscript. PB & LJL: conception and design of the study and revising the manuscript critically. All authors confirm that the manuscript has been read and approved to be published.

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Safety and fesibility study of using Polyphosphate (PolyP) in alveolar cleft repair: A pilot study

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Authors' contributions:

DSNK: data collection, quality control of the data, data analysis and interpretation, and drafting the manuscript. SAA: supervising clinical procedures, data collection, data analysis and interpretation, and drafting the manuscript. AF & AT: data collection and supervising clinical procedures. RN: data collection and quality control of the data. WEGM, HCS & XW: conception and design of the study, and revising the manuscript critically. TF: conception and design of the study, providing research materials, and revising the manuscript critically. MNH & MR: conception and design of the study, analysis and interpretation of data, quality control of the data, and revising the manuscript critically. All authors have read and agreed to the submitted version of the manuscript.

Chapter 9 was prepared as:

A prospective control clinical trial using microfragmented fat (MFAT) and BCP for the treatment of alveolar clefts

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"Which, then, of your Lord's blessings do you both deny?"

– Qur'an, Ar-Rahman

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"Better than a thousand days of diligent study is one day with a great teacher."

– Japanese Proverb

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"Never forget where you came from. It's what made you the person you are today."

– Anonymous

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" You don't choose your family. They're God's gift to you, as you are to them."

– Desmond Tutu

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CURRICULUM VITAE

Diandra Sabrina Natsir Kalla was born on 15 March 1989 in Ujung Pandang (now known as Makassar), South Sulawesi, Indonesia. In 2013 she received her MD Degree in Medicine from Faculty of Medicine of Hasanuddin University Makassar. After graduating, she interned as a general practitioner at Kota Community Health Center and Prof. Dr. H. M. Anwar Makkatutu Hospital in Bantaeng, South Sulawesi until 2015. In 2015, she was also accepted as a permanent lecturer at the Faculty of Medicine, Hasanuddin University. In June 2015, she got a Ph.D. scholarship from the Indonesia Endowment Fund for Education (LPDP), Ministry of Finance, the Republic of Indonesia. Diandra started her Ph.D. program in November 2015 at the Oral & Maxillofacial Surgery/Oral Pathology department at VU University Medical Center, Amsterdam which resulting in the creation of this thesis. Currently, she is establishing a private medical practice in Makassar.

PUBLICATIONS

Natsir Kalla DS, Alkaabi SA, Hendra FN, Nasrun NE, Ruslin M, Forouzanfar T, Helder MN. **Stem Cell-Based Tissue Engineering for Cleft Defects: Systematic Review and Meta-Analysis**. The Cleft Palate Craniofacial Journal. 2023 May 18:10556656231175278.

Natsir Kalla DS, Ruslin M, Aartman IHA, Helder MN, Forouzanfar T, Gilijamse M. **Postoperative Daycare as a Safe and Cost-Effective Option for Secondary Alveolar Bone Graft (SABG) Surgery: A Retrospective Comparative Cohort Study.** Cleft Palate Craniofac J. 2023 Apr 25:10556656231171210.

Natsir-Kalla DS, Ruslin M, Alkaabi SA, Yusuf AS, Tajrin A, Forouzanfar T, Siswanto, Kuswanto H, Boffano P, Lo LJ. **Influence of patient-related factors on intraoperative blood loss during double opposing Z-plasty Furlow palatoplasty and buccal fat pad coverage: A prospective study.** J Clin Exp Dent. 2022 Aug 1;14(8):e608-e614.

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.

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.

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.

Hendra FN, **Natsir Kalla DS,** Van Cann EM, de Vet HC, Helder MN, Forouzanfar T. **Radical vs conservative treatment of intraosseous ameloblastoma: Systematic review and meta-analysis**. Oral diseases. 2019 Oct;25(7):1683-96.

INTERNATIONAL MEETINGS ATTENDED

2022 International Federation for Adipose Therapeutics and Science (IFATS) 19th Annual Meeting, USA (oral speaker)

2021 International Webinar "Cutting Edge of Anti-Aging Medicine and Stem Cell Technology", Medical Faculty, Bosowa University, Makassar, Indonesia

2020 The 3rd International Conference on Biophysical Technology in Dentistry (ICoBTD), Faculty of Dentistry Hasanuddin University Makassar, Indonesia (oral speaker)

2019 The 2nd International Conference on Biophysical Technology in Dentistry (ICoBTD), Faculty of Dentistry Hasanuddin University Makassar, Indonesia (oral speaker)

2018 The 1st International Conference on Biophysical Technology in Dentistry (ICoBTD), Faculty of Dentistry Hasanuddin University Makassar, Indonesia (oral speaker)

2017 The 5th Belgian Symposium on Tissue Engineering, Promotheus – The Division of Skeletal Tissue Engineering of KU Leuven

NATIONAL MEETINGS ATTENDED

2021 4th Annual Research Meeting Amsterdam Movement Sciences

2019 2nd Annual Meeting Indonesia Association of Tissue Engineering and Cell Theraphy (REJASELINDO) in conjunction with National Congress and Annual Meeting Indonesian Stem Cell Association (ASPI) and 5th Annual Meeting Indonesian Tissue Bank Association (PERBAJI) (poster)

2018 The 27th Annual Meeting Netherlands Society for Biomaterials and Tissue Engineering (poster)