The Role of a Novel Biosynthetic Graft in Nasal Reconstruction

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Statement of Originality

This is to certify that the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Approval for chapter 3 (an in-vivo large animal study) was obtained from the University of Sydney Animal Ethics Committee (Project number 2021/2003).

Dr Beeshman Nandakumar

Date: 4th of December 2023

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Publications and Presentations Arising from Thesis

Publications

- Nandakumar BS, Mukherjee P, Yung AE, Nirmalananda A, Ch'ng S. Grafts in septorhinoplasty: a systematic review and future directions. *Australian Journal of Otolaryngology 2022;5:28*. (Accepted).
- Nandakumar BS, Yung AE, Mukherjee P, Boughton P, Boughton E, Nirmalananda A, Ch'ng S. In-vivo evaluation of a novel biosynthetic graft (actv-graft®) for nasal reconstruction. *Biomaterials*. (Under Review).

Presentations

- Nandakumar BS, Mukherjee P, Yung AE, Nirmalananda A, Ch'ng S. Alloplastic and Autologous Implants – A Systematic Review. *Poster presentation at the 15th Asia Oceania ORL-HNS Congress (73rd ASOHNS ASM 2023)*.
- Nandakumar BS, Mukherjee P, Yung AE, Nirmalananda A, Ch'ng S. Tissue engineering in Rhinology – a review of experience thus-far and future directions. *Poster presentation at the 15th Asia Oceania ORL-HNS Congress (73rd ASOHNS ASM 2023)*.

Authorship Attribution Statement

Chapter 2 of this thesis has been published in the Australian Journal of Otolaryngology and presented at the 15th Asia Oceania ORL-HNS Congress (73rd ASOHNS ASM 2023) as detailed in the "Publications and Presentations Arising from Thesis" section. Chapter 3 of this thesis has been submitted to Biomaterials for publication and is currently under consideration. I designed the aforementioned studies with my supervisors, collected the data, analysed the data, and wrote the manuscripts. Furthermore, I understand that if this thesis is successful, it will be lodged with the University Librarian and made available for immediate use.

Dr Beeshman Nandakumar

Date: 4th of December 2023

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct. Furthermore, as lead supervisor, it is my opinion that the thesis is sufficiently well presented to be examined and does not exceed the prescribed word limit.

Associate Professor Sydney Ch'ng

Date: 4th of December 2023

Signature: [REDACTED]

List of Tables and Graphs

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Glossary

ACC	Autologous Costal Cartilage
BG-PCL	Bioglass®-Polycaprolactone
CT	Computed Tomography
FDA	Food and Drug Administration
GSI	Global Surgical Innovations™
IHCC	Irradiated Homologous Cadaveric Cartilage
LAS	Laboratory Animal Services
LLC	Lower Lateral Cartilage
MSC	Mesenchymal Stem Cells
NOSE	Nasal Obstruction Septoplasty Effectiveness scale
OCT	Optimal Cutting Temperature
ROBIS	Risk of Bias in Systematic Reviews
SBF	Simulated Body Fluid
SD	Standard Deviation
SNOT-20	Sinonasal Outcome Test-20
SMAS	Superficial Musculo-Aponeurotic System
ULC	Upper Lateral Cartilage

Thesis Abstract

Background: Septorhinoplasty is a common procedure, and when native septal cartilage is insufficient for grafting during reconstruction, a variety of alternatives are currently used with varying success. Thus, the aims of this thesis are threefold: 1) summarise the evidence for current alternative grafts, 2) identify emerging alternatives, and 3) assess the feasibility of a novel hybrid graft in nasal reconstruction.

Methods: The first chapter of this thesis establishes a foundational understanding of the anatomy and function of the nose and septorhinoplasty. This contextualises the second chapter which utilises a systematic review of the literature to qualitatively evaluate complication rates of current alternative grafts. Furthermore, emerging alternatives are summarised. One of these emerging grafts, a hybrid bioactive-polymer scaffold (actv-graft®), is then used in a feasibility study in three sheep after ethics approval and grant acquisition in chapter 3.

Results: The results of six systematic reviews were qualitatively synthetised in chapter 2. Biologic grafts were generally associated with higher rates of warping, revision surgery, donor-site morbidity, and resorption. Synthetic grafts were more commonly associated with extrusion, infection, and removal surgery. No single alternative graft was ideal. Tissue-engineering and hybrid bioactive-polymer scaffolds were identified as two emerging fields. In chapter 3, actv-graft® was found to be biocompatible, biodegradable, have favourable volume augmentation over time, and stimulate host infiltration within the graft.

Conclusion: Cost-effective, easily procured, patient-specific, bioactive, and biodegradable scaffolds are required in septorhinoplasty. This thesis establishes the feasibility of a promising graft. However, a longer and larger cohort animal study would aid in characterising the safety profile, chondrogenic potential, and degradation rate of actv-graft® prior to human trials.

Chapter One – Introduction

Anatomy and Function of the Nose

The nose is a midline structure that is pyramidal in shape and serves respiratory, olfactory, speech and aesthetic purposes(1). Embryologically, it has contributions from the mesoderm, neural crest, and ectoderm(2). Its cartilage is hyaline and derived from neural crest mesenchyme(3). The external nose is comprised of skin, supporting tissue and an osseocartilaginous framework(4). The latter osseocartilaginous framework is the focus of this chapter as its structure and function are what grafts attempt to recreate or support during septorhinoplasty. The osseocartilaginous framework of the nose is separated from the skin by four layers from deep to superficial: 1) the periosteum or perichondrium, 2) the deep fatty layer in which the vasculature that supplies the nose courses through, 3), the fibromuscular layer or superficial musculo-aponeurotic system (SMAS), and 4) the superficial fatty layer (**Figure 1**)(4-6). The osseocartilaginous framework can be divided into three parts or vaults with the septum traversing these three regions internally (**Figure 2**)(7,8).

The upper one-third is known as the bony vault and is comprised of bilateral nasal bones and the frontal process of the maxilla(8). In the midline, its upper border is the nasion at the frontonasal suture, and its lower border is the rhinion at the caudal end of the nasal bones(9). Between the upper and middle third is the keystone region of the nose(10). The middle one-third, or upper cartilaginous vault, contains the upper lateral cartilage (ULC) which is continuous medially with the dorsal septum and creates a scroll region with the lower lateral cartilage (LLC)(11). The internal nasal valve exists in this region. The lower one-third, or lower cartilaginous vault, is characterised by the LLC and tip of the nose, and gives rise to the external nasal valve(12). The LLC is divided into lateral, intermediary, and medial crura. The septum acts to divide the nasal cavity whilst providing structure and support during the varying aerodynamic forces of respiration. The septum is comprised of the quadrangular cartilage which may be harvested for grafting, perpendicular plate of the ethmoid bone, vomer, and nasal crest of the maxilla and palatine bones (**Figure 3**)(13). Caudally the septum contributes to the columella which divides the nostrils and contributes to tip support.

The keystone region of the nose is an osseocartilaginous unit comprised of bilateral nasal bones, ULCs, and the dorsal nasal septum at the osseocartilaginous junction of the perpendicular plate of ethmoid and quadrangular cartilage(14). Its importance to the structure and stability of the nose is held in its name, however it also has relevance to the aesthetics of the nose as it contributes to the nasofrontal angle. The scroll region is created at the junction of the ULC and LLC as the caudal aspect of the ULC curls up to meet the downward curl of the cephalic aspect of the LLC(15). This junction is also home to the intercartilaginous ligament. The scroll region acts to support both the internal and external nasal valve and contributes to the aesthetic of the nose.

The internal and external nasal valves are crucial considerations to airflow dynamics during respiration(16). When these regions are anatomically weak and collapse, they create turbulence and can limit airflow. Together, the septum and ULC create the medial and lateral walls of the upper cartilaginous vault respectively. The region between these walls and the anterior aspect of the inferior turbinate creates the internal nasal valve. The external nasal valve is bounded by the LLC, columella, and the nasal spine of the maxillary crest(17).

Rhinoplasty and Septoplasty

Rhinoplasty and septoplasty are reconstructive procedures that alter the form and/or function of the nose. Rhinoplasty is the term often used to refer to external reconstructive attempts, whilst septoplasty focusses on reconstruction of the septum internally. At times, these terms are combined as septorhinoplasty when reconstructive work is required both internally and externally. Congenital and acquired pathologies of the nose can impair its function and form, necessitating septorhinoplasty(18). Abnormal growth of the frontonasal and maxillary prominences during gestation can lead to congenital pathology such as cleft palate or changes in the septal orientation leading to septal deviation(2). Trauma, malignancy, and iatrogenic injury constitute the majority of acquired causes of nasal deformity(19). Of the acquired causes, trauma is the most common with the nasal bones or septum being the major sites affected. Whilst nasal bone trauma can affect the stability of the keystone region, fractures to the septum often dislocate it off the maxilla, leaving the keystone as its primary structural attachment(14). The most common site of skin cancer on the face is the nose, and by its disease progress or in its treatment, this malignancy can alter the shape and thus function of the nose(20).

Although nasal obstruction in the context of nasal deformity is rarely life-threatening, its ramifications are widespread. With the nasal passage contributing over half of the total resistance within the upper airway, chronic nasal airway obstruction can contribute to obstructive sleep apnoea which itself has further impacts on a patient's quality of life and health(21-23). Symptomology from nasal obstruction can be evaluated by tools such as the Nasal Obstruction Septoplasty Effectiveness scale (NOSE)(24) or Sinonasal Outcome Test-20 (SNOT-20)(25). Furthermore, the shape and position of the nose on the face has a significant impact on perceived facial symmetry and thus an individual's perception of their appearance which influences their social interactions(26,27). Through surveys of patients with facial deformities, key social metrics such as trustworthiness, employability, and interpersonal relationships have been shown to be negatively affected by their facial deformities(28). Thus, both aesthetic and functional reasons are considered as indications for septorhinoplasty.

In the reconstruction of the nose, a multitude of grafting techniques are employed and can be best thought of by location (**Table 1**)(29). An in-depth review of all grafting techniques during septorhinoplasty is outside the scope of this chapter. However, the most relevant grafting technique to this thesis is the dorsal onlay graft which is employed in chapter 3. Dorsal onlay grafts are placed longitudinally along the dorsal osseocartilaginous border of the nose to augment and alter its shape without bearing significant load (**Figure 4**)(30). Although septal cartilage can be harvested to act as the graft in this technique, this adds extra morbidity and detracts from the stability of the nose. Alternative grafts to native septal cartilage in septorhinoplasty are broadly classified as biologic or synthetic, however there remains significant limitations associated with each graft(31).

Conclusion

The anatomy of the nose is complex, and reconstruction requires an intimate knowledge of not only its anatomy, but also its function. Septorhinoplasty aims to restore function and form to the nose, and whilst there are multiple described techniques, a common issue faced is which graft material to use when native septal cartilage is insufficient. The following chapter details the common alternative grafts in septorhinoplasty and contextualises their clinical utility by describing their complication rates from multiple systematic reviews. In doing so, it becomes evident that no single ideal graft exists, and promising future directions are reviewed. Consequently, chapter 3 explores one of these future avenues - a novel hybrid biosynthetic graft in the nasal dorsum of sheep to determine its feasibility in nasal reconstruction.

Tables and Figures

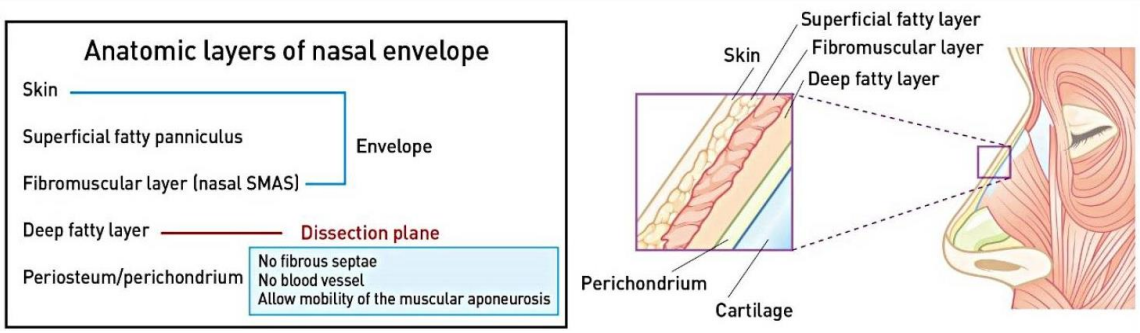

Figure 1: The five layers of the nose from Jeong and Kim 2018(5).

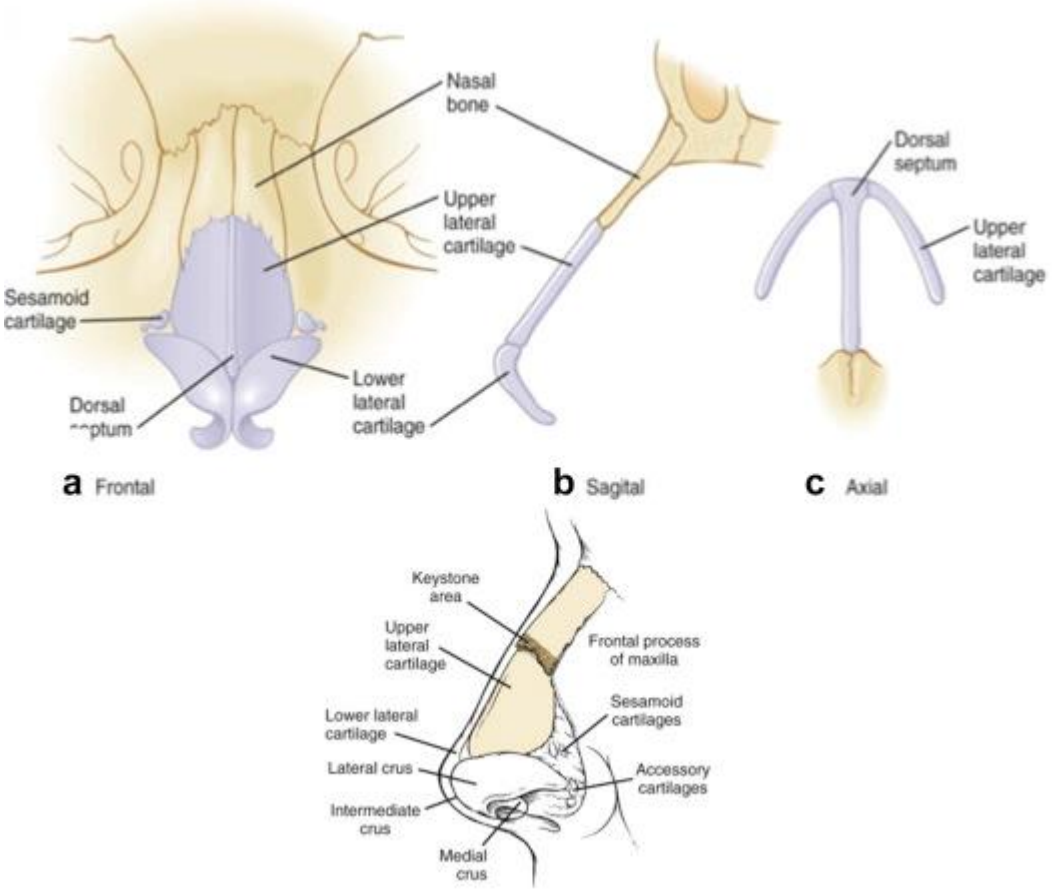

Figure 2: Anatomy of the nasal thirds in Frontal (a), Sagittal (b) and Axial (c) views. Adapted from Hsu and Suh 2018(8).

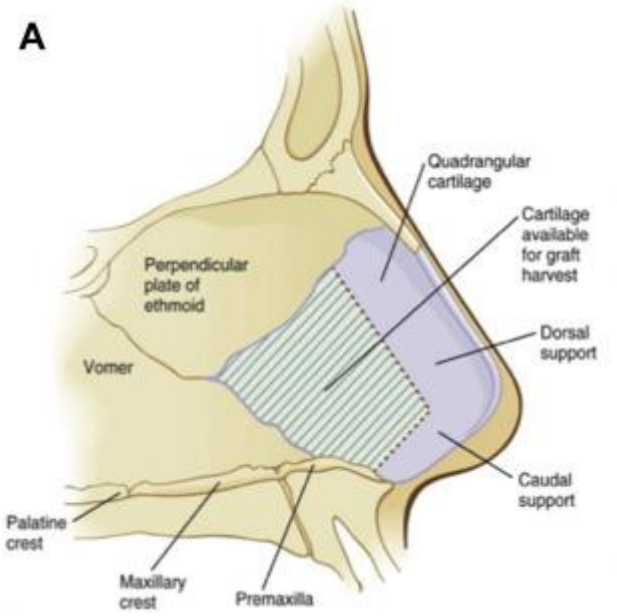

Figure 3: Anatomy of the nasal septum adapted from Hsu and Suh 2018(8).

Dorsum	Tip	Alar Region	Base
Dorsal onlay graft	Anchor graft	Alar batten graft	Alar base graft
Dorsal sidewall onlay graft (lateral nasal wall graft)	Cap graft	Alar contour graft (alar rim graft)	Columellar plumping grafts(s)
Radix graft	Columellar strut (floating/fixed-floating)	Alar spreader graft (lateral crural spanning graft)	Premaxillary graft
Spreader grafts	Columellar strut (fixed)	Composite alar rim graft	
Septal extension grafts	Extended columellar strut-tip graft (extended shield graft)	Lateral crural onlay graft	
	Onlay tip graft	Lateral crural strut graft	
	Shield graft (Sheen or infralobular graft)	Lateral crural turnover graft	
	Subdomal graft		
	Umbrella graft		

Table 1: Types of grafting techniques based on location from Gunter et al. 2006(29).

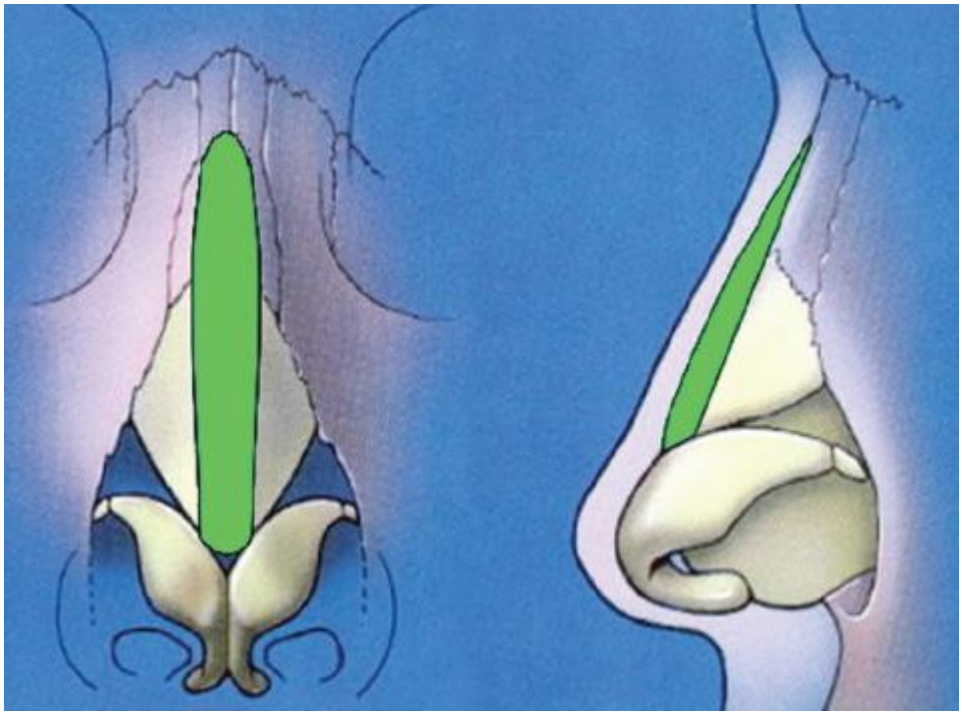

Figure 4: Dorsal onlay graft positioning from Gunter et al. 1990(30).

Chapter Two – Grafts in septorhinoplasty: a systematic review and future directions

Overview

Septorhinoplasty is a common procedure that aims to improve nasal function or cosmesis for patients. If native septal cartilage is insufficient for grafting material during septorhinoplasty, surgeons face the dilemma of which type of graft should be used. Multiple possibilities currently exist in the form of autologous costal cartilage (ACC), irradiated homologous cadaveric cartilage (IHCC), and synthetic grafts. Given the ubiquity of septorhinoplasty, multiple systematic reviews have analysed these grafts with varying conclusions. Furthermore, advances in tissue-engineering and hybrid grafts have provided promising avenues for future grafts that may overcome the difficulties encountered when using the aforementioned grafts.

The primary aim of this second chapter is to present a systematic review of the literature on current grafts in septorhinoplasty and their associated complication rates. Consequently, it becomes evident that no single graft is ideal in all situations. Thus, the secondary aim of this chapter is to explore the evolving landscape of nascent graft technology, specifically with regards to hybrid grafts and tissue-engineering. This chapter is presented as published in the Australian Journal of Otolaryngology.

Title: Grafts in septorhinoplasty: a systematic review and future directions.

Running Title: Systematic Review of grafts in septorhinoplasty.

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Abstract

Background: When there is insufficient septal cartilage graft for nasal reconstruction, current alternatives include the autologous costal cartilage (ACC) graft, irradiated homologous cadaveric cartilage (IHCC) graft, and synthetic (alloplastic) polymer scaffolds. Newer alternatives such as hybrid bioactive-biocompatible polymer scaffolds and tissue-engineered cartilage grafts have also emerged. With the widespread use of alternative grafts in septorhinoplasty, multiple systematic reviews have analysed their complication rates with varying results. This systematic review aims to summarise the current body of systematic reviews analysing complication rates for ACC, IHCC, and alloplastic grafts in septorhinoplasty. In addition, it aims to provide an overview of the recent progress of newer grafts and potential avenues for future research.

Methods: A comprehensive search of EMBASE, Medline, Google Scholar and EBMR was conducted for systematic reviews with more than 10 primary studies analysing complication rates of ACC, IHCC, and alloplastic grafts in septorhinoplasty. The Preferred Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed, and the Risk of Bias in Systematic Reviews (ROBIS) tool was used. Outcomes for qualitative evaluation included infection, warping, resorption, extrusion, hypertrophic scarring, pneumothorax, and revision and implant removal surgery.

Results: Six systematic reviews were included that analysed ACC with alloplastic grafts (n=2), ACC with IHCC grafts (n=1), alloplastic grafts only (n=2), and ACC grafts only (n=1). ACC grafts were associated with warping, resorption, revision surgery and donor-site morbidity. IHCC grafts had similar complication rates to ACC grafts in specific situations. Infection, extrusion, and removal surgery rates were generally higher amongst all alloplastic grafts, although rates varied between specific alloplastics. The Corrected Covered Area was calculated as 10.63% limiting direct comparison of results between reviews.

Conclusions: Whilst it is evident that biologic and alloplastic grafts do not fulfil the criteria of the ideal graft described in the literature, newer alternatives such as tissue-engineering and bioactive-biocompatible polymer composites still require more research to characterise their complication rates.

Introduction

The presence or treatment of congenital, traumatic, and oncological defects of the nose can compromise nasal airway function and cosmesis. Congenital and traumatic septal deviation is present in up to 80% of the general population(3), and facial skin cancer most commonly affects the nose(3). A myriad of reconstructive surgical procedures has been described to address congenital and acquired nasal deformity, ranging from simple nasal bone fracture reduction, skin graft and local flaps to septorhinoplasty and complex microsurgical composite reconstruction. Septorhinoplasty is the third most common procedure in the United States, accounting for over $1.1 billion in 2016 alone(32).

Nasal reconstruction poses unique challenges for a variety of reasons in addition to the obvious cosmetic ramifications. The nose is a tri-lamellar structure that is difficult to duplicate synthetically. The external skin-soft tissue envelope protecting the internal nasal structures can be thin, and is in close proximity with the nasal mucosa which commonly harbours pathogens(33). The outcome of any reconstructive procedure must withstand the normal aerodynamic forces of airflow during respiration that filters and humidifies air, in addition to air expulsion during sneezing(3).

Commonly, the native nasal septum alone is sufficient as an autologous cartilage graft donor site for addition of structure to the nasal skeleton and volume to the nose. However, when there is insufficient septal cartilage for harvest, alternative grafts are necessary. Current alternative grafts to native septal cartilage are either biologic or synthetic/alloplastic(34). Biologic grafts are predominantly autologous (i.e., autograft), and to a much lesser extent allogeneic (i.e., allograft). Autografts are harvested from one anatomical location and used elsewhere on the same individual, making the patient both donor and recipient. Traditionally, the autologous costal cartilage (ACC) graft is the gold standard when an individual's nasal cartilage is insufficient during septorhinoplasty. An alternative is the auricular cartilage; however it is curved, thin, and scarce(3,34). Allografts, derived from another within the same species, are commonly cadaveric irradiated homologous costal cartilage (IHCC) grafts. The most common commercially available alloplastic grafts are Medpor (porous high-density polyethylene), Gore-Tex (expanded polytetrafluoroethylene), and Silicone.

The literature describes the ideal graft in septorhinoplasty on microscopic and macroscopic levels(3,34). Biocompatibility, bioactivity, and biodegradability are essential for graft success on a microscopic level. Biocompatibility is influenced by the immunogenicity of a graft(3). Grafts with low immunogenicity elicit lesser host immune responses, resulting in greater biocompatibility and generally lower infection rates. Bioactive factors promote integration by increasing chondrogenesis and/or osteogenesis without fibrous tissue encapsulation sealing the implant off(35,36). Biodegradation ideally occurs at a balanced rate whereby the graft maintains its properties and mechanical strength for an adequate time to allow sufficient host response to form the desired tissue before degrading into biocompatible waste products(36). Whilst greater porosity influences cellular migration, ingrowth and vascularisation, excessive pore size does hinder strength(36). Generally, porosity above 50 μm is recommended as many studies have shown that greater porosity is associated with increasing cellular concentration, DNA content, glycosaminoglycan (GAG) and collagen synthesis(37).

Macroscopically, grafts that mimic the biomechanical and functional properties of the target tissue without being a vector for disease are ideal(34). In addition to maintaining volume without extrusion over time, grafts should resist external and internal physiological forces. Ideal grafts are expediently produced or procured inexpensively on-demand to patient-specific requirements, or are intra-operatively customisable, and are not associated with donor-site morbidity. Infusing grafts with antimicrobial agents is preferred in limiting peri-operative infection.

Considering how common septorhinoplasty is, many studies have analysed complication rates of biologic and alloplastic grafts resulting in multiple systematic reviews with varying results. This systematic review aims to summarise the complication rates of commonly used septorhinoplasty grafts from systematic reviews and provide an overview of emerging alternatives. We present the following article in accordance with the PRISMA reporting checklist (available at https://www.theajo.com/article/view/10.21037/ajo-22-12/rc)(38).

Methods

Eligibility Criteria

Systematic reviews with or without meta-analyses including more than 10 primary studies were included whilst narrative reviews, case reports, and abstracts were excluded. Furthermore, reviews without pooled analysis of complication rates, not full text, not written in English, and not pertaining to humans were excluded.

Information Sources

Two independent reviewers (B.S.N and A.E.Y) conducted a systematic literature review of four databases (Medline, EMBASE, Google Scholar and EBMR) up to the 8th of November 2021 using the below search strategy. References of retrieved articles were also manually searched for additional articles missed in the original literature review.

Search Methods

A search strategy was generated using the PICO structure: Population – patients receiving septorhinoplasty for functional or aesthetic reasons; Intervention – ACC grafts in septorhinoplasty; Comparison – allogeneic or alloplastic grafts in septorhinoplasty; Outcome – complication rates post-operatively. The following search strategy was used "(nasal augmentation or nasal reconstruction or septoplasty or rhinoplasty or septorhinoplasty) AND ([rib graft or autologous costal cartilage graft or cadaveric cartilage graft] OR [alloplastic implant or silicone or Med*por or Gore*Tex])". Two independent reviewers (B.S.N and A.E.Y) selected studies against the pre-defined inclusion criteria and any disagreements were resolved by discussion.

Data Collection and Analysis

Two independent reviewers (B.S.N and A.E.Y) extracted data regarding study characteristics, complication rates and required data to complete a Risk of Bias in Systematic Reviews (ROBIS) assessment. Any disagreements were resolved by discussion between reviewers and, where required, with senior authors (P.M and S.C). Data extracted and tabulated is as follows:

- Study Characteristics included study design, implants (i.e., interventions) analysed, level of evidence and included study types, number of included studies and their year range, number of included patients, degree of overlap of included primary studies, length of follow-up, databases searched, and risk of bias assessment tool used.
- Complications included infection, warping, resorption, extrusion, hypertrophic scarring, pneumothorax, and revision and removal surgery.

The degree of overlap of primary studies between included systematic reviews was calculated using the Corrected Covered Area (CCA) method by two independent reviewers (B.S.N and A.E.Y)(39). Considering the degree of overlap of primary studies and heterogeneity between included systematic reviews, results of complication rates were described narratively as further meta-analysis was not feasible(40).

Results

Search Results

The initial literature search yielded 494 articles of which 87 were excluded as duplicates. 407 unique titles and abstracts were then screened using pre-defined inclusion and exclusion criteria yielding five systematic reviews, which were included. Two systematic reviews were excluded in this process as they included 10 or less primary studies and thus did not meet inclusion criteria(41,42). Further manual searching of the citations of included reviews resulted in one further systematic review being included (**Figure 1**)(38,43). The six included systematic reviews were published between 2008 and 2020 and analysed ACC with alloplastic grafts (n=2)(32,44), ACC with IHCC grafts (n=1)(45), alloplastic grafts only (n=2)(46,47), and ACC grafts only (n=1)(43) (**Table 1**).

Quality of Included Studies

In total, 170 primary studies were cited in the six systematic reviews with 111 of these being unique primary studies yielding a CCA of 10.63% which is considered moderate to high overlap(39). **Table 2** is a citation matrix representing the number of overlapping primary studies between any two given systematic reviews. Overall, Liang et al. had the most overlap of included primary studies with all other reviews (50 citations), however Hudise et al. had the highest degree of overlap of any one systematic review (76.7%) (**Table 1**)(32,44).

Using the ROBIS tool, two reviews were considered to have a high overall risk of bias representing 30% of the included reviews(46-48). The most common domain introducing bias was in synthesis and findings (50%) (**Table 3**). Peled et al. did not have pre-defined eligibility criteria, searched a single database, did not use a standardised risk of bias assessment tool, and did not employ funnel plots or sensitivity analysis in the synthesis of their results(47). Similarly, Hoang et al. did not employ a formal assessment of risk of bias or heterogeneity(46).

Qualitative Synthesis

Complications associated with graft use were thematically organised: overall complication, infection, warping, resorption, extrusion, hypertrophic scarring, pneumothorax, and revision and removal surgery. Complication results in each systematic review for ACC, IHCC, and alloplastic grafts are listed in **Table 4**.

Overall complication

Overall complication rates at the recipient site for ACC grafts were 11.4%(43) and 14%(32), and at the donor site were 3.2%(43) and 7%(32). One systematic review(44) reported overall complication rates irrespective of donor or recipient site as 6.9% for ACC grafts. Overall complication rates for alloplastic grafts were found to be 8%(32) and 7.8%(44). No systematic review provided overall complication rates for IHCC grafts.

Infection

All six systematic reviews(32,43-47) analysed infection rates by graft type. Gore-Tex was found to have varying infection rates of 1.3%(32), 1.4%(46), and 4%(47). Medpor demonstrated rates of 2.3%(32) and 6%(47). Silicone had reported rates of 1.9%(46), 2.1%(32), and 3.7%(47). Combined alloplastic graft infection rates were 3.7%(44) and 4.6%(32). ACC grafts were found to cause infection at the donor site in 0.6%(43) of cases, and at the recipient site at rates of 2%(45), 2.1%(44), 2.5%(43) and 6.8%(32). IHCC grafts demonstrated an infection rate of 3%(45).

Warping and Resorption

Of the four systematic reviews(32,43-45) reporting rates of warping amongst ACC grafts, only two(32,44) documented comparative alloplastic warping rates. One systematic review found the combined alloplastic warping rate as 1.6%(44), whilst another differentiated warping rates by alloplastic with Silicone having the highest warping rate at 5.1%, Gore-Tex the lowest at 0.9%, and no statistically significant data for Medpor(32). Warping was generally higher with ACC grafts (5.2%(43), 5.6%(32), and 6%(45)), however one systematic review reported rates as low as 1.5%(44).

One systematic review comparing ACC and alloplastic resorption rates found higher resorption rates for ACC grafts (3.9%) compared with Silicone (1.9%), Medpor (2.5%), and Gore-Tex (0.69%)(32). Contrastingly, three systematic reviews that analysed resorption rates for ACC grafts found varied rates (0.9%(43), 1%(45), and 4.1%(44)). IHCC graft warping and resorption rates were 5% and 4% respectively(45).

Extrusion

Three systematic reviews(32,46,47) documented extrusion rates. ACC grafts generally extruded at lower rates (0.6%(43) and 1.5%(32)). Medpor had higher rates of extrusion (2%(47) and 5.1%(32)) than Gore-Tex (1.1%(47), 1.2%(46), and 2.9%(32)). Silicone had varying rates of extrusion at 0.7%(46), 1.6%(32), and 3.2%(47).

Hypertrophic scarring and Pneumothorax

There systematic reviews(32,43,44) reported hypertrophic scarring rates which were generally higher for ACC (1.8%(44), 2.9%(43), and 3.8%(32)) than alloplastic grafts (1.5%(44)). Pneumothorax was solely associated with ACC grafts, with varying rates between 0.1%(43) and 3.1%(32).

Revision and Removal surgery

Six systematic reviews(32,43-47) analysed alloplastic, IHCC and ACC grafts with regards to rates of surgical revision or removal. One systematic review showed ACC grafts had lower removal rates (1.9%) and higher revision rates (8%)(32). Contrastingly, in the same study alloplastics generally had higher removal than revision rates: Silicone's removal and revision rate was 12% and 3.9% respectively, Medpor 4.5% and 5.7%, and Gore-Tex 3.6% and 2%(32). Another systematic review showed similar revision surgery rates for alloplastic (3.7%) and ACC (4.2%) grafts(44). IHCC grafts had higher revision rates (7%(45)) compared to ACC grafts (5%(45)) which was also reflected in another systematic review where the ACC revision rate was 5.4%(43). One systematic review found removal rates to be 6.5% for Silicone, and 3.1% for Medpor and Goretex(47). Another systematic review demonstrated revision rates of 6.8% for Silicone, and 2.4% for Gore-tex(46).

Discussion

This review identified six systematic reviews with or without meta-analysis that show neither ACC, allogeneic, or alloplastic grafts are superior in every clinical situation during septorhinoplasty. Warping, resorption, revision surgery and donor-site morbidity were generally higher with ACC graft use. Infection, extrusion, and removal surgery were generally higher amongst all alloplastic grafts compared to ACC grafts. However, there was variation of these rates between different types of alloplastic grafts. IHCC grafts had similar complication rates to ACC grafts with regards to infection, warping, resorption, and secondary surgery when used as dorsal onlay grafts only.

Biologic grafts

ACC grafts are popular for their intra-operative versatility and low infection risk. In addition to being relatively abundant in supply, ACC grafts are sufficiently rigid to provide structural support, or be morselised as a filler(34). Being autologous, ACC grafts have inherently low immunogenicity and thus low infection rates(3,45). When an infection is present, intravenous antibiotics have greater penetrance in ACC grafts compared with synthetic alternatives, thus reducing requirements for secondary surgery for ACC grafts due to infection(42). Despite its popularity, there are multiple challenges with ACC grafts; donor-site morbidity, longer operating times for harvest, revision surgery and warping(43).

IHCC grafts are a potential solution for the drawbacks of ACC grafts as they have low infection rates, no donor-site morbidity, and are readily available eliminating long operating times. Although IHCC grafts are irradiated to lower infection risk, this process causes decellularisation limiting in-vivo remodelling thereby promoting greater resorption and revision surgery rates(3). Despite this, a recent meta-analysis by Vila et al. demonstrated similar rates of warping between IHCC and ACC grafts(45). However, their study only included IHCC dorsal onlay grafts where structural support was not required(45). Traditionally, IHCC grafts are limited to situations where structural support isn't crucial to compensate for their varying warping rates and poorer retention(45,49). Considering this, IHCC

grafts are generally less popular than ACC grafts, contributing to a paucity of long-term follow-up studies(49).

Alloplastic grafts

Another alternative to ACC grafts is the alloplastic graft. Alloplastics can be cost-effectively produced providing an abundance of supply without donor-site morbidity. Whilst some alloplastics can be intra-operatively carved to specific dimensions, others can be pre-fabricated to generic shapes such as alar battens or L-struts(34). However, there is no readily available alloplastic graft suitable in every septorhinoplasty operation(34) and no studies to date have quantitatively compared the biomechanical properties of alloplastics with septal or costal cartilage(3,50). Despite this, they have been used extensively in select groups(51) .

Of the three common alloplastics, the softness of Gore-Tex makes it appropriate for dorsal onlay grafting but too weak for structural support(34). With intermediate porosity ranging from 10-30 μm(51), Gore-Tex allows limited tissue ingrowth for stabilisation without promoting significant ingrowth(34). Medpor possesses the highest porosity of the three alloplastics (125-250 μm(51)), promoting significant tissue ingrowth. Combined with its superior rigidity, this makes Medpor suitable for structural support(34). However, this also contributes to Medpor's extrusion and removal rates, with the removal operation being more difficult considering the tissue ingrowth(32). Silicone is cheap and easily carved intra-operatively, promoting its use in dorsal onlay techniques(32,34). As silicone is non-porous, there is significant fibrous encapsulation and subsequent calcification over time promoting the highest complication and removal surgery rates of the three alloplastics (32). Interestingly however, the rates of silicone extrusion and displacement amongst Asians are lower compared with Caucasians. Asian patients have a thicker skin-soft tissue envelope that likely creates a more forgiving environment for silicone implants compared with Caucasian patients(34). Consequently, silicone is more popular with Asian surgeons, resulting in greater experience and refined surgical techniques(52).

In comparing alloplastic and ACC grafts, Liang et al. demonstrated higher removal rates for alloplastics compared with higher revision rates for ACC grafts(32). This is likely attributable to ACC grafts warping at higher rates but being autologous in nature, often only requiring revision rather than removal. Contrastingly, alloplastic grafts extrude at higher rates and are less adept to manipulation for revision, therefore requiring removal.

Limitations

The major limitation of this systematic review is the heterogeneity of included systematic reviews and significant overlap of included primary studies rendering meta-analysis or comparison of results between studies unfeasible(39,53). Furthermore, all reviews relied heavily on retrospective studies making their level of evidence IV at best. Two of the included systematic reviews were also considered to have a high risk of bias which limits interpretation of their results.

Future Directions

Tissue-engineered grafts in septorhinoplasty have emerged in the hope of eliminating the drawbacks of donor-site morbidity and longer operating times associated with biologic grafts whilst retaining their inherently low immunogenicity and tissue versatility. A tissue-engineered graft aims to produce biomimetic tissue by utilising the optimal combination of a cellular source, stimuli, and scaffolding(54).

Cellular source in tissue-engineering neo-cartilage is primarily either chondrocytes or mesenchymal stem cells (MSCs)(3,54). Although chondrocytes harvested from bovine(55), rabbit(56), and ovine(57) sources have been investigated, only autologous sources have been approved for human use and thus the issue of donor-site morbidity persists(58). Autologous or allogeneic MSCs can grow rapidly but produce fibrocartilage that lacks the loading and stretching properties that hyaline septal cartilage possesses(54). Genetic modifications present a future avenue for research to obtain appropriate hyaline cartilaginous tissue from MSCs(59,60).

Stimulation whilst culturing chondrocytes through either 3D culture systems(61), growth factors(62), or MSCs(54) is important as chondrocytes dedifferentiate to adopt fibroblastic characteristics after

three monolayer culture passages(54). To combat the significant quantity of chondrocytes required to engineer the volume of cartilage required in septorhinoplasty, methods such as incorporating autologous MSCs(55) and using photocrosslinkable bioinks that can bioprint chondrocytes instead of seeding them have been studied(63). However, further research is required to overcome issues of time and cost to allow production at scale to be viable.

Clinically, tissue-engineered grafts have demonstrated promising but limited results(3). There are no systematic reviews, meta-analyses, large animal studies, or standardised animal models for tissue-engineered grafts in septorhinoplasty(3,60-62,64). Only five uncontrolled cohort studies in small human populations have been conducted to date without any report of significant adverse events(65-69).

Bioactive-biocompatible polymer hybrid scaffolds combine porous biodegradable biocompatible polymeric scaffolds with bioactive materials to improve integration and reduce extrusion rates when compared to alloplastics(70-72). Whilst no hybrid scaffolds have been approved in septorhinoplasty, the success of hybrid scaffolds like Chondrotissue® (Biotissue) in replacing articular cartilage is promising(73,74). Wu et al. have demonstrated in-vivo that the combination of a bioactive ceramic silica-based glass (Bioglass®) with a polymer scaffold exhibited greater hydrophilicity, adherence, and stimulated thicker cartilage-like tissue with better biomechanics than Bioglass® alone(71). Yao et al. also demonstrated a hybrid Bioglass®-polymer scaffold had degradation rates and mechanical strength superior to Bioglass® alone(72). Characterising the ability of Bioglass®-polymer scaffold composites to stimulate chondrogenesis in vivo requires further research.

Conclusion

Our systematic review of six systematic reviews suggests alloplastic, ACC and IHCC grafts are viable alternatives when native septal cartilage is insufficient during septorhinoplasty. When selecting grafts, surgeons should consider the purpose and complications associated with each graft and educate patients appropriately. Whilst ACC grafts remain the preferred alternative, it is evident that the 'ideal' graft has yet to be achieved. Tissue-engineering and bioactive-biocompatible polymer composites hold promise but require further research to overcome their limitations and nascence.

Tables and Figures

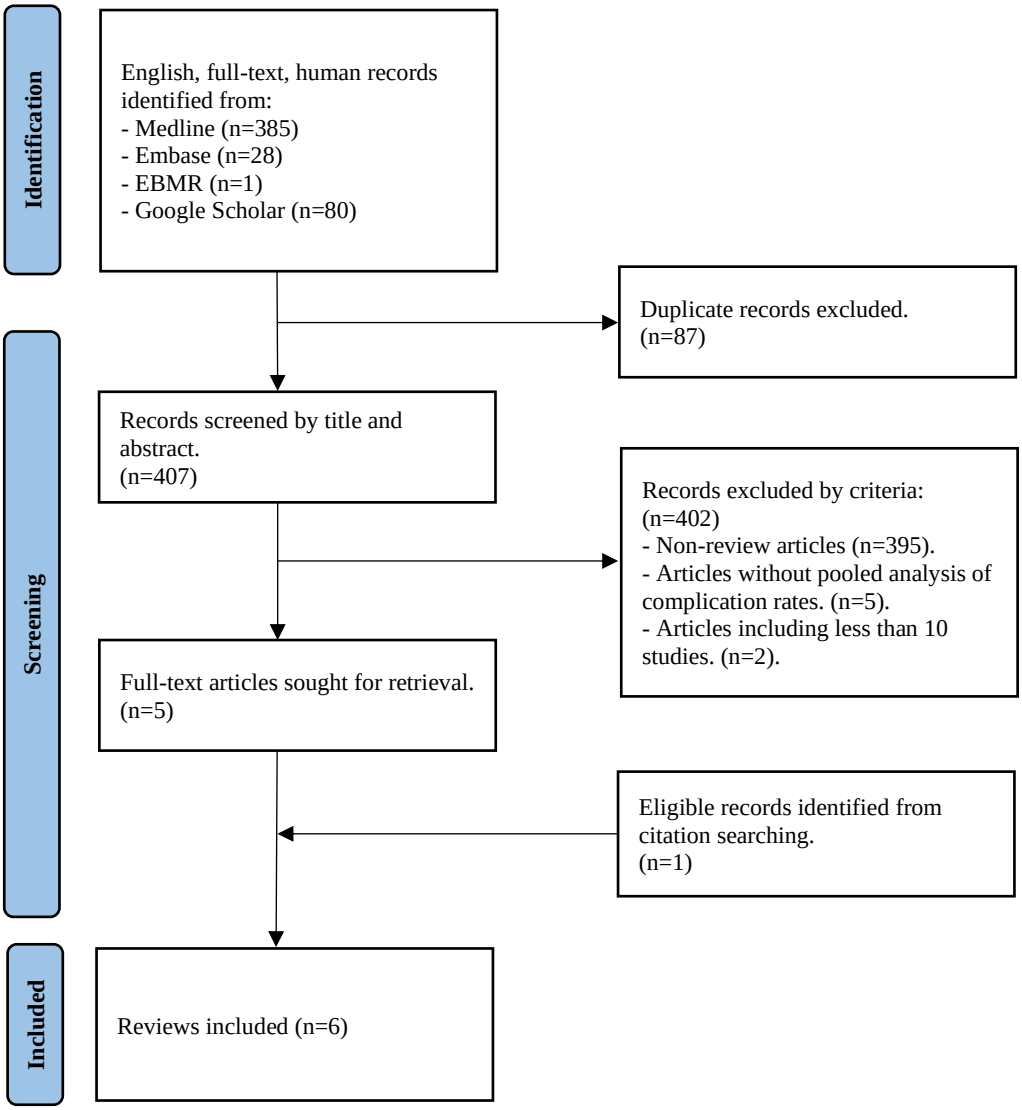

Figure 1: PRISMA flowchart.

Author, Year	Implant Used	Study Design	Level of Evidence	No. of studies (year)	No. of Patients	Overlap (Overlap/Total No. of Studies)	Follow up	Databases Searched	Risk of Bias Assessment Tool
Peled et al, 2008(47)	Silicone Medpor Gore-tex	Meta-analysis & Systematic Review	IV	20 (1966-2005)	4264	35% (7/20)	4 weeks – 11 years	MEDLINE	N/A
Hoang et al, 2018(46)	Silicone Gore-tex	Meta-analysis & Systematic Review	IV	18 (1988-2016)	7759	66.7% (12/18)	6 days – 17 years	MEDLINE, PubMed, Cochrane	N/A
Varadharajan et al, 2014(43)	ACC	Systematic Review	IV	21 (1980-2014)	1545	53.4% (11/21)	3 – 73 months	MEDLINE, PubMed, Cochrane, EMBASE	ASPS critical appraisal checklist
Hudise et al, 2020(44)	ACC Silicone Medpor Gore-tex	Meta-analysis & Systematic Review	IV	30 (1999-2018)	1013	76.7% (23/30)	2.5 – 106 months	PubMed, Cochrane Library, Web of Science, Virtual Health Library, Google Scholar, SCOPUS	ROBINS-1
Liang et al, 2018(32)	ACC Silicone Medpor Gore-tex	Meta-analysis & Systematic Review	IV	53 (1973-2017)	5415	67.9% (36/53)	3 – 72 months	EMBASE, PubMed	MINORS
Vila et al, 2020(45)	ACC IHCC	Meta-analysis & Systematic Review	IV	28 (1990-2017)	1041	50% (14/28)	6 – 48 months	MEDLINE, EMBASE, Scopus, CENTRAL, ClinicalTrials.gov	Cochrane Collaboration Risk of Bias Tool

Table 1: Summary of Study Characteristics. ACC, Autologous Costal Cartilage; IHCC, Irradiated Homologous Costal Cartilage; ASPS, American Society of Plastic Surgeons; ROBINS-1, Risk of Bias In Non-randomised Studies; MINORS, Methodological Index for Non-Randomised Studies.

Review	Peled(47)	Hoang(46)	Varadharajan(43)	Hudise(44)	Liang(32)	Vila(45)
Peled(47)	N/A	5	0	1	3	0
Hoang(46)	N/A	N/A	0	0	8	0
Varadharajan(43)	N/A	N/A	N/A	1	7	5
Hudise(44)	N/A	N/A	N/A	N/A	21	5
Liang(32)	N/A	N/A	N/A	N/A	N/A	11
Vila(45)	N/A	N/A	N/A	N/A	N/A	N/A

Table 2: Citation Matrix of Overlap between Systematic Reviews. Numbers in each cell represent the total number of overlapping primary studies between any two systematic reviews.

Review	Phase 2				Phase 3
	1. STUDY ELIGIBILITY CRITERIA	2. IDENTIFICATION AND SELECTION OF STUDIES	3. DATA COLLECTION AND STUDY APPRAISAL	4. SYNTHESIS AND FINDINGS	RISK OF BIAS IN THE REVIEW
Peled(47)	☹	☹	☹	☹	☹
Hoang(46)	☹	☺	☹	☹	☹
Varadharajan(43)	☺	☺	☺	☹	☺
Hudise(44)	☹	☺	☺	☺	☺
Liang(32)	☺	☺	☺	☺	☺
Vila(45)	☺	☺	☺	☺	☺

Table 3: Risk of Bias (ROBIS) by Review. ☺ = low risk, ☹ = high risk.

	ACC			IHCC	Alloplastic			
	% of Donor Site [CI]	**% of Recipient Site [CI]**	**% of Combined Sites [CI]**		**% of Silicone [CI]**	**% of Med-por [CI]**	**% of Gore-Tex [CI]**	**% of Combined Alloplastics [CI]**
Overall Complication	3.2(43), 7 [4-11.6](32)	11.4(43), 14 [10-19](32)	6.9 [4.3-9.6] (44)	NS	9.2(46)	NS	5.3(46)	7.8 [4-11.5](44), 8 [5-11](32)
Infection	0.6(43)	2.5(43)	2 [0-5](45), 2.1 [1-3.3](44), 6.8 [4.7-9.7](32)	3 [1-8] (45)	1.9(46), 2.1 [0.6-7.3](32), 3.7(47)	2.3 [1-8](32), 6(47)	1.3[0.6-3](32), 1.4(46), 4(47)	3.7 [0.4-7](44), 4.6 [0.3-44.8](32)
Warping	N/A	1.5 [0.3-2.8](44), 5.2(43), 5.6 [4-7.7](32), 6 [2-11](45)	N/A	5 [1-12] (45)	5.1 [2-11.6](32)	NS	0.9 [0.6-1.7] (32)	1.6 [0.5-3.7](44)
Resorption	N/A	0.9(43), 1 [0-2](45), 3.9 [2.6-5.8](32), 4.1 [2.5-5.6](44)	N/A	4 [0-13] (45)	1.9 [0.1-24](32)	2.5 [0.2-30] (32)	0.7 [0.1-3.6] (32)	NS
Extrusion	N/A	0.6(43), 1.5 [0.6-3.8](32)	N/A	NS	0.7(46), 1.6 [0.5-5.2](32), 3.2(47)	2(47), 5.1 [2.5-10] (32)	1.1(47), 1.2(46), 2.9 [2.2-4](32)	1.1 [0.2-5.2](32)
Hypertrophic scar	2.9(43), 3.8 [1.7-8](32)	NS	1.8 [0.2-3.4] (44)	NS	NS	NS	NS	1.5 [0.4-3.5](44)
Pneumothorax	0.1(43), 3.1 [1.5-6.5] (32)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Revision Surgery	N/A	4.2 [2.4-6](44), 5 [1-10](45), 5.4(43), 8 [4.8-12.9](32)	N/A	7 [3-12] (45)	3.9 [1-14.2](32), 6.8(46)	5.7[2.8-11.3] (32)	2 [0.3-14](32), 2.4(46)	5 [1.8-13](32), 3.7[1.0-6.4](44)
Removal Surgery	N/A	1.9 [1-3.6](32)	N/A	NS	6.5(47), 12 [6.5-19.9](32)	3.1(47), 4.5 [2-10](32)	3.1(47), 3.6 [2-6.2](32)	2.2 [0.4-10.5](32)

Table 4: Complication Rates by Graft Type. CI, Confidence Interval; ACC, Autologous Costal Cartilage; IHCC, Irradiated Homologous Costal Cartilage; NS, Not Stated; N/A, Not Applicable.

Chapter Three - In-vivo evaluation of a novel biosynthetic graft (actv-graft®) for nasal reconstruction

Overview

In chapter 2, the lack of a clearly ideal alternative graft to native septal cartilage during septorhinoplasty was established. Future directions including tissue-engineering and hybrid scaffolds were explored. The field of tissue-engineering has grown rapidly recently, and although there are promising results being generated from this work, limitations such as donor-site morbidity associated with cellular source harvest, production time and cost, and chondrocyte dedifferentiation remain key barriers. Within this context, evidence in the literature for hybrids of Bioglass® and Polycaprolactone are established in chapter 3. A commercially available composite (actv-graft®) is subsequently identified with pre-clinical evidence in small animal studies.

Thus, the primary aim of this chapter is to present a large animal feasibility study where actv-graft® was implanted in the nasal dorsum of three sheep for 3 months. Our study demonstrated that actv-graft® was well tolerated without clinical signs of infection. It maintained volume augmentation over time both clinically and radiologically. Although partial degradation was demonstrated radiologically, integration was suggested by increasing radiodensity over the 3-month period. This was confirmed histologically. This chapter is currently under consideration for publication in Biomaterials.

Title: In-vivo evaluation of a novel biosynthetic graft (actv-graft®) for nasal reconstruction.

Running Title: In-vivo nasal reconstruction with a novel biosynthetic graft.

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Abstract

Background: When native septal cartilage is insufficient during septorhinoplasty, alternative grafts are required. Current alternatives have their own drawbacks limiting widespread clinical utility of any one graft. Hybrid bioactive-polymer scaffolds have emerged as a potential solution to this problem. Bioglass®-Polycaprolactone (BG-PCL) is a hybrid graft that is a commercially available composite (actv-graft®) with promising pre-clinical results in small animals. This study aims to establish the feasibility of actv-graft® for nasal reconstruction in an ovine model by assessing its ability to integrate into and stimulate the surrounding host tissue over 3 months.

Methods: A BG-PCL composite (actv-graft®) was implanted in the nasal dorsum of three sheep for 3 months with ethics approval. Macroscopic computed tomography (CT) scans and clinical photographs were obtained on the day of surgery and at 3 months prior to harvest. Grafts and the surrounding tissue were removed, analysed with microscopic CT, then examined histologically (haematoxylin and eosin, picrosirius red, and Masson's trichrome) for neovascularisation, and collagen formation and type.

Results: Clinically, all sheep maintained volume augmentation at 3 months with no signs of infection. Macroscopic CT analysis showed persistent volume augmentation at 3 months with majority retention of graft volume (average 91±5%) on microscopic CT. Graft radiodensity values increased over time, indicating host infiltration within the graft. This was confirmed histologically with rich cellular infiltrate into the graft and hyalinised collagen formation which was predominantly type III rather than type I under polarised light microscopy.

Conclusion: The BG-PCL hybrid scaffold used sustained volume augmentation, stimulated cellular infiltrate, and degraded safely over time in the nasal dorsum of large animals. Future research into its effectiveness and long-term stability in a human population is required.

Introduction

Cartilaginous defects of the nose can be congenital or arise from trauma, previous surgery, or cancer. The subsequent potential impairment on airflow or cosmesis can necessitate surgical reconstruction of the nose in the form of fracture reduction, septoplasty, and/or rhinoplasty. With up to 80% of the population having a deviated nasal septum, it is not unsurprising that septorhinoplasty was the third most common procedure in the United States in 2016, costing $1.1 billion(3,32). When reconstructing the structure, shape, and volume of the nose, native nasal septal cartilage can be insufficient, necessitating alternative graft material. Key features of alternative grafts are biocompatibility, bioactivity, and biodegradability(3). Grafts that elicit a greater immune response such as infection are less biocompatible, whilst grafts that stimulate a greater host integration response are considered more bioactive(36). Grafts that degrade into harmless by-products that can be easily resorbed by the host are considered biodegradable(36).

Commonly used alternatives include biologic and alloplastic (synthetic) grafts(34). Biologic grafts include the autologous costal cartilage (ACC) and irradiated homologous cadaveric cartilage (IHCC) graft. ACC grafts are considered the gold standard for their inherently low immunogenicity and their intra-operative versatility, being used as structural support or morselised as filler(34). However, the prolonged operative time for harvest, associated donor-site morbidity, and higher rates of revision surgery and warping create a necessity for other alternatives(75). IHCC grafts are readily available, have similar infection rates to ACC grafts, and do not have any donor morbidity(45). However, their varying warping and retention rates limit their use in patients requiring structural support, meaning their use is largely confined to dorsal onlay grafts(45). A variety of alloplastic grafts are used in septorhinoplasty, with each having unique characteristics such as strength and porosity. Whilst alloplastics are cost-effectively procured, cause no donor morbidity, and are versatile (through pre-operative fabrication or intra-operative carving), they generally have higher rates of infection, extrusion, and removal surgery compared to biologic grafts(75).

It is evident that the ideal graft does not yet exist in septorhinoplasty, and thus alternatives such as tissue-engineered grafts and hybrid bioactive-polymer scaffolds have emerged. Tissue-engineered

grafts promise low immunogenicity, versatility in prefabricated patient-specific implants, and less donor morbidity than ACC grafts(3). Nevertheless, cost of production, time to culture, and limitations in chondrocyte dedifferentiation have emerged as key barriers to their immediate clinical implementation.

In an attempt to improve integration and reduce extrusion rates when compared to alloplastics, biocompatible polymer scaffolds have been combined with bioactive materials which stimulate biological cues at the host-scaffold interface(35,36,70-72,76,77). Bioglass® (BG) is a bioactive ceramic silica-based glass that reacts with its host environment to form interfacial bonds and promote healing by limiting infection and stimulating angiogenesis(36,71,78,79). Whilst it has established osteoinductive properties(36), it has recently gained attention for its chondrogenic potential, although its use in scaffolding is limited by its brittleness(76). Polycaprolactone (PCL) is a biocompatible polymer scaffold that has favourable mechanical stability, controllable porosity, and can biodegrade into harmless products within 2 years(3,72,80,81). Furthermore, it has been shown to stimulate the production of type II collagen which is favourable for cartilage formation after 3 months in rabbits(82), and chondrocytes after 6 months in pigs(81). Lebourg et al. also demonstrated that by varying the porosity of PCL structures, its compressive elastic modulus could be targeted to similar values exhibited by cartilage(83).

In order to overcome the brittleness of BG, its particles can be dispersed in a biocompatible polymer such as PCL(84). Both BG and PCL have been approved for human use by the United States Food and Drug Administration (FDA)(81). Additionally, composites of BG-PCL have been demonstrated in-vitro and in small animal studies to have favourable biomechanics and biodegradation rates(72,84). Kim et al. recently implanted a BG-PCL composite in craniofacial bone defects of seven humans with reasonable bone-binding properties and clinical safety(85). However, to date no large animal studies have been conducted to investigate the feasibility of a BG-PCL composite as a graft during septorhinoplasty.

A novel 45S5 BG-PCL composite (actv-graft®) is produced by Global Surgical Innovations™ (GSI) and has demonstrated neovascularisation, granulation, and epithelial tissue growth without formation

of a fibrous capsule when it was implanted subcutaneously in rats for 6 weeks(86). Furthermore, Boughton et al. established that actv-graft® had macropores from 300-500µm and mesopores from 2-40µm within the pore struts, creating a favourable environment for chondrogenesis(87).

The objectives of this study are to firstly establish the feasibility of actv-graft® for nasal reconstruction in an ovine model. Secondly, through radiological and histological assessment, characterise the ability of implanted actv-graft® to integrate into and stimulate the surrounding host tissue over 3 months.

Methods

Material Preparation

Nasal implants were manufactured and supplied by GSI™. GSI™ employ a novel method of Rapid Batch Coagulation to customise the precursor macro-, micro-, and nano-structure before a PCL solution is introduced into the gaps of the precursor and coagulated with Bioactive Glass (45S5)(86). The precursor is then removed leaving behind the customised BG-PCL graft, now called actv-graft®. For this study, a "soft" 5mm-thick sheet of actv-graft® was supplied (**Figure 1**).

Clinical Application

The methods used in this study were approved by the University of Sydney Animal Ethics Committee (Project number 2021/2003). Ovis aries sheep were acquired, transported, anaesthetised, operated on, and cared for according to local standard operating procedures.

Three sheep underwent general anaesthesia by the Laboratory Animal Services (LAS) Veterinarians at the Charles Perkins Centre at the University of Sydney. Whilst under anaesthetic, each sheep underwent a macroscopic computed tomography (CT) scan of the head prior to surgery to serve as a baseline for comparison post-operatively. CT was favoured over magnetic resonance imaging due to availability, cost, time and ability to correlate with microscopic CT. Additionally, a profile view clinical photograph of each sheep head was obtained pre-operatively whilst under anaesthetic. In each sheep, a 2cm horizontal incision was made over the nasal bridge and an approximately 2x2cm subcutaneous pocket was dissected over the bony and cartilaginous nasal dorsum. A "soft" 5mm-thick actv-graft® was inserted as an onlay graft in contact with cartilage and bone and sutured to the subcutaneous tissue. Finally, the skin incision was closed with sutures and clinical photographs and macroscopic CT scans were taken immediately post-operatively.

Three months after surgery, a macroscopic CT scan was conducted of each sheep head with all grafts in situ. Clinical photographs were also taken for macroscopic assessment. Sheep were then sacrificed according to local protocols and the grafts removed en-bloc with surrounding tissue. Microscopic CT scans were taken of each explanted graft. Transverse slices of each explant were obtained and flash

frozen in optimal cutting temperature (OCT) compound, with the remainder of the explant preserved in 10% phosphate-buffered formalin.

Volume Retention

Volume retention was assessed over time by comparing the CT images pre-operatively, immediately post-operatively, and at 3 months for each sheep. Two methods were employed to assess dorsal nasal augmentation and graft volume changes over time using 3D Slicer 5.2.2 to translate CT images into a three-dimensional model(88).

To assess maintenance of dorsal nasal augmentation, the skull was first rendered using thresholding to identify bone on macroscopic CT images. Three points along the nasal dorsum were identified on the three-dimensional model of the skull - the first point was made to be the most caudal point of the nasal bone, the second point was placed on the dorsal nasal bone 50mm cephalic from the first point, and the third point was created at the midpoint of the straight line connecting the first and second points (**Figure 2**). The volume of all tissue within a sphere of diameter 80mm with its centre at the third point was calculated. Volumes were compared across the pre-operative, post-operative and 3-month CT scans to assess the host tissue-graft volume response over time at the nasal dorsum. In one sheep, due to technical difficulties on the day of surgery, peri-operative macroscopic CT scans could not be obtained, and thus plain X-rays were captured instead. As such, these specimens could not be included in this method of graft volume analysis.

In assessing graft volume retention over time, dimensions of the explanted graft on microscopic CT were compared to pre-implantation graft measurements. To create a three-dimensional model of the graft and surrounding tissue on the microscopic CT images of each sheep, thresholding was used to create a segment containing both bone and remnant BG-containing struts of the graft (**Figure 3**). On this model, the maximum dimensions (length, width, and height) of the graft were measured. This was then compared to the equivalent dimensions of each graft before implantation, as measured from clinical photographs taken with a scale prior to implantation (**Figure 1**), which provided an evaluation of graft degradation over time. Given our small sample size, statistical analysis was not performed.

Graft-Tissue Integration: Radiological Assessment

On both immediate post operative macroscopic CT and 3-month microscopic CT images, five regions of interest were randomly selected in each of the three tissue types – within the scaffold (including any cellular infiltrate contained within), in soft tissue, and in bone. A region of interest was defined as a two-dimensional circle of diameter 2mm and included all tissue within this region. The mean voxel intensity across the subsequent 15 regions of interest was calculated on macroscopic CT images to provide a baseline. Comparison was then made to values obtained from microscopic CT images as an indicator of soft tissue integration into the graft over time.

Graft-Tissue Integration: Histological Assessment

For the histological analysis of the explanted grafts, the flash-frozen specimens were cryosectioned at 10μm thickness and mounted on glass slides. The slides were fixed with 75% ethanol for 30 seconds and then stained with haematoxylin and eosin, picrosirius red, and Masson's trichrome according to standard protocols. The outcomes of interest included presence of neovascularisation, formation and thickness of a collagenous host-implant interface, and quality of this collagenous interface. This was indicated by the ratio of type I versus type III collagen in the interface on picrosirius red staining, which was determined by the ratio of red versus green seen under polarised light microscopy respectively(89,90).

Results

Macroscopic assessment

Clinically, no significant infective response or animal distress was noted at 3 months. Volume augmentation in the nasal dorsum was evident post-surgery, and was maintained, albeit decreased, at 3 months (**Figure 4**).

Whilst the graft was palpable and easily identifiable macroscopically within explanted specimens, it was noted that blunt dissection around the graft was challenging, suggesting a degree of integration with the surrounding tissue (**Figure 5**). On macroscopic CT imaging immediately post-operatively, the graft was clearly visible as a radiopaque material filled with radiolucent air pockets. At 3 months, the graft was still visible however the clear distinction between host tissue and graft present after surgery had subjectively reduced, suggesting a degree of integration or partial degradation (**Figure 6**).

Volume Retention

Consistent dorsal nasal volume augmentation was demonstrated over 3 months in both included samples as measured on macroscopic CT images. One sheep exhibited an increase from 123% immediately post-operatively to 126% at 3 months, whilst the other sheep demonstrated a slight volume reduction from 109% to 107% (**Graph 1**).

On microscopic CT images, graft retention at 3 months was on average 91±5% of original volume indicating partial degradation over time (**Graph 2**).

Graft-Tissue Integration: Radiological Assessment

The mean ratio of radiodensity values obtained from the implanted graft and surrounding soft tissue increased from an average of 1.2±0.1 immediately post-surgery to an average of 3.1±0.2 3 months post-surgery (**Graph 3**). Similarly, the mean radiodensity ratio of the implanted graft and surrounding bone increased from an average of 0.2±0.01 immediately post-surgery to 0.2±0.01 3 months post-

surgery (**Graph 4**). The increase in radiodensity despite reduction in graft volume suggests infiltration within the graft itself. The radiodensity values for sheep 1 immediately post-surgery were not available due to difficulty obtaining a macroscopic CT at that time.

Graft-Tissue Integration: Histological Assessment

All grafts demonstrated a rich cellular infiltrate with moderate fibroblastic and inflammatory contents (**Figure 7 and 8**). An interface rich in hyalinised collagen was formed between the graft and surrounding tissues. Thickness of the host tissue-graft interface ranged from 0 to 1mm. The specimens exhibited type III collagen predominance in the interface, as assessed on picrosirius red staining under polarised light microscopy. Two specimens exhibited 90% collagen type III and 10% collagen type I, and the final specimen demonstrated 70% collage type III and 30% collagen type I (**Figure 9**). No neovascularisation was demonstrated within the samples explanted at 3 months.

Discussion

This study investigated the suitability of a BG-PCL composite (actv-graft®) in a large animal ovine model for nasal reconstruction. Biocompatibility, graft infiltration, and biodegradability were demonstrated along with favourable volume augmentation over time.

Balancing volume augmentation with a graft's biodegradability over time is crucial to ensure adequate time for the host to create the appropriate tissue whilst maintaining a desired clinical appearance. Classically, PCL degrades over approximately 2 years through a two-step process. Firstly, water uptake initiates non-enzymatic hydrolytic cleavage of ester links, stimulating bulk degradation(81). The second phase involves bioresorption through host metabolic pathways into biocompatible by-products(91). Higher molecular weight implants tend to exhibit longer degradation times, introducing a degree of control over PCL degradation rates when initial molecular weight is varied(92). However, when BG is combined with PCL, an accelerated degradation rate has been described(84,93). BG-PCL composites immersed in simulated body fluid (SBF) demonstrated a 13.2% weight loss over 8 weeks in-vitro(84). Bossard et al. suggest the hydrophilicity of BG increased the surface wettability of the composite, thereby accelerating the usual first phase degradation of PCL(84). Furthermore, they hypothesised that the production of hydroxide ions induced by BG would increase the local pH catalysing ester link cleavage and promoting degradation. Ji et al. found similar changes to degradation when BG was added to PCL, suggesting the quicker consumption of BG created a rougher PCL surface with greater surface area leading to more rapid degradation(93). Our results comparing the volume of the pre-implanted graft to the microscopic CT images of the explanted graft demonstrated comparable volume reduction over 3 months to other results in the literature (84,93). However, there were limitations with our approach. Given the degree of integration of the surrounding tissue into the graft and the thin tissue-graft interface measurements, it was difficult even on microscopic CT images to determine the exact host tissue-graft interface, introducing potential error to our results.

Assessing volume augmentation over time in animal studies is a difficult metric to objectively quantify, with multiple studies opting for subjective macroscopic assessments(71,81,82,94). Kim et al.

utilised 1mm CT scans to grossly comment on retention of the initial shape of their chondrocyte seeded 3D-printed PCL grafts implanted in the nasal dorsum of rabbits after 12 weeks(95). However, no other formal volume assessment was conducted. When implanting PCL grafts in the nasal dorsum of rabbits, Park et al. provided a degree of objectivity in assessing nasal augmentation by comparing pre- and post-operative nasal tip angle, showing significant reduction over 12 weeks indicating retained nasal tip projection(96). To our knowledge, our study represents the first of its kind to employ a radiologically based volume assessment using bony reference points that can reliably and repeatably assess volume changes over time. We demonstrated volume augmentation over 3 months in both included samples. However, one sample showed a 2% reduction in volume from immediately post-operatively to 3 months. Immediate post-operative swelling that reduced over time likely explains this reduction in volume.

Our study demonstrated infiltration within the graft through histological and radiological methods. Histologically, a rich cellular infiltrate into the graft with hyalinised collagen at the tissue-graft interface was found. Contrary to prior findings of actv-graft® in-vivo, we did not show neovascularisation(86). This could be due to our sectioning technique, as similar timeframes(95) and implant locations(81) have revealed neovascularisation within a PCL scaffold previously. It is difficult to directly compare our results of a BG-PCL composite for in-vivo nasal reconstruction to the literature, as most prior studies have focused on bone regeneration rather than nasal reconstruction(84,97-99). One study implanted a BG-PCL composite in the subcutaneous tissue of the back of rats and demonstrated fibrous connective tissue and adipose tissue infiltrate, however they did not comment on neovascularisation or collagen type formation(100). Radiologically, we demonstrated infiltration within the graft as there was an increase in radiodensity over the life of the graft despite the expected reduction in radiodensity as the glass beads of BG broke down(101).

When comparing picrosirius polarisation, samples from each of our three sheep demonstrated higher green than red levels, indicating higher levels of collagen type III than type I(90). Whilst collagen type III is crucial in early wound healing(102), it has also been shown to positively affect the elastic modulus of cartilage, aid in orchestrating fibril assembly, and improve the biomechanical function of

cartilage(103). Wu et al. suggest a reason for this being that collagen type III modifies the covalent fibril network between collagen type II, acting as a “molecular glue” to improve inter-fibrillar strength(104). Furthermore, in the pericellular matrix between chondrocytes and the extracellular matrix, type III collagen may mediate early type II collagen fibrillogenesis and chondrocyte mechanotransduction(103). These are key requirements in neocartilage formation, especially within the nose where type II cartilage predominates(105). Whilst higher levels of type III collagen may be beneficial to neocartilage formation, as our study did not directly assess for type II collagen and the implant was over bone and cartilage, it is difficult to draw conclusions about chondrogenesis.

Our study was limited by its duration and cohort size. As aforementioned, PCL can take around 2 years to degrade, and monitoring long-term host changes as the PCL degrades is difficult in a 3-month timeframe. Logistical and financial limitations meant a 3-month duration was chosen over 6 months. Furthermore, we faced unexpected perioperative difficulty CT scanning one sheep which affected volume and radiodensity assessment. During implantation, we noted that bone partially overlay cartilage in sections of the nasal dorsum. Subsequently, the implanted graft was in contact with both the bone and cartilage of the nasal dorsum. Whilst this allowed us to assess its reaction to both tissue types, it limited its contact with cartilage alone, and may have consequently limited chondrogenesis. Immunohistochemistry analysis would have been beneficial in further characterising the inflammatory reaction we saw histologically, in addition to assessing the presence of type II collagen as a surrogate for chondrogenesis. This remains a future avenue for our research and grant approval has been secured to ensure this. A pre-clinical trial with a larger cohort and longer timeframe would be beneficial in creating a stronger assessment of the long-term profile of actv-graft®.

Conclusion

Our pre-clinical evaluation of actv-graft®, a BG-PCL composite, demonstrated safe and sustained volume augmentation with cellular infiltrate and favourable collagen formation over 3 months in the nasal dorsum of three sheep. Therefore, actv-graft® has clinical potential to become a viable alternative to commonly used grafts in rhinoplasty. However, further research is needed to confirm its effectiveness and long-term stability in a human population.

Tables and Figures

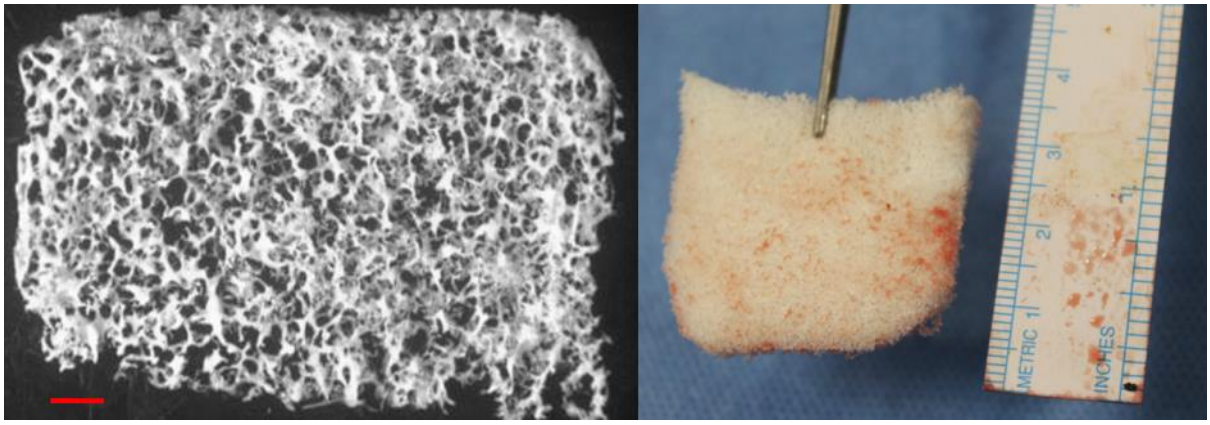


Figure 1: Raw form of the actv-graft® implant demonstrating porosity (left) (red line reflects 1mm) (modified from Boughton 2011(86)). actv-graft® implant used in study with ruler for scale (right).

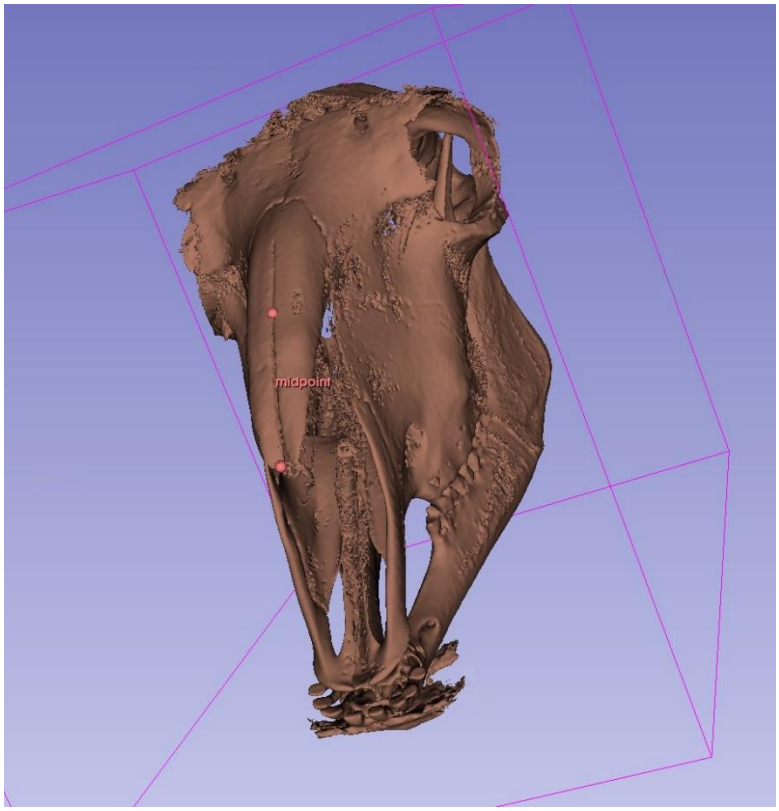


Figure 2: 3D reconstruction in Slicer 5.2.2 of macroscopic CT images for dorsal augmentation assessment. Orange ball points indicate caudal and cephalic endpoints with the midpoint marked.

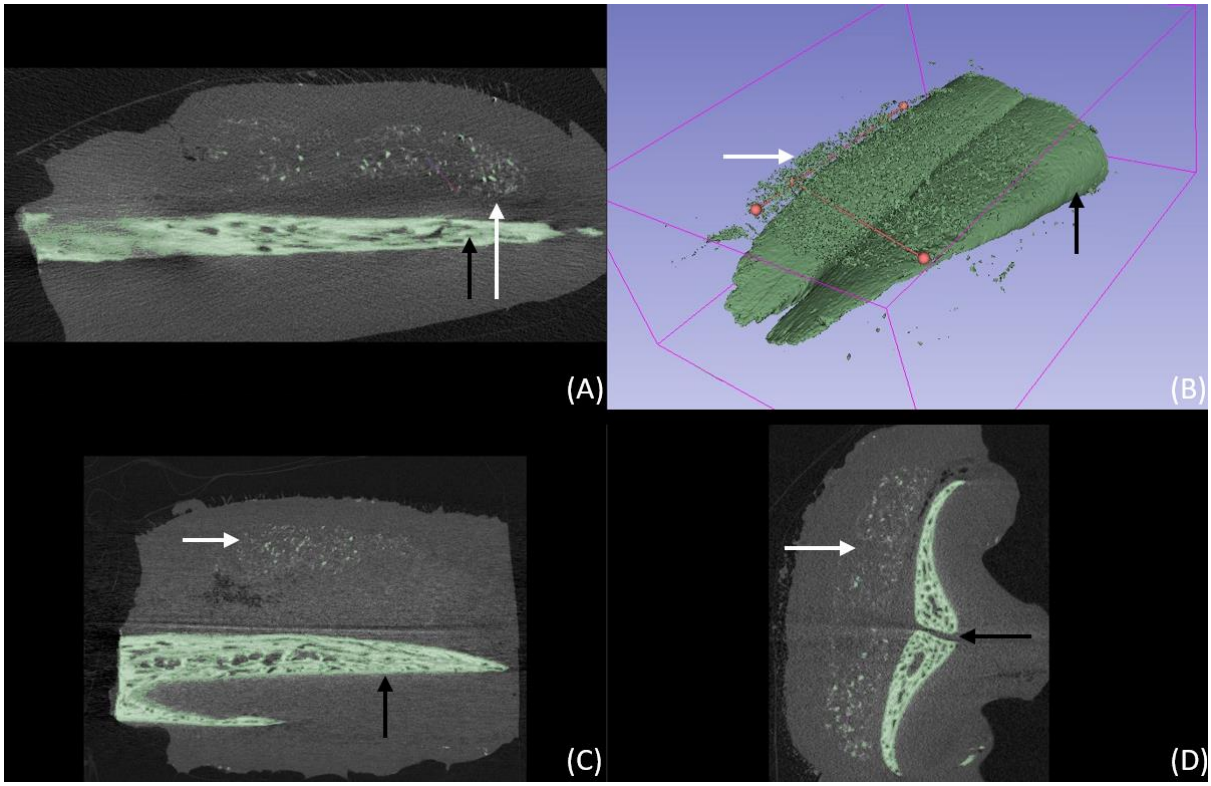


Figure 3: Microscopic CT images with 3D reconstruction in Slicer 5.2.2 for volume retention assessment. White arrows point to the graft, and black arrows point to bone. (A) Axial microscopic CT slice, (B) 3D reconstruction with orange ball-ended lines outlining dimensions of graft, (C) Sagittal microscopic CT slice, (D) Coronal microscopic CT slice.

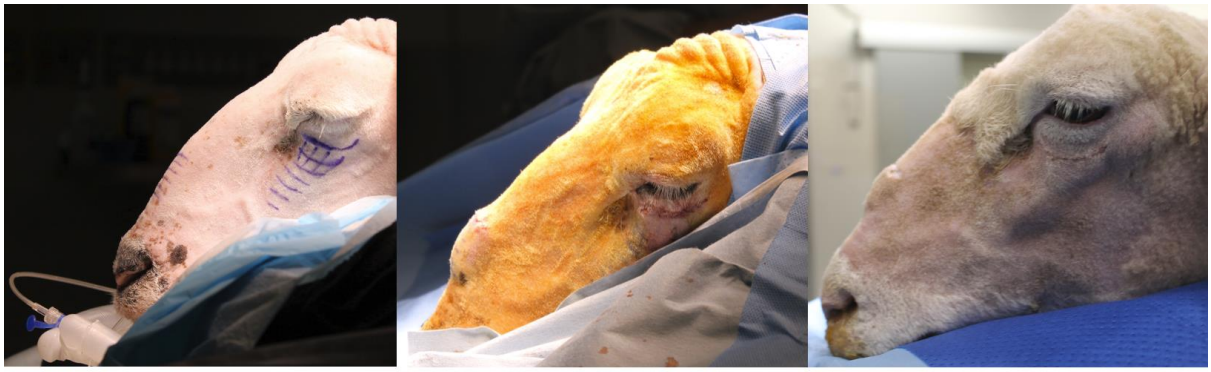


Figure 4: Clinical Photographs taken at various time-points. (A) Pre-surgery, (B) Immediately post-surgery, and (C) 3 months post-surgery.

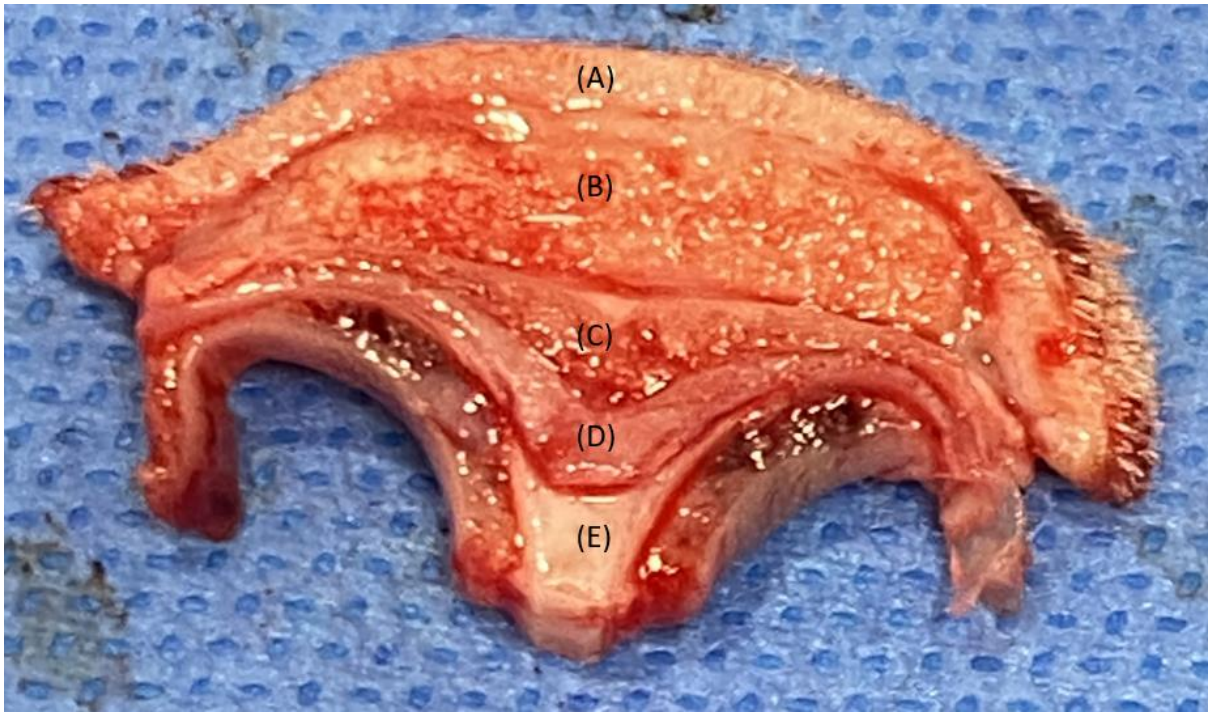


Figure 5: Section of explanted actv-graft® with surrounding tissue after 3 months. Note this particular section demonstrates a segment of the nasal dorsum where bone was both superficial and deep to cartilage. Other sections demonstrated actv-graft® directly over cartilage. (A) Skin and soft tissue, (B) actv-graft®, (C) Bone, (D) Cartilage, (E) Bone.

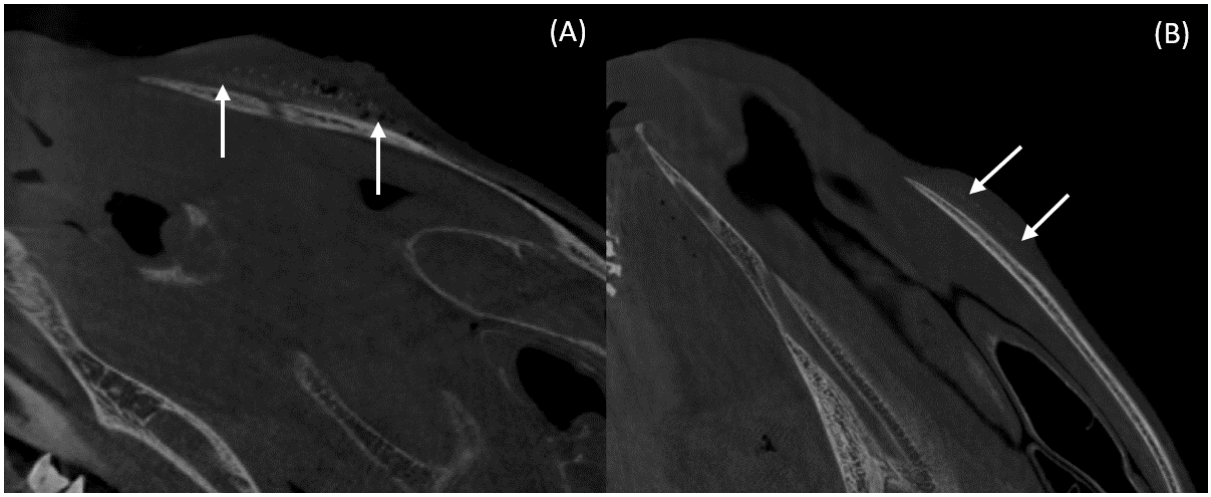
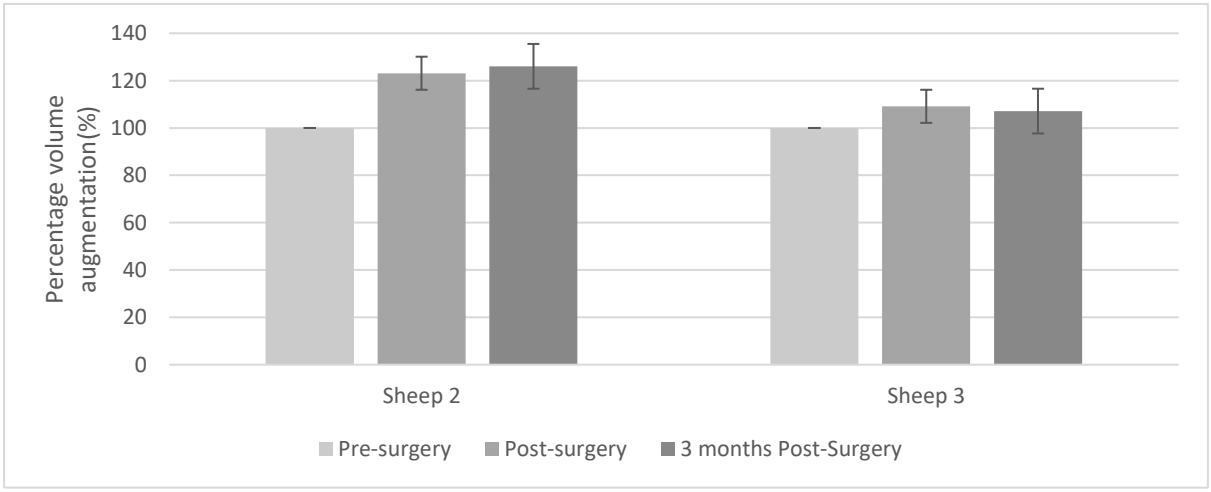
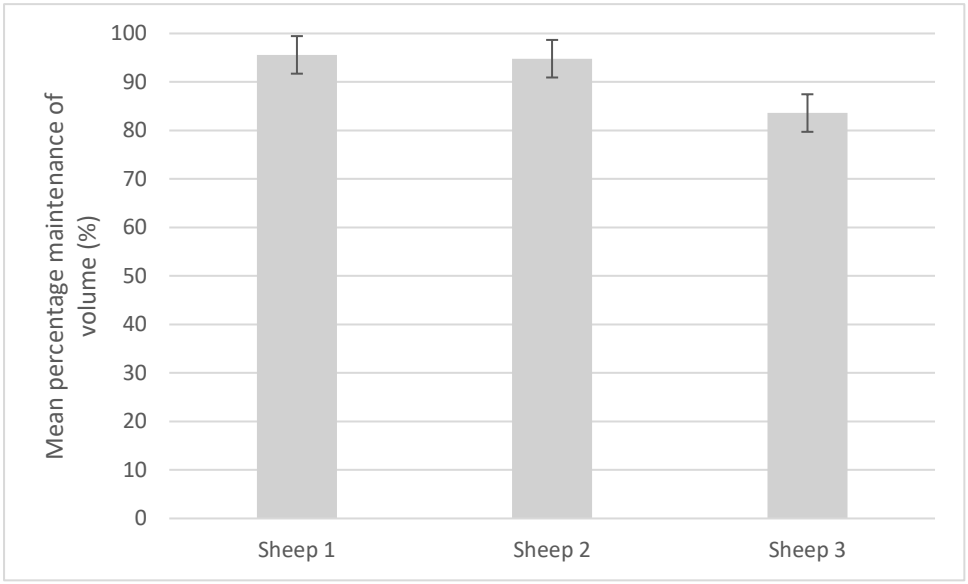


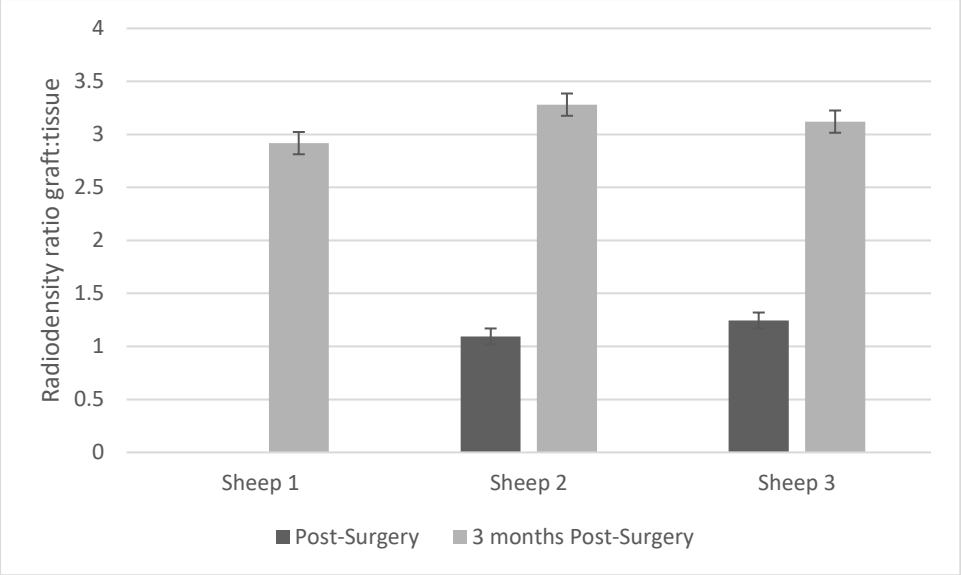
Figure 6: Sagittal macroscopic CT images of actv-graft® in-situ (white arrows) at different timepoints. (A) Immediately post-surgery, (B) 3 months post-surgery.



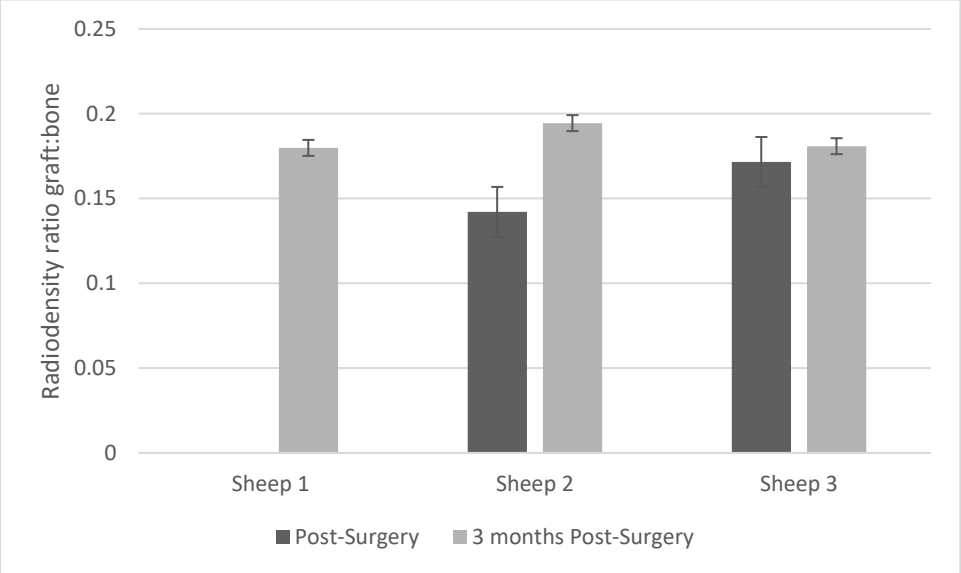
Graph 1: Volume Augmentation over time with standard error bars.



Graph 2: Graft volume retention after 3 months with standard error bars.



Graph 3: Graft to surrounding tissue radiodensity ratio from immediately post-surgery to 3 months post-surgery with standard error bars.



Graph 4: Graft to bone radiodensity ratio from immediately post-surgery to 3 months post-surgery with standard error bars.

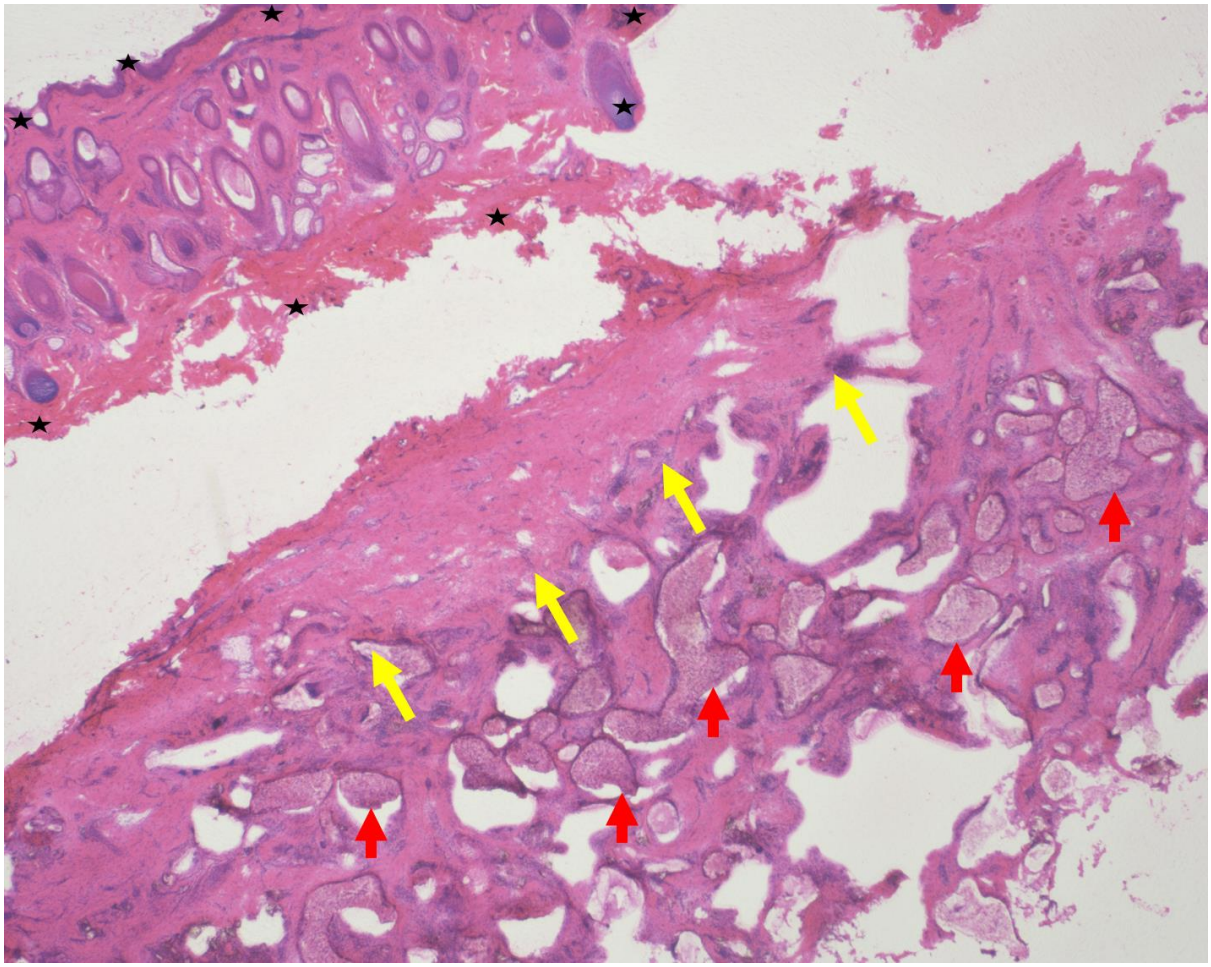


Figure 7: Section of the implant and surrounding tissue stained with haematoxylin and eosin. Region bounded by stars is epidermis and superficial dermis containing numerous hair follicles. Red arrows represent the graft and yellow the graft-host interface.

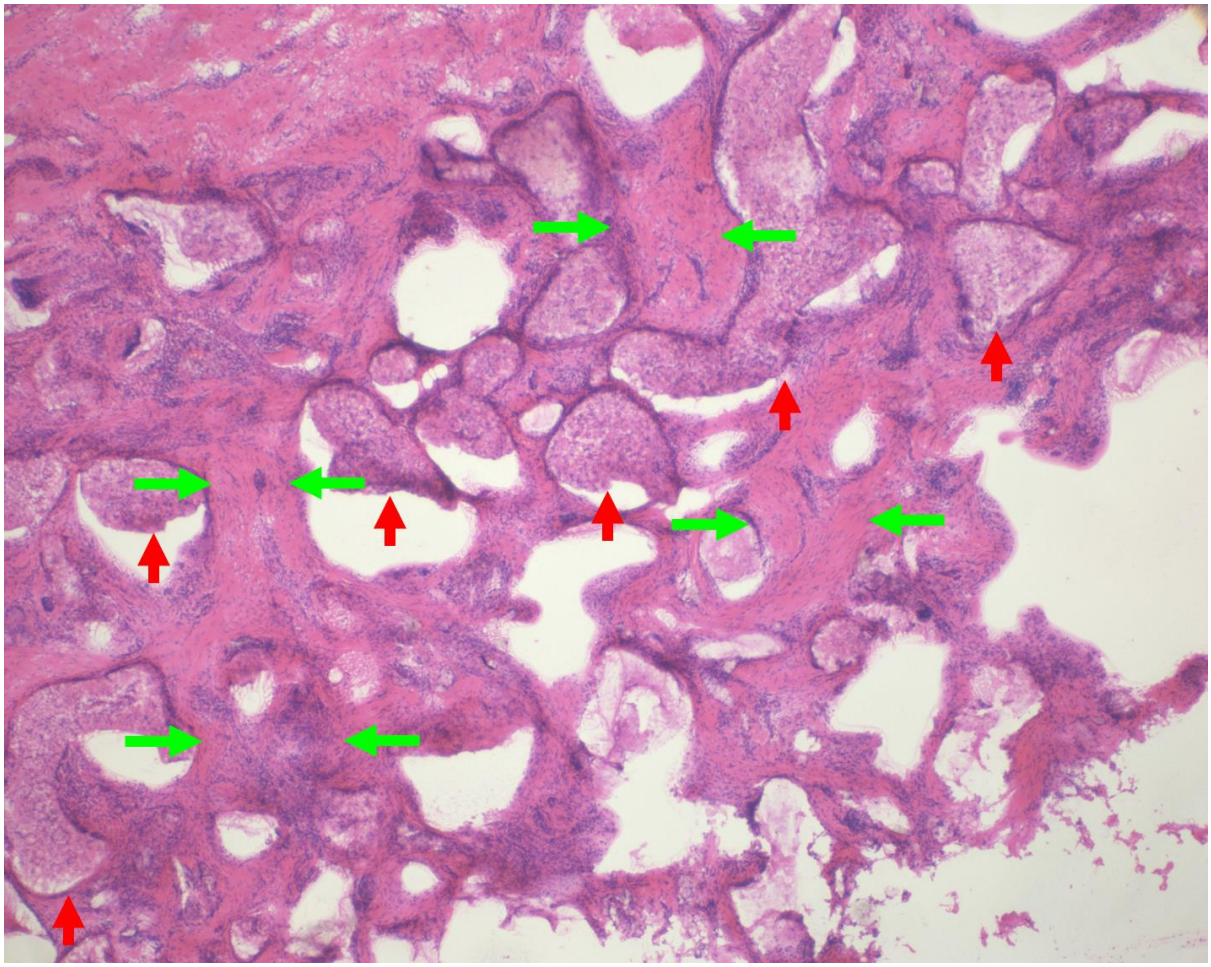


Figure 8: Section of the implant and surrounding tissue stained with haematoxylin and eosin. Red arrows represent the graft and green the collagen and fibrosis.

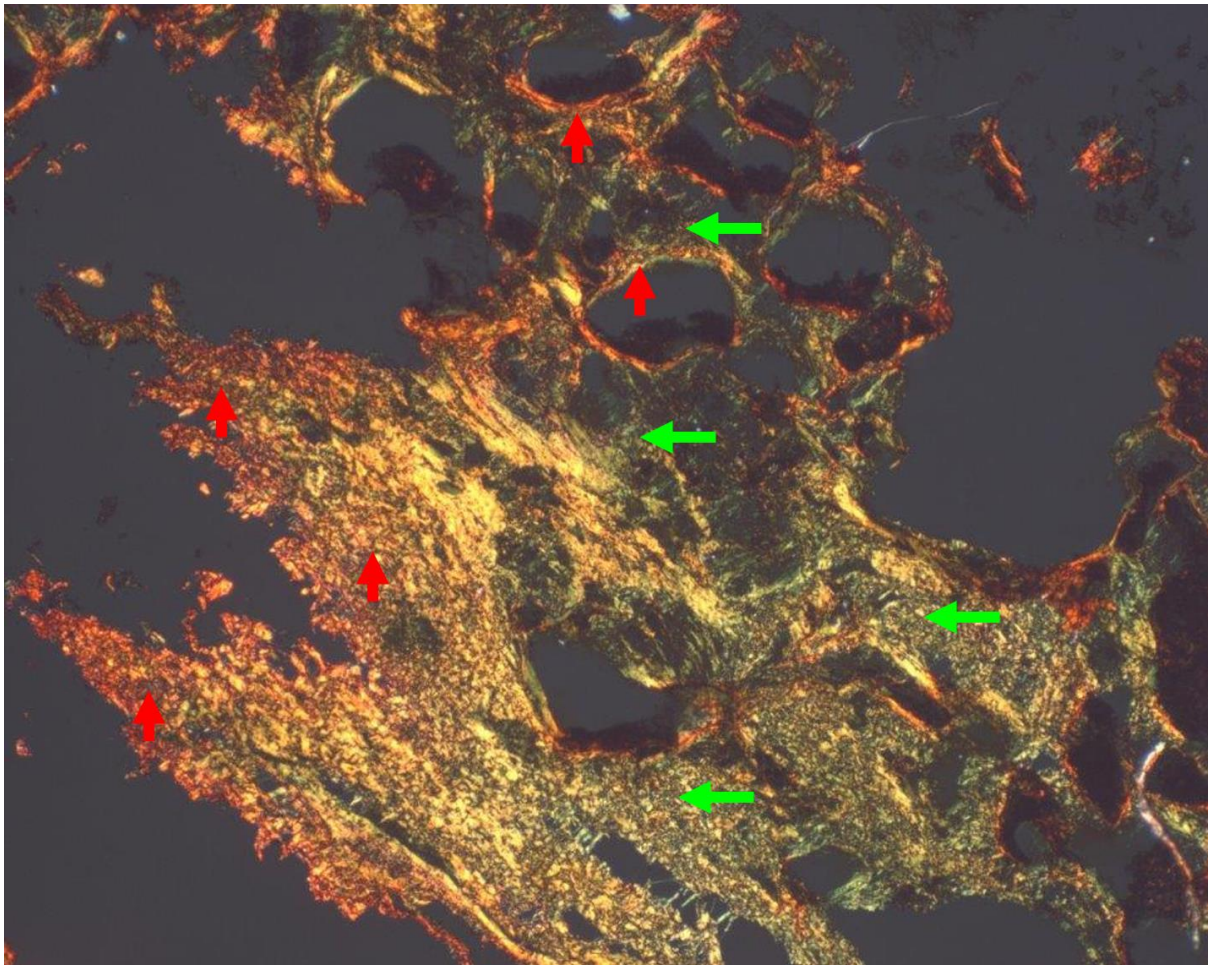


Figure 9: Cross section of the implant and surrounding tissue stained with picrosirius red under polarised light microscopy. Red arrows represent collagen type I and green collagen type III.

Chapter Four – Conclusions and Future Directions

Summary of Findings and Limitations

Septorhinoplasty is a fundamental and common surgical procedure that aims to improve the function and cosmesis of the nose. Native septal cartilage is often the graft of choice due to convenience and compatibility. However, there is a limit to the amount of septal graft that can be harvested before the structural and functional integrity of the septum becomes compromised. Thus ACC, IHCC and synthetic grafts are commonly used in these procedures as alternatives to native septal cartilage. However, in clinical practice each of these grafts pose unique challenges for the surgeon and potential complications for the patient, creating the genesis of this thesis. The aims of the thesis were to identify the evidence for each of these current alternatives, explore emerging alternatives, and assess the feasibility of a novel graft in nasal reconstruction.

The second chapter of this thesis utilised a systematic review of the literature to establish the clinical effectiveness of ACC, IHCC, and synthetic grafts. Six systematic reviews with more than 10 included primary studies that detailed graft complication rates were included. By qualitatively synthesising the results from these six systematic reviews, varying conclusions were found. ACC and IHCC grafts were generally associated with higher rates of warping, revision surgery, donor-site morbidity, and resorption. Contrastingly, synthetic grafts were more commonly associated with extrusion, infection, and removal surgery. This established that no single current alternative graft to native septal cartilage is ideal. The major limitations of this chapter were the heterogeneity of systemic reviews and moderate overlap of included primary studies between systematic reviews. This precluded statistical comparison and meta-analysis. Additionally, the included systematic reviews relied predominantly on level IV evidence.

Out of the necessity for a graft similar to native septal cartilage without the drawbacks of current alternatives, nascent fields such as tissue-engineered and hybrid grafts have emerged. Tissue-engineering as a field has gained significant traction, especially with the popularisation of three-dimensional printing and bioprinting alleviating some of the field's inherent limitations. Despite this,

only five clinical trials have been conducted thus-far on limited human populations. Whilst all have demonstrated reasonable outcomes, none are robust evidence as they are all case series. Some key limitations in the field of tissue-engineering include donor-site morbidity associated with cellular source harvest, production cost, chondrocyte dedifferentiation and requisite time for culture that precludes on-demand potential, and appropriate scaffolding material.

Hybrid grafts conventionally combine a bioactive material with a biocompatible polymer scaffold to create a favourable biomechanical structure that can stimulate the surrounding host environment to form target tissue before degrading into biocompatible products. Two materials that individually have FDA approval and evidence of chondrogenic potential are BG and PCL. Furthermore, when BG and PCL are combined, a desirable reduction in BG's brittleness has been observed, creating a composite with favourable biomechanics and biodegradation rates. A commercially available form of the BG-PCL composite graft exists in actv-graft® that can be easily procured and customised to patient-specific requirements without need for co-culture.

The third chapter of this thesis detailed a pre-clinical feasibility study conducted after grant acquisition and ethics approval. actv-graft® was implanted in the nasal dorsum of three sheep and harvested after 3 months. No sheep exhibited signs of infection, and all sheep clinically demonstrated volume augmentation immediately after surgery and at 3 months. A novel method of objectively analysing volume augmentation with macroscopic CT was developed during the study as no method was found in the literature. This reproducible method showed persistent volume augmentation over time, confirming the clinical evaluation. Furthermore, another novel method of radiologically assessing graft degradation after 3 months in-vivo was formulated and implemented. This indicated that there was partial degradation of the graft but at a favourable rate which was comparable to the literature. The absence of clinical infection during the study suggested the partial degradation actv-graft® underwent was not harmful, indicating its potential biodegradability. Graft integration was demonstrated radiologically and histologically. Mean radiodensity values were obtained for the graft, surrounding soft tissue, and bone. Ratios were then calculated immediately after surgery and at 3 months, indicating an increase in radiodensity within the graft suggesting host infiltration and

integration. This was confirmed histologically through cellular infiltrate with hyalinised collagen formation that was primarily type III than type I under polarised light microscopy.

Whilst the results of chapter 3 suggest actv-graft® may be a feasible alternative graft, a significant limitation in interpreting these results lies in the relatively short duration of the study, and its size. Additionally, as cartilage was the target tissue, immunohistochemistry to assess the presence of type II collagen as an indicator of chondrogenesis would have been useful. Funding has been acquired to complete this analysis.

Future Directions and Clinical Implication

Utilising the protocols employed in chapter 3, a larger cohort animal study over a longer period of time would aid in characterising the safety profile, chondrogenic potential, and degradation rate of actv-graft® in-vivo prior to human trials. Clinically, a BG-PCL composite has been used with relative success in craniofacial bony reconstruction in seven humans(85). BG-PCL composites such as actv-graft® have the potential to alter the landscape of grafting as they can be easily obtained, tailored both pre-operatively and intra-operatively to be patient-specific, and biodegrade safely. If bioactivity through chondrogenesis and osteogenesis without significant immunogenicity are established, this represents a graft that has widespread clinical potential.

References

1. Geurkink N. Nasal anatomy, physiology, and function. Journal of allergy and clinical immunology 1983;72:123-8.

2. Neskey D, Eloy JA, Casiano RR. Nasal, septal, and turbinate anatomy and embryology. Otolaryngol Clin North Am 2009;42:193-205, vii.

3. Lavernia L, Brown WE, Wong BJF, et al. Toward tissue-engineering of nasal cartilages. Acta Biomaterialia 2019;88:42-56.

4. Kairo AK, Sarkar S, Nayak A, et al. Rhinoplasty Anatomy and Procedures. In: Verma H, Thakar A, editors. Essentials of Rhinology. Singapore: Springer Singapore; 2021. p. 31-47.

5. Jeong JY, Kim TK. Rebuilding nose: rhinoplasty for Asians. Uijeongbu: Medic Medicine 2018.

6. Kim TK, Jeong JY. Surgical anatomy for Asian rhinoplasty. Arch Craniofac Surg 2019;20:147-57.

7. Daniel RK, Letourneau A. Rhinoplasty: nasal anatomy. Ann Plast Surg 1988;20:5-13.

8. Hsu DW, Suh JD. Anatomy and physiology of nasal obstruction. Otolaryngologic Clinics of North America 2018;51:853-65.

9. Lazovic GD, Daniel RK, Janosevic LB, et al. Rhinoplasty: The Nasal Bones – Anatomy and Analysis. Aesthetic Surgery Journal 2015;35:255-63.

10. Afrooz PN, Rohrich RJ. The keystone: consistency in restoring the aesthetic dorsum in rhinoplasty. Plastic and reconstructive surgery 2018;141:355-63.

11. Öztürk G. Scroll ligament preservation and improvement in nasal tip with the room concept. Aesthetic Plastic Surgery 2020;44:491-500.

12. Wexler DB, Davidson TM. The nasal valve: a review of the anatomy, imaging, and physiology. American journal of rhinology 2004;18:143-50.

13. Miles BA, Petrisor D, Kao H, et al. Anatomical variation of the nasal septum: analysis of 57 cadaver specimens. Otolaryngology—Head and Neck Surgery 2007;136:362-8.

14. Simon PE, Lam K, Sidle D, et al. The nasal keystone region: an anatomical study. JAMA Facial Plast Surg 2013;15:235-7.

15. Bitik O, Uzun H, Konaş E. Scroll Reconstruction: Fine Tuning of the Interface Between Middle and Lower Thirds in Rhinoplasty. Aesthet Surg J 2019;39:481-94.

16. Gelardi M, Ciprandi G. The clinical importance of the nasal valve. Acta Biomed 2019;90.

17. Nigro CEN, de Aguiar Nigro JF, Mion O, et al. Nasal valve: anatomy and physiology. Brazilian Journal of Otorhinolaryngology 2009;75:305-10.

18. Rohrich RJ, Ahmad J. Rhinoplasty. Plastic and Reconstructive Surgery 2011;128.

19. Roblin DG, Eccles R. What, if any, is the value of septal surgery? Clin Otolaryngol Allied Sci 2002;27:77-80.

20. Andrews SHJ, Kunze M, Mulet-Sierra A, et al. Strategies to Mitigate Variability in Engineering Human Nasal Cartilage. Scientific Reports 2017;7:6490.

21. Michels Dde S, Rodrigues Ada M, Nakanishi M, et al. Nasal involvement in obstructive sleep apnea syndrome. Int J Otolaryngol 2014;2014:717419.

22. Proctor DF. The upper airways. I. Nasal physiology and defense of the lungs. Am Rev Respir Dis 1977;115:97-129.

23. Yeghiazarians Y, Jneid H, Tietjens JR, et al. Obstructive Sleep Apnea and Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation 2021;144:e56-e67.

24. Stewart MG, Witsell DL, Smith TL, et al. Development and validation of the Nasal Obstruction Symptom Evaluation (NOSE) scale. Otolaryngol Head Neck Surg 2004;130:157-63.

25. Pynnonen MA, Kim HM, Terrell JE. Validation of the Sino-Nasal Outcome Test 20 (SNOT-20) domains in nonsurgical patients. Am J Rhinol Allergy 2009;23:40-5.

26. Hern J, Hamann J, Tostevin P, et al. Assessing psychological morbidity in patients with nasal deformity using the CORE questionnaire. Clin Otolaryngol Allied Sci 2002;27:359-64.

27. Kucur C, Kuduban O, Ozturk A, et al. Psychological Evaluation of Patients Seeking Rhinoplasty. Eurasian J Med 2016;48:102-6.

28. Rankin M, Borah GL. Perceived functional impact of abnormal facial appearance. Plast Reconstr Surg 2003;111:2140-6; discussion 7-8.

29. Gunter JP, Landecker A, Cochran CS. Frequently Used Grafts in Rhinoplasty: Nomenclature and Analysis. Plastic and Reconstructive Surgery 2006;118.

30. Gunter JP, Rohrich RJ. Augmentation rhinoplasty: dorsal onlay grafting using shaped autogenous septal cartilage. Plastic and reconstructive surgery 1990;86:39-45.
31. Lin G, Lawson W. Complications using grafts and implants in rhinoplasty. Operative Techniques in Otolaryngology-Head and Neck Surgery 2007;18:315-23.
32. Liang X, Wang K, Malay S, et al. A systematic review and meta-analysis of comparison between autologous costal cartilage and alloplastic materials in rhinoplasty. J Plast Reconstr Aesthet Surg 2018;71:1164-73.
33. Karnes J, Salisbury M, Schaeferle M, et al. Porous High-density Polyethylene Implants (Medpor) for Nasal Dorsum Augmentation. Aesthetic Surgery Journal 2000;20:26-30.
34. Ansari K, Asaria J, Hilger P, et al. Grafts and implants in rhinoplasty—Techniques and long-term results. Operative Techniques in Otolaryngology-Head and Neck Surgery 2008;19:42-58.
35. Asselin A, Hattar S, Oboeuf M, et al. The modulation of tissue-specific gene expression in rat nasal chondrocyte cultures by bioactive glasses. Biomaterials 2004;25:5621-30.
36. Jones JR. Review of bioactive glass: From Hench to hybrids. Acta Biomaterialia 2013;9:4457-86.
37. Nava MM, Draghi L, Giordano C, et al. The effect of scaffold pore size in cartilage tissue engineering. J Appl Biomater Funct Mater 2016;14:e223-9.
38. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71.
39. Pieper D, Antoine SL, Mathes T, et al. Systematic review finds overlapping reviews were not mentioned in every other overview. J Clin Epidemiol 2014;67:368-75.
40. Higgins JP, Thomas J, Chandler J, et al. Cochrane handbook for systematic reviews of interventions. John Wiley & Sons; 2019.
41. Alhussain OH, Alhussain GO, Rajkhan AA, et al. Efficacy of autologous vs. homologous costal cartilage grafts in dorsal augmentation rhinoplasty: a systematic review and meta-analysis. Annals of Medical and Health Sciences Research 2020;10.
42. Wee JH, Park M-H, Oh S, et al. Complications Associated With Autologous Rib Cartilage Use in Rhinoplasty. JAMA Facial Plastic Surgery 2015;17:49-55.

43. Varadharajan K, Sethukumar P, Anwar M, et al. Complications Associated With the Use of Autologous Costal Cartilage in Rhinoplasty: A Systematic Review. Aesthet Surg J 2015;35:644-52.
44. Hudise JY, Aldhabaan SA, Aldosari BF. Complications of the nasal dorsum reconstruction using autologous or alloplastic grafts: evidence from systematic review and meta-analysis. Brazilian Journal of Otorhinolaryngology 2020.
45. Vila PM, Jeanpierre LM, Rizzi CJ, et al. Comparison of Autologous vs Homologous Costal Cartilage Grafts in Dorsal Augmentation Rhinoplasty: A Systematic Review and Meta-analysis. JAMA Otolaryngol Head Neck Surg 2020;146:347-54.
46. Hoang HLT, Januszyk M, Boyd JB. A systematic review of complications associated with nasal augmentation implants: expanded polytetrafluoroethylene (Gore-Tex) versus silicone. Journal of Cosmetic Medicine 2018;2:27-31.
47. Peled ZM, Warren AG, Johnston P, et al. The use of alloplastic materials in rhinoplasty surgery: a meta-analysis. Plast Reconstr Surg 2008;121:85e-92e.
48. Whiting P, Savović J, Higgins JPT, et al. ROBIS: A new tool to assess risk of bias in systematic reviews was developed. Journal of clinical epidemiology 2016;69:225-34.
49. Wee JH, Mun SJ, Na WS, et al. Autologous vs Irradiated Homologous Costal Cartilage as Graft Material in Rhinoplasty. JAMA Facial Plast Surg 2017;19:183-8.
50. Griffin MF, Premakumar Y, Seifalian AM, et al. Biomechanical characterisation of the human nasal cartilages; implications for tissue engineering. J Mater Sci Mater Med 2016;27:11.
51. Mella J, Christophel J, Park S. Are Alloplastic Implants Safe in Rhinoplasty? Laryngoscope 2020;130:1854-6.
52. Shirakabe Y, Suzuki Y, Lam SM. A systematic approach to rhinoplasty of the Japanese nose: a thirty-year experience. Aesthetic Plast Surg 2003;27:221-31.
53. Glenny AM, Altman DG, Song F, et al. Indirect comparisons of competing interventions. Health Technol Assess 2005;9:1-134, iii-iv.
54. Kim M-S, Kim H-K, Kim D-W. Cartilage tissue engineering for craniofacial reconstruction. Archives of plastic surgery 2020;47:392-403.

55. Pleumeekers MM, Nimeskern L, Koevoet WLM, et al. Cartilage Regeneration in the Head and Neck Area: Combination of Ear or Nasal Chondrocytes and Mesenchymal Stem Cells Improves Cartilage Production. Plast Reconstr Surg 2015;136:762e-74e.

56. Kushnaryov A, Yamaguchi T, Briggs KK, et al. Evaluation of Autogenous Engineered Septal Cartilage Grafts in Rabbits: A Minimally Invasive Preclinical Model. Advances in Otolaryngology 2014;2014:415821.

57. Oseni AO, Butler PE, Seifalian AM. Optimization of chondrocyte isolation and characterization for large-scale cartilage tissue engineering. J Surg Res 2013;181:41-8.

58. Phull A-R, Eo S-H, Abbas Q, et al. Applications of Chondrocyte-Based Cartilage Engineering: An Overview. BioMed Research International 2016;2016:1879837.

59. Lee WY-w, Wang B. Cartilage repair by mesenchymal stem cells: Clinical trial update and perspectives. Journal of Orthopaedic Translation 2017;9:76-88.

60. Tan AR, Hung CT. Concise Review: Mesenchymal Stem Cells for Functional Cartilage Tissue Engineering: Taking Cues from Chondrocyte-Based Constructs. Stem Cells Transl Med 2017;6:1295-303.

61. Caron MM, Emans PJ, Coolsen MM, et al. Redifferentiation of dedifferentiated human articular chondrocytes: comparison of 2D and 3D cultures. Osteoarthritis Cartilage 2012;20:1170-8.

62. Alexander TH, Sage AB, Chen AC, et al. Insulin-like growth factor-I and growth differentiation factor-5 promote the formation of tissue-engineered human nasal septal cartilage. Tissue engineering Part C, Methods 2010;16:1213-21.

63. Zhang YS, Yue K, Aleman J, et al. 3D Bioprinting for Tissue and Organ Fabrication. Ann Biomed Eng 2017;45:148-63.

64. Di Gesù R, Acharya AP, Jacobs I, et al. 3D printing for tissue engineering in otolaryngology. Connective Tissue Research 2020;61:117-36.

65. Ceccarelli G, Gentile P, Marcarelli M, et al. In Vitro and In Vivo Studies of Alar-Nasal Cartilage Using Autologous Micro-Grafts: The Use of the Rigenera(®) Protocol in the Treatment of an Osteochondral Lesion of the Nose. Pharmaceuticals (Basel, Switzerland) 2017;10:53.

66. Fulco I, Miot S, Haug MD, et al. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. Lancet 2014;384:337-46.
67. Gentile P, Scioli MG, Bielli A, et al. Reconstruction of Alar Nasal Cartilage Defects Using a Tissue Engineering Technique Based on a Combined Use of Autologous Chondrocyte Micrografts and Platelet-rich Plasma: Preliminary Clinical and Instrumental Evaluation. Plastic and reconstructive surgery Global open 2016;4:e1027-e.
68. Hoshi K, Fujihara Y, Saijo H, et al. Implant-type Tissue-engineered Cartilage for Secondary Correction of Cleft Lip-nose Patients: An Exploratory First-in-human Trial. Journal of clinical trials 2017;7:1-9.
69. Yanaga H, Imai K, Yanaga K. Generative surgery of cultured autologous auricular chondrocytes for nasal augmentation. Aesthetic plastic surgery 2009;33:795-802.
70. Deliormanlı AM, Atmaca H. Biological Response of Osteoblastic and Chondrogenic Cells to Graphene-Containing PCL/Bioactive Glass Bilayered Scaffolds for Osteochondral Tissue Engineering Applications. Appl Biochem Biotechnol 2018;186:972-89.
71. Wu J, Xue K, Li H, et al. Improvement of PHBV scaffolds with bioglass for cartilage tissue engineering. PLoS One 2013;8:e71563.
72. Yao Q, Nooeaid P, Detsch R, et al. Bioglass®/chitosan-polycaprolactone bilayered composite scaffolds intended for osteochondral tissue engineering. J Biomed Mater Res A 2014;102:4510-8.
73. Siclari A, Mascaro G, Kaps C, et al. A 5-year follow-up after cartilage repair in the knee using a platelet-rich plasma-immersed polymer-based implant. The open orthopaedics journal 2014;8:346-54.
74. Wasyłeczko M, Sikorska W, Chwojnowski A. Review of Synthetic and Hybrid Scaffolds in Cartilage Tissue Engineering. Membranes 2020;10:348.
75. Nandakumar BS, Mukherjee P, Yung AE, et al. Grafts in septorhinoplasty: a systematic review and future directions. Australian Journal of Otolaryngology; Vol 5 (November 2022): Australian Journal of Otolaryngology 2022.
76. Gögele C, Wiltzsch S, Lenhart A, et al. Highly porous novel chondro-instructive bioactive glass scaffolds tailored for cartilage tissue engineering. Materials Science and Engineering: C 2021;130:112421.

77. Lei B, Guo B, Rambhia KJ, et al. Hybrid polymer biomaterials for bone tissue regeneration. Front Med 2019;13:189-201.

78. Hench L, editor. Bioceramics: From Concept to Clinic1991.

79. Lese I, Graf DA, Tsai C, et al. Bioactive nanoparticle-based formulations increase survival area of perforator flaps in a rat model. PLOS ONE 2018;13:e0207802.

80. Terzopoulou Z, Baciu D, Gounari E, et al. Composite Membranes of Poly(ε-caprolactone) with Bisphosphonate-Loaded Bioactive Glasses for Potential Bone Tissue Engineering Applications. Molecules (Basel, Switzerland) 2019;24:3067.

81. Wiggenhauser PS, Balmayor ER, Rotter N, et al. In vivo evaluation of a regenerative approach to nasal dorsum augmentation with a polycaprolactone-based implant. European journal of medical research 2019;24:6-.

82. Martinez-Diaz S, Garcia-Giralt N, Lebourg M, et al. In vivo evaluation of 3-dimensional polycaprolactone scaffolds for cartilage repair in rabbits. Am J Sports Med 2010;38:509-19.

83. Lebourg M, Sabater Serra R, Más Estellés J, et al. Biodegradable polycaprolactone scaffold with controlled porosity obtained by modified particle-leaching technique. Journal of Materials Science : Materials in Medicine 2008;19:2047-53.

84. Bossard C, Granel H, Wittrant Y, et al. Polycaprolactone / bioactive glass hybrid scaffolds for bone regeneration. Biomedical Glasses 2018;4:108-22.

85. Kim YC, Yoon IA, Woo SH, et al. Complications arising from clinical application of composite polycaprolactone/bioactive glass ceramic implants for craniofacial reconstruction: A prospective study. Journal of Cranio-Maxillofacial Surgery 2022;50:863-72.

86. Boughton EA. Development of Bioactive Soft Tissue Scaffold Systems: University of Sydney; 2011.

87. Lien SM, Ko LY, Huang TJ. Effect of pore size on ECM secretion and cell growth in gelatin scaffold for articular cartilage tissue engineering. Acta Biomater 2009;5:670-9.

88. Fedorov A, Beichel R, Kalpathy-Cramer J, et al. 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magn Reson Imaging 2012;30:1323-41.

89. Klinge U, Dietz U, Fet N, et al. Characterisation of the cellular infiltrate in the foreign body granuloma of textile meshes with its impact on collagen deposition. Hernia 2014;18:571-8.
90. Liu J, Xu MY, Wu J, et al. Picrosirius-Polarization Method for Collagen Fiber Detection in Tendons: A Mini-Review. Orthop Surg 2021;13:701-7.
91. Woodruff MA, Hutmacher DW. The return of a forgotten polymer—Polycaprolactone in the 21st century. Progress in Polymer Science 2010;35:1217-56.
92. Chen DR, Bei JZ, Wang SG. Polycaprolactone microparticles and their biodegradation. Polymer Degradation and Stability 2000;67:455-9.
93. Ji L, Wang W, Jin D, et al. In vitro bioactivity and mechanical properties of bioactive glass nanoparticles/polycaprolactone composites. Materials Science and Engineering: C 2015;46:1-9.
94. Farhadi J, Fulco I, Miot S, et al. Precultivation of engineered human nasal cartilage enhances the mechanical properties relevant for use in facial reconstructive surgery. Annals of surgery 2006;244:978-85.
95. Kim YS, Shin YS, Park DY, et al. The Application of Three-Dimensional Printing in Animal Model of Augmentation Rhinoplasty. Ann Biomed Eng 2015;43:2153-62.
96. Park SH, Yun BG, Won JY, et al. New application of three-dimensional printing biomaterial in nasal reconstruction. The Laryngoscope 2017;127:1036-43.
97. da Fonseca Gabriela F, Avelino Sarah de Oliveira M, Mello Daphne de Camargo R, et al. Scaffolds of PCL combined to bioglass: synthesis, characterization and biological performance. Journal of Materials Science : Materials in Medicine 2020;31.
98. Gómez-Cerezo N, Casarrubios L, Saiz-Pardo M, et al. Mesoporous bioactive glass/ε-polycaprolactone scaffolds promote bone regeneration in osteoporotic sheep. Acta Biomater 2019;90:393-402.
99. Rajzer I, Dziadek M, Kurowska A, et al. Electrospun polycaprolactone membranes with Zn-doped bioglass for nasal tissues treatment. J Mater Sci Mater Med 2019;30:80.
100. Poh PSP, Hutmacher DW, Holzapfel BM, et al. In vitro and in vivo bone formation potential of surface calcium phosphate-coated polycaprolactone and polycaprolactone/bioactive glass composite scaffolds. Acta Biomater 2016;30:319-33.

101. de Souza Cruz EL, de Souza FJ, Jr., de Menezes LM, et al. Bioactive glass granules S53P4 in osteotomy Le Fort I. Sci Rep 2020;10:12690.
102. Singh D, Rai V, Agrawal DK. Regulation of Collagen I and Collagen III in Tissue Injury and Regeneration. Cardiol Cardiovasc Med 2023;7:5-16.
103. Wang C, Brisson BK, Terajima M, et al. Type III collagen is a key regulator of the collagen fibrillar structure and biomechanics of articular cartilage and meniscus. Matrix Biol 2020;85-86:47-67.
104. Wu JJ, Weis MA, Kim LS, et al. Type III collagen, a fibril network modifier in articular cartilage. J Biol Chem 2010;285:18537-44.
105. Urlić I, Ivković A. Cell Sources for Cartilage Repair-Biological and Clinical Perspective. Cells 2021;10.

Appendix

Ethics Application

THE UNIVERSITY OF SYDNEY

RESEARCH INTEGRITY & ETHICS ADMINISTRATION

Animal Research Authority (ARA)

This ARA must be kept in the facility/farm where animals are housed or carried with you during field work

Animal Research Authority

Project title:	In-vivo evaluation of a novel bioactive synthetic graft for skeletal and soft tissue reconstructive surgery.
Project number:	2021/2003
Chief investigator:	Sydney Ch'ng
ARA approval period:	23 November 2021 – 23 November 2022 An annual report must be submitted and approved prior to the ARA expiration date above.
Project type:	Experimental (non-wildlife)

In compliance with Section 27 of the NSW *Animal Research Act 1985*, this Animal Research Authority (ARA) remains in force for a period of 12 months, unless cancelled sooner.

Renewal of the ARA is conditional upon submission of a satisfactory annual report to the AEC in accordance with the *Australian code for the care and use of animals for scientific purposes 8th Edition 2013*.

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CRICOS 00026A

This ARA must be kept in the facility/farm where animals are housed or carried with you during field work

Tuesday, 23 November 2021

Assoc Prof Sydney Ch'ng
Central Clinical School: Medicine; Faculty of Medicine and Health
The University of Sydney

Email: Sydney.chng@sydney.edu.au

Dear Assoc Prof Ch'ng

I am pleased to inform you that the University of Sydney Animal Ethics Committee (AEC) has approved your project entitled **"In-vivo evaluation of a novel bioactive synthetic graft for skeletal and soft tissue reconstructive surgery."**.

Project duration: 23 November 2021 – 23 November 2024
At the end of the project duration (3 years) a completion report must be submitted.

ARA expiry date: 23 November 2022

Authorised investigators: Ch'ng Sydney; Wise Innes; Mukherjee Payal; Yung Amanda; Nessiem Andrew; Nandakumar Beeshman S.; Williams Zoe; Burrows Chris; Kolakshyapati Pranish;

Project description:
Grafts are used to provide structure in reconstructive surgery for cancer, congenital abnormalities, and trauma. Grafts ideally become integrated and promote host-tissue regeneration. Commonly, grafts are either from the same person (autologous graft i.e. autograft), or a biocompatible synthetic polymer (i.e. alloplastic graft) (Ansari et al. 2008).
The literature describes the ideal graft as one that has low infection rates whilst being intra-operatively versatile without warping or extruding or inciting donor-site morbidity. Each graft type has its advantages and disadvantages, however the 'ideal' graft has yet to be achieved. Whilst autografts are better at stimulating host regeneration (i.e. bioactivity) and integrating with host tissue, they require longer operating time to be harvested from the patient and cause donor site morbidity. Alloplastics can be custom produced on-demand and provide versatile biomechanical structure and strength, but are poorer at integrating and promoting host regeneration than autografts. This results in higher complication rates including graft extrusion, migration, and infection.
Actv-graft is a promising novel graft that is a combination of bioactive glass (Bioglass) and a biocompatible polymer similar to polycaprolactone (PCL). Both of these materials have extensive data proving their in-vivo efficacy and safety. In small-animal studies, PCL-Bioglass composites (i.e. Actv-graft) were shown to be superior to PCL scaffolds in accelerating vascularisation and tissue ingrowth.
Our project looks to use minimally invasive techniques to implant Actv-graft in three areas of the mid-face of sheep that each pose unique tissue environments – the cartilage of the nose, bone of the eye socket, and soft tissue of the cheek.

Documents approved:

Date	Type	Document
27/10/2021	Standard Operating Procedure	SOP emergency_procedures
27/10/2021	Standard Operating Procedure	SOP husbandry Mcmasters
27/10/2021	WH&S Risk Assessment	Risk assessment intraop
27/10/2021	WH&S Risk Assessment	Risk assessment post op
27/10/2021	Flowchart	Sequence flow chart

27/10/2021	Standard Operating Procedure	SOP-anaesthesia analgesia
27/10/2021	Standard Operating Procedure	SOP euthanasia
27/10/2021	Standard Operating Procedure	SOP husbandry
27/10/2021	Other	Supplementary figures
27/10/2021	Standard Operating Procedure	SOP animal transfer
27/10/2021	Standard Operating Procedure	SOP anaesthetic recovery

Animals approved:
Please refer to the document at the end of this letter, which details your approved animal usage and location(s).

Conditions of approval

1. An annual progress report or completion report must be submitted and approved before the anniversary of approval of the project.

2. The following documentation must be kept in the facility where your animals are housed or with you when undertaking fieldwork:
   - A copy of this ARA
   - Emergency contact details in case of an animal emergency
   - Approved monitoring records

3. This project must be conducted according to the application approved by the AEC, including continuing compliance with the conditions outlined in this ARA and with the relevant state/territory animal welfare legislation, the Australian code for the care and use of animals for scientific purposes 8th edition 2013 (the Code), the University of Sydney Animal Ethics Procedures and all other relevant legislation.

4. Animals in captivity must be monitored by approved investigators (or animal care staff under a formal agreement with facility management) at least twice weekly (with even spacing throughout the week). Where applicable, more frequent monitoring must be adhered to in accordance with the approved project. The husbandry and environmental conditions of captive animals must be monitored daily. This may be performed by the facility animal care staff.

5. Any changes to the project must receive written approval from the AEC before they are implemented.
   This includes obtaining AEC approval for any changes to experimental procedures, animal numbers, personnel, source of animals or location of animals.

6. All unexpected adverse events that may impact on the wellbeing of an animal must be reported to the AEC within 48 hours, as per Clause 2.1.5 [v] [d] and 2.4.34 [ii] in the Code.

7. Personnel working on this project must be sufficiently qualified for their role by education, training and experience, or adequately supervised.
   All new investigators must successfully complete the Introduction to Animal Research (ITAR) course.

8. All animal enclosures (e.g. pens, cages and containers) must be clearly identified with chief investigator name, number of animals, DOB if provided and date of arrival, sex and strain.

9. The AEC must be notified if rodents are required to be singly housed for more than 4 weeks.

10. Research data must be retained and stored in accordance with the relevant legislation and University guidelines.

11. Any drugs to be used for procedures involving animals must be within date (not expired) and stored appropriately as per the manufacturer's recommendations. It is the responsibility of the Chief Investigator to ensure that relevant and current authority for the use of restricted drugs is obtained.

Please contact the Ethics Office at animal.ethics@sydney.edu.au should you require further information.

Yours sincerely

[REDACTION]

Professor Michael D'Occhio
Chair
Animal Ethics Committee
On behalf of the University of Sydney

The University of Sydney AECs are constituted and operate in accordance with the NSW Animal Research Act 1985 and its associated Regulations, the Australian code for the care and use of animals for scientific purposes 8th Edition 2013 and the Australian Code for the Responsible Conduct of Research 2018. All personnel named on the project should be conversant with these documents.

THE UNIVERSITY OF SYDNEY

RESEARCH INTEGRITY &
ETHICS ADMINISTRATION

Animal Research Authority (ARA)

This ARA must be kept in the facility/farm where animals are housed or carried with you during field work

Animals approved:

Country	State	Invasiveness	Location	Classification one	Classification two	Common/strain name	Approved	Total used to date
Australia	NSW	5. Major surgery with recovery	Charles Perkins Centre (D17 McMaster Annex (B02)	Domestic mammals	Sheep	Ovis aries	3	0

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Grant Application Outcome

Thank you for your application for funding through Perpetual's 2021 IMPACT Philanthropy Application Program.

We are pleased to inform you that The University of Sydney has been successful in receiving funding of $25,000 for application IPAP2021/0900 - In-vivo evaluation of a novel bioactive synthetic graft for skeletal and soft tissue reconstructive surgery..

Funding has been provided on behalf of the following client/s:

Baxter Charitable Foundation	$25,000
Total	**$25,000**

Congratulations on receiving this grant. Perpetual is honoured to assist our clients in providing funding to organisations that align to the areas they wish to support through their philanthropy.

Published Systematic Review

Review Article

Grafts in septorhinoplasty: a systematic review and future directions

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Contributions: (I) Conception and design: All authors; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: BS Nandakumar, AE Yung; (V) Data analysis and interpretation: BS Nandakumar, AE Yung; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Background: When there is insufficient septal cartilage graft for nasal reconstruction, current alternatives include the autologous costal cartilage (ACC) graft, irradiated homologous costal cartilage (IHCC) graft, and synthetic (alloplastic) polymer scaffolds. Newer alternatives such as hybrid bioactive-biocompatible polymer scaffolds and tissue-engineered cartilage grafts have also emerged. With the widespread use of alternative grafts in septorhinoplasty, multiple systematic reviews have analyzed their complication rates with varying results. This systematic review aims to summarize the current body of systematic reviews analyzing complication rates for ACC, IHCC, and alloplastic grafts in septorhinoplasty. In addition, it aims to provide an overview of the recent progress of newer grafts and potential avenues for future research.

Methods: A comprehensive search of EMBASE, Medline, Google Scholar and EBMR was conducted for systematic reviews with more than 10 primary studies analyzing complication rates of ACC, IHCC, and alloplastic grafts in septorhinoplasty. The Preferred Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed, and the Risk of Bias in Systematic Reviews (ROBIS) tool was used. Outcomes for qualitative evaluation included overall complication rate, infection, warping, resorption, extrusion, hypertrophic scarring, pneumothorax, and revision and implant removal surgery.

Results: Six systematic reviews were included that analyzed ACC with alloplastic grafts (n=2), ACC with IHCC grafts (n=1), alloplastic grafts only (n=2), and ACC grafts only (n=1). ACC grafts were associated with warping, resorption, revision surgery and donor-site morbidity. IHCC grafts had similar complication rates to ACC grafts in specific situations. Infection, extrusion, and removal surgery rates were generally higher amongst all alloplastic grafts, although rates varied between specific alloplastics. The Corrected Covered Area (CCA) was calculated as 10.63% limiting direct comparison of results between reviews.

Conclusions: Whilst it is evident that biologic and alloplastic grafts do not fulfil the criteria of the ideal graft described in the literature, newer alternatives such as tissue-engineered grafts and bioactive-biocompatible polymer composites still require more research to characterize their complication rates.

Keywords: Rhinoplasty; autologous costal cartilage (ACC); alloplastic; tissue engineering; biosynthetic

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Introduction

The presence or treatment of congenital, traumatic, and oncological defects of the nose can compromise nasal airway function and cosmesis. Congenital and traumatic septal deviation is present in up to 80% of the general population (1), and facial skin cancer most commonly affects the nose (1). A myriad of reconstructive surgical procedures has been described to address congenital and acquired nasal deformity, ranging from simple nasal bone fracture reduction, skin graft and local flaps to septorhinoplasty and complex microsurgical composite reconstruction. Septorhinoplasty is the third most common procedure in the United States, accounting for over $1.1 billion in 2016 alone (2).

Nasal reconstruction poses unique challenges for a variety of reasons in addition to the obvious cosmetic ramifications. The nose is a tri-lamellar structure that is difficult to duplicate synthetically. The external skin-soft tissue envelope protecting the internal nasal structures can be thin, and is in close proximity with the nasal mucosa which commonly harbors pathogens (3). The outcome of any reconstructive procedure must withstand the normal aerodynamic forces of airflow during respiration that filters and humidifies air, in addition to air expulsion during sneezing (1).

Commonly, the native nasal septum alone is sufficient as an autologous cartilage graft donor site for addition of structure to the nasal skeleton and volume to the nose. However, when there is insufficient septal cartilage for harvest, alternative grafts are necessary. Current alternative grafts to native septal cartilage are either biologic or synthetic/alloplastic (4). Biologic grafts are predominantly autologous (i.e., autograft), and to a much lesser extent allogeneic (i.e., allograft). Autografts are harvested from one anatomical location and used elsewhere on the same individual, making the patient both donor and recipient. Traditionally, the autologous costal cartilage (ACC) graft is the gold standard when an individual's nasal cartilage is insufficient during septorhinoplasty. An alternative is the auricular cartilage; however it is curved, thin, and scarce (1,4). Allografts, derived from another within the same species, are commonly cadaveric irradiated homologous costal cartilage (IHCC) grafts. The most common commercially available alloplastic grafts are Medpor (porous high-density polyethylene), Gore-Tex (expanded polytetrafluoroethylene), and Silicone.

The literature describes the ideal graft in septorhinoplasty on microscopic and macroscopic levels (1,4). Biocompatibility, bioactivity, and biodegradability are essential for graft success on a microscopic level. Biocompatibility is influenced by the immunogenicity of a graft (1). Grafts with low immunogenicity elicit lesser host immune responses, resulting in greater biocompatibility and generally lower infection rates. Bioactive factors promote integration by inducing chondrogenesis and/or osteogenesis without fibrous tissue encapsulation sealing the implant off (5,6). Biodegradation ideally occurs at a balanced rate whereby the graft maintains its properties and mechanical strength for an adequate time to allow sufficient host response to form the desired tissue before degrading into biocompatible waste products (6). Whilst greater porosity influences cellular migration, ingrowth and vascularization, excessive pore size hinders strength (6). Generally, porosity above 50 μm is recommended as many studies have shown that greater porosity is associated with increasing cellular concentration, DNA content, glycosaminoglycan (GAG) and collagen synthesis (7).

Macroscopically, grafts that mimic the biomechanical and functional properties of the target tissue without being a vector for disease are ideal (4). In addition to maintaining volume without extruding over time, grafts should resist external and internal physiological forces. Ideal grafts are expediently produced or procured inexpensively on-demand to patient-specific requirements, or are intra-operatively customizable, and are not associated with donor-site morbidity. Infusing grafts with antimicrobial agents is preferred in limiting perioperative infection.

Considering how common septorhinoplasty is, many studies have analyzed complication rates of biologic and alloplastic grafts resulting in multiple systematic reviews with varying results. This systematic review aims to summarize the complication rates of commonly used septorhinoplasty grafts from systematic reviews and provide an overview of emerging alternatives. We present the following article in accordance with the PRISMA reporting checklist (available at https://www.theajo.com/article/view/10.21037/ajo-22-12/rc) (8).

Methods

Eligibility criteria

Systematic reviews with or without meta-analysis including more than 10 primary studies were included whilst narrative reviews, case reports, and abstracts were excluded. Furthermore, reviews without pooled analysis of complication rates, not full text, not written in English, and

not pertaining to humans were excluded.

Information sources

Two independent reviewers (BSN and AEY) conducted a systematic literature review of four databases (Medline, EMBASE, Google Scholar and EBMR) up to the 8th of November 2021 using the below search strategy. References of retrieved articles were also manually searched for additional articles missed in the original literature review.

Search methods

A search strategy was generated using the PICO structure: Population—patients receiving septorhinoplasty for functional or aesthetic reasons; Intervention—ACC grafts in septorhinoplasty; Comparison—allogeneic or alloplastic grafts in septorhinoplasty; Outcome—complication rates post-operatively. The following search strategy was used "(nasal augmentation or nasal reconstruction or septoplasty or rhinoplasty or septorhinoplasty) AND ([rib graft or autologous costal cartilage graft or cadaveric cartilage graft] OR [alloplastic implant or silicone or Med*por or Gore*Tex])". Two independent reviewers (B.S.N and A.E.Y) selected studies against the pre-defined inclusion criteria and any disagreements were resolved by discussion.

Data collection and analysis

Two independent reviewers (BSN and AEY) extracted data regarding study characteristics, complication rates and required data to complete a Risk of Bias in Systematic Reviews (ROBIS) assessment. Any disagreements were resolved by discussion between reviewers and, where required, with senior authors (PM and SC). Data extracted and tabulated is as follows:

(I) Study Characteristics included study design, implants (i.e., interventions) analyzed, level of evidence and included study types, number of included studies and their year range, number of included patients, degree of overlap of included primary studies, length of follow-up, databases searched, and risk of bias assessment tool used;

(II) Complications included overall complication rate, infection, warping, resorption, extrusion, hypertrophic scarring, pneumothorax, and revision and removal surgery.

The degree of overlap of primary studies between included systematic reviews was calculated using the Corrected Covered Area (CCA) method by two independent reviewers (BSN and AEY) (9). Considering the degree of overlap of primary studies and heterogeneity of included systematic reviews, results of complication rates were described narratively as further meta-analysis was not feasible (10).

Results

Search results

The initial literature search yielded 494 articles of which 87 were excluded as duplicates. Four hundred and seven unique titles and abstracts were then screened using pre-defined inclusion and exclusion criteria yielding five systematic reviews, which were included. Two systematic reviews were excluded in this process as they included 10 or less primary studies and thus did not meet inclusion criteria (11,12). Further manual searching of the citations of included reviews resulted in one further systematic review being included (*Figure 1*) (8,13). The six included systematic reviews were published between 2008 and 2020 and analyzed ACC with alloplastic grafts (n=2) (2,14), ACC with IHCC grafts (n=1) (15), alloplastic grafts only (n=2) (16,17), and ACC grafts only (n=1) (13) (*Table 1*).

Quality of included systematic reviews

In total, 170 primary studies were cited in the six systematic reviews with 111 of these being unique primary studies yielding a CCA of 10.63% which is considered moderate to high overlap (9). *Table 2* is a citation matrix representing the number of overlapping primary studies between any two given systematic reviews. Overall, Liang *et al.* had the most overlap of included primary studies with all other reviews (50 citations), however Hudise *et al.* had the highest degree of overlap of any one systematic review (76.7%) (*Table 1*) (2,14).

Using the ROBIS tool, two reviews were considered to have a high overall risk of bias representing 30% of the included reviews (16-18). The most common domain introducing bias was in synthesis and findings (50%) (*Table 3*). Peled *et al.* did not have pre-defined eligibility criteria, searched a single database, did not use a standardized risk of bias assessment tool, and did not employ funnel plots or sensitivity analysis in the synthesis of their results (17). Similarly, Hoang *et al.* did not employ a formal assessment of risk of bias or heterogeneity (16).

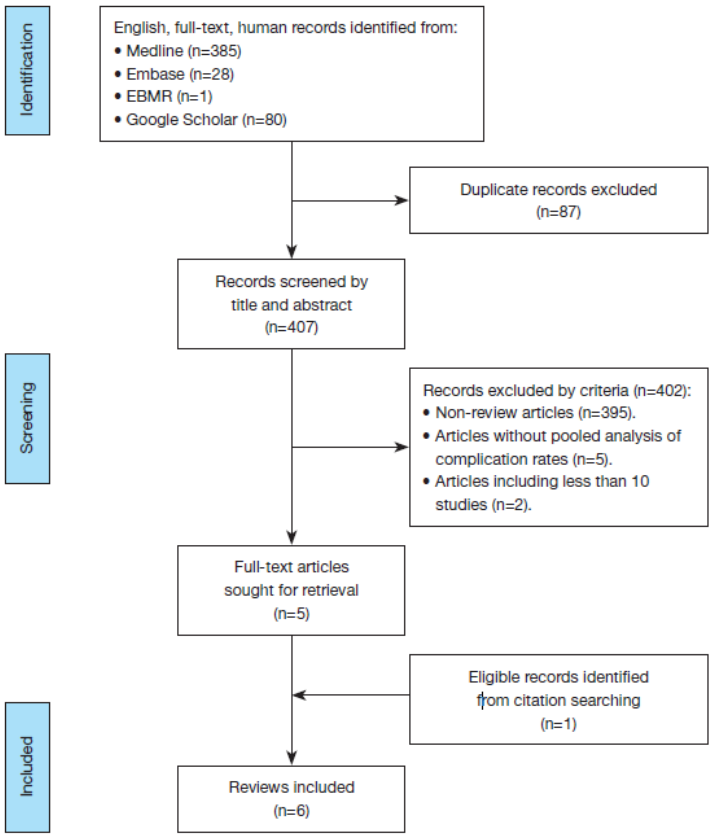

Figure 1 PRISMA flowchart.

Qualitative synthesis

Complications associated with graft use were thematically organized: overall complication rate, infection, warping, resorption, extrusion, hypertrophic scarring, pneumothorax, and revision and removal surgery. Results of complication rates from each systematic review for ACC, IHCC, and alloplastic grafts are listed in *Table 4*.

Overall complication rate

Overall complication rates at the recipient site for ACC grafts were 11.4% (13) and 14% (2), and at the donor site were 3.2% (13) and 7% (2). One systematic review (14) reported overall complication rates irrespective of donor or recipient site as 6.9% for ACC grafts. Overall complication rates for alloplastic grafts were found to be 8% (2) and 7.8% (14). No systematic review provided overall complication rates for IHCC grafts.

Infection

All six systematic reviews (2,13-17) analyzed infection rates by graft type. Gore-Tex was found to have varying infection rates of 1.3% (2), 1.4% (16), and 4% (17). Medpor demonstrated rates of 2.3% (2) and 6% (17). Silicone had reported rates of 1.9% (16), 2.1% (2), and 3.7% (17). Combined alloplastic graft infection rates were 3.7% (14) and 4.6% (2). ACC grafts were found to cause infection at the donor site in 0.6% (13) of cases, and at the recipient site at rates of 2% (15), 2.1% (14), 2.5% (13) and 6.8% (2). IHCC grafts demonstrated an infection rate of 3% (15).

Table 1 Summary of study characteristics

Author, year	Implant used	Study design	Level of evidence	No. of studies (year)	No. of patients	Overlap (overlap/ total No. of studies)	Follow up	Databases searched	Risk of bias assessment tool
Peled *et al.*, 2008 (17)	Silicone; Medpor; Gore-tex	Meta-analysis & Systematic Review	IV	20 (1966–2005)	4,264	35% (7/20)	4 weeks – 11 years	Medline	N/A
Hoang *et al.*, 2018 (16)	Silicone; Gore-tex	Meta-analysis & Systematic Review	IV	18 (1988–2016)	7,759	66.7% (12/18)	6 days – 17 years	Medline, PubMed, Cochrane	N/A
Varadharajan *et al.*, 2014 (13)	ACC†	Systematic Review	IV	21 (1980–2014)	1,545	53.4% (11/21)	3–73 months	Medline, PubMed, Cochrane, EMBASE	ASPS critical appraisal checklist
Hudise *et al.*, 2020 (14)	ACC; Silicone; Medpor; Gore-tex	Meta-analysis & Systematic Review	IV	30 (1999–2018)	1,013	76.7% (23/30)	2.5–106 months	PubMed, Cochrane Library, Web of Science, Virtual Health Library, Google Scholar, SCOPUS	ROBINS-1
Liang *et al.*, 2018 (2)	ACC; Silicone; edpor; Gore-tex	Meta-analysis & Systematic Review	IV	53 (1973–2017)	5,415	67.9% (36/53)	3–72 months	EMBASE, PubMed	MINORS
Vila *et al.*, 2020 (15)	ACC; IHCC‡	Meta-analysis & Systematic Review	IV	28 (1990–2017)	1,041	50% (14/28)	6–48 months	Medline, EMBASE, Scopus, CENTRAL, ClinicalTrials.gov	Cochrane Collaboration Risk of Bias Tool

†, Autologous Costal Cartilage; ‡, Irradiated Homologous Costal Cartilage. ASPS, American Society of Plastic Surgeons; ROBINS-1, Risk of Bias In Non-randomized Studies; MINORS, Methodological Index for Non-Randomized Studies; N/A, not applicable.

Table 2 Citation matrix of overlap between systematic reviews

Review	Peled (17)	Hoang (16)	Varadharajan (13)	Hudise (14)	Liang (2)	Vila (15)
Peled (17)	N/A	5	0	1	3	0
Hoang (16)	N/A	N/A	0	0	8	0
Varadharajan (13)	N/A	N/A	N/A	1	7	5
Hudise (14)	N/A	N/A	N/A	N/A	21	5
Liang (2)	N/A	N/A	N/A	N/A	N/A	11
Vila (15)	N/A	N/A	N/A	N/A	N/A	N/A

Numbers in each cell represent the total number of overlapping primary studies between any two systematic reviews. N/A, not applicable.

Table 3 Risk of bias (ROBIS) by review

Review	Phase 2				Phase 3
	Study eligibility criteria	Identification and selection of studies	Data collection and study appraisal	Synthesis and findings	Risk of bias in the review
Peled (17)	☹	☹	☹	☹	☹
Hoang (16)	☹	☺	☹	☹	☹
Varadharajan (13)	☺	☺	☺	☹	☺
Hudise (14)	☹	☺	☺	☺	☺
Liang (2)	☺	☺	☺	☺	☺
Vila (15)	☺	☺	☺	☺	☺

☺ = low risk; ☹ = high risk.

Warping and resorption

Of the four systematic reviews (2,13-15) reporting rates of warping amongst ACC grafts, only two (2,14) documented comparative alloplastic warping rates. One systematic review found the combined alloplastic warping rate as 1.6% (14), whilst another differentiated warping rates by alloplastic with Silicone having the highest warping rate at 5.1%, Gore-Tex the lowest at 0.9%, and no statistically significant data for Medpor (2). Warping was generally higher with ACC grafts [5.2% (13), 5.6% (2), and 6% (15)], however one systematic review reported rates as low as 1.5% (14).

One systematic review comparing ACC and alloplastic resorption rates found higher resorption rates for ACC grafts (3.9%) compared with Silicone (1.9%), Medpor (2.5%), and Gore-Tex (0.69%) (2). Contrastingly, three systematic reviews that analyzed resorption rates for ACC grafts found varied rates [0.9% (13), 1% (15), and 4.1% (14)]. IHCC graft warping and resorption rates were 5% and 4% respectively (15).

Extrusion

Three systematic reviews (2,16,17) documented extrusion rates. ACC grafts generally extruded at lower rates [0.6% (13) and 1.5% (2)]. Medpor had higher rates of extrusion [2% (17) and 5.1% (2)] than Gore-Tex [1.1% (17), 1.2% (16), and 2.9% (2)]. Silicone had varying rates of extrusion at 0.7% (16), 1.6% (2), and 3.2% (17).

Hypertrophic scarring and pneumothorax

Three systematic reviews (2,13,14) reported hypertrophic scarring rates which were generally higher for ACC [1.8% (14), 2.9% (13), and 3.8% (2)] than alloplastic grafts [1.5% (14)]. Pneumothorax was solely associated with ACC grafts, with varying rates between 0.1% (13) and 3.1% (2).

Revision and removal surgery

Six systematic reviews (2,13-17) analyzed alloplastic, IHCC

Table 4 Complication rates by graft type with CI in brackets where provided

	ACC*			IHCC‡	Alloplastic			
	% of donor site [CI]	% of recipient site [CI]	% of combined sites [CI]		% of silicone [CI]	% of Med-por [CI]	% of Gore-Tex [CI]	% of combined alloplastics [CI]
Overall complication	3.2 (13), 7 [4–11.6] (2)	11.4 (13), 14 [10–19] (2)	6.9 [4.3–9.6] (14)	NS§	9.2 (16)	NS	5.3 (16)	7.8 [4–11.5] (14), 8 [5–11] (2)
Infection	0.6 (13)	2.5 (13)	2 [0–5] (15), 2.1 [1–3.3] (14), 6.8 [4.7–9.7] (2)	3 [1–8] (15)	1.9 (16), 2.1 [0.6–7.3] (2), 3.7 (17)	2.3 [1–8] (2), 6 (17)	1.3 [0.6–3] (2), 1.4 (16), 4 (17)	3.7 [0.4–7] (14), 4.6 [0.3–44.8] (2)
Warping	N/A	1.5 [0.3–2.8] (14), 5.2 (13), 5.6 [4–7.7] (2), 6 [2–11] (15)	N/A	5 [1–12] (15)	5.1 [2–11.6] (2)	NS	0.9 [0.6–1.7] (2)	1.6 [0.5–3.7] (14)
Resorption	N/A	0.9 (13), 1 [0–2] (15), 3.9 [2.6–5.8] (2), 4.1 [2.5–5.6] (14)	N/A	4 [0–13] (15)	1.9 [0.1–24] (2)	2.5 [0.2–30] (2)	0.7 [0.1–3.6] (2)	NS
Extrusion	N/A	0.6 (13), 1.5 [0.6–3.8] (2)	N/A	NS	0.7 (16), 1.6 [0.5–5.2] (2), 3.2 (17)	2 (17), 5.1 [2.5–10] (2)	1.1 (17), 1.2 (16), 2.9 [2.2–4] (2)	1.1 [0.2–5.2] (2)
Hypertrophic scar	2.9 (13), 3.8 [1.7–8] (2)	NS	1.8 [0.2–3.4] (14)	NS	NS	NS	NS	1.5 [0.4–3.5] (14)
Pneumothorax	0.1 (13), 3.1 [1.5–6.5] (2)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Revision surgery	N/A	4.2 [2.4–6] (14), 5 [1–10] (15), 5.4 (13), 8 [4.8–12.9] (2)	N/A	7 [3–12] (15)	3.9 [1–14.2] (2), 6.8 (16)	5.7[2.8–11.3] (2)	2 [0.3–14] (2), 2.4 (16)	5 [1.8–13] (2), 3.7[1.0–6.4] (14)
Removal Surgery	N/A	1.9 [1–3.6] (2)	N/A	NS	6.5 (17), 12 [6.5–19.9] (2)	3.1 (17), 4.5 [2–10] (2)	3.1 (17), 3.6 [2–6.2] (2)	2.2 [0.4–10.5] (2)

*, autologous costal cartilage; ‡, irradiated homologous costal cartilage; §, not stated. CI, confidence interval; N/A, not applicable.

and ACC grafts with regards to rates of surgical revision or removal. One systematic review showed ACC grafts had lower removal rates (1.9%) and higher revision rates (8%) (2). Contrastingly, in the same study alloplastics generally had higher removal than revision rates: Silicone's removal and revision rate was 12% and 3.9% respectively, Medpor 4.5% and 5.7%, and Gore-Tex 3.6% and 2% (2). Another systematic review showed similar revision surgery rates for alloplastic (3.7%) and ACC (4.2%) grafts (14). IHCC grafts had higher revision rates [7% (15)] compared to ACC grafts [5% (15)] which was also reflected in another systematic review where the ACC revision rate was 5.4% (13). One systematic review found removal rates to be 6.5% for Silicone, and 3.1% for Medpor and Goretex (17). Another systematic review demonstrated revision rates of 6.8% for Silicone, and 2.4% for Gore-tex (16).

Discussion

This review identified six systematic reviews with or without meta-analysis that show neither ACC, allogeneic, or alloplastic grafts are superior in every clinical situation during septorhinoplasty. Warping, resorption, revision surgery and donor-site morbidity were generally higher with ACC graft use. Infection, extrusion, and removal surgery were generally higher amongst all alloplastic grafts compared to ACC grafts. However, there was variation of these rates between different types of alloplastic grafts. IHCC grafts had similar complication rates to ACC grafts with regards to infection, warping, resorption, and secondary surgery when used as dorsal onlay grafts only.

Biologic grafts

ACC grafts are popular for their intra-operative versatility and low infection risk. In addition to being relatively abundant in supply, ACC grafts are sufficiently rigid to provide structural support, or be morselized as a filler (4). Being autologous, ACC grafts have inherently low immunogenicity and thus low infection rates (1,15). When an infection is present, intravenous antibiotics have greater penetrance in ACC grafts compared with synthetic alternatives, thus reducing requirements for secondary surgery for ACC grafts due to infection (12). Despite its popularity, there are multiple challenges with ACC grafts; donor-site morbidity, longer operating times for harvest, revision surgery and warping (13).

IHCC grafts are a potential solution for the drawbacks of ACC grafts as they have low infection rates, no donor-site morbidity, and are readily available eliminating long operating times. Although IHCC grafts are irradiated to lower infection risk, this process causes decellularization limiting *in vivo* remodeling thereby promoting greater resorption and revision surgery rates (1). Despite this, a recent meta-analysis by Vila *et al.* demonstrated similar rates of warping between IHCC and ACC grafts (15). However, their study only included IHCC dorsal onlay grafts where structural support was not required (15). Traditionally, IHCC grafts are limited to situations where structural support isn't crucial to compensate for their varying warping rates and poorer retention (15,19). Considering this, IHCC grafts are generally less popular than ACC grafts, contributing to a paucity of long-term follow-up studies (19).

Alloplastic grafts

Another alternative to ACC grafts is the alloplastic graft. Alloplastics can be cost-effectively produced providing an abundance of supply without donor-site morbidity. Whilst some alloplastics can be intra-operatively carved to specific dimensions, others can be pre-fabricated to generic shapes such as alar battens or L-struts (4). However, there is no readily available alloplastic graft suitable in every septorhinoplasty operation (4) and no studies to date have quantitatively compared the biomechanical properties of alloplastics with septal or costal cartilage (1,20). Despite this, they have been used extensively in select groups (21).

Of the three common alloplastics, the softness of Gore-Tex makes it appropriate for dorsal onlay grafting but too weak for structural support (4). With intermediate porosity ranging from 10–30 µm (21), Gore-Tex allows limited tissue ingrowth for stabilization (4). Medpor possesses the highest porosity of the three alloplastics [125–250 µm (21)], promoting significant tissue ingrowth. Combined with its superior rigidity, this makes Medpor suitable for structural support (4). However, this also contributes to Medpor's extrusion and removal rates, with the removal operation being more difficult considering the tissue ingrowth (2). Silicone is cheap and easily carved intra-operatively, promoting its use in dorsal onlay techniques (2,4). As silicone is non-porous, there is significant fibrous encapsulation and subsequent calcification over time promoting the highest complication and removal surgery rates of the three alloplastics (2). Interestingly however, the rates of silicone extrusion and displacement amongst

Asians are lower compared with Caucasians. Asian patients have a thicker skin-soft tissue envelope that likely creates a more forgiving environment for silicone implants compared with Caucasian patients (4). Consequently, silicone is more popular with Asian surgeons, resulting in greater experience and refined surgical techniques (22).

In comparing alloplastic and ACC grafts, Liang *et al.* demonstrated higher removal rates for alloplastics compared with higher revision rates for ACC grafts (2). This is likely attributable to ACC grafts warping at higher rates but being autologous in nature, often only requiring revision rather than removal. Contrastingly, alloplastic grafts extrude at higher rates and are less adept to manipulation for revision, therefore requiring removal.

Limitations

The major limitation of this systematic review is the heterogeneity of included systematic reviews and significant overlap of included primary studies rendering meta-analysis or comparison of results between studies unfeasible (9,23). Furthermore, all reviews relied heavily on retrospective studies making their level of evidence IV at best. Two of the included systematic reviews were also considered to have a high risk of bias which limits interpretation of their results.

Future directions

Tissue-engineered grafts in septorhinoplasty have emerged in the hope of eliminating the drawbacks of donor-site morbidity and longer operating times associated with biologic grafts whilst retaining their inherently low immunogenicity and tissue versatility. A tissue-engineered graft aims to produce biomimetic tissue by utilizing the optimal combination of a cellular source, stimuli, and scaffolding (24).

Cellular source in tissue-engineering neo-cartilage is primarily either chondrocytes or mesenchymal stem cells (MSCs) (1,24). Although chondrocytes harvested from bovine (25), rabbit (26), and ovine (27) sources have been investigated, only autologous sources have been approved for human use and thus the issue of donor-site morbidity persists (28). Autologous or allogeneic MSCs can grow rapidly but produce fibrocartilage that lacks the loading and stretching properties that hyaline septal cartilage possesses (24). Genetic modifications present a future avenue for research to obtain appropriate hyaline cartilaginous tissue from MSCs (29,30).

Stimulation whilst culturing chondrocytes through either 3D culture systems (31), growth factors (32), or MSCs (24) is important as chondrocytes dedifferentiate to adopt fibroblastic characteristics after three monolayer culture passages (24). To combat the significant quantity of chondrocytes required to engineer the volume of cartilage required in septorhinoplasty, methods such as incorporating autologous MSCs (25) and using photocrosslinkable bioinks that can bioprint chondrocytes instead of seeding them have been studied (33). However, further research is required to overcome issues of time and cost to allow production at scale to be viable.

Clinically, tissue-engineered grafts have demonstrated promising but limited results (1). There are no systematic reviews, meta-analyses, large animal studies, or standardized animal models for tissue-engineered grafts in septorhinoplasty (1,30-32,34). Only five uncontrolled cohort studies in small human populations have been conducted to date without any report of significant adverse events (35-39).

Bioactive-biocompatible polymer hybrid scaffolds combine porous biodegradable biocompatible polymeric scaffolds with bioactive materials to improve integration and reduce extrusion rates when compared to alloplastics (40-42). Whilst no hybrid scaffolds have been approved in septorhinoplasty, the success of hybrid scaffolds like Chondrotissue® (Biotissue) in replacing articular cartilage is promising (43,44). Wu *et al.* have demonstrated *in vivo* that the combination of a bioactive ceramic silica-based glass (Bioglass®) with a polymer scaffold exhibited greater hydrophilicity, adherence, and stimulated thicker cartilage-like tissue with better biomechanics than Bioglass® alone (41). Yao *et al.* also demonstrated a hybrid Bioglass®-polymer scaffold had degradation rates and mechanical strength superior to Bioglass® alone (42). Characterizing the ability of Bioglass®-polymer scaffold composites to stimulate chondrogenesis *in vivo* requires further research.

Conclusions

Our systematic review of six systematic reviews suggests alloplastic, ACC and IHCC grafts are viable alternatives when native septal cartilage is insufficient during septorhinoplasty. When selecting grafts, surgeons should consider the purpose and complications associated with each graft and educate patients appropriately. Whilst ACC grafts remain the preferred alternative, it is evident that the 'ideal' graft has yet to be achieved. Tissue-engineered grafts and

bioactive-biocompatible polymer composites hold promise but require further research to overcome their limitations and nascence.

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Footnote

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References

1. Lavernia L, Brown WE, Wong BJF, et al. Toward tissue-engineering of nasal cartilages. Acta Biomater 2019;88:42-56.
2. Liang X, Wang K, Malay S, et al. A systematic review and meta-analysis of comparison between autologous costal cartilage and alloplastic materials in rhinoplasty. J Plast Reconstr Aesthet Surg 2018;71:1164-73.
3. Karnes J, Salisbury M, Schaeferle M, et al. Porous High-density Polyethylene Implants (Medpor) for Nasal Dorsum Augmentation. Aesthetic Surgery Journal 2000;20:26-30.
4. Ansari K, Asaria J, Hilger P, et al. Grafts and implants in rhinoplasty—Techniques and long-term results. Operative Techniques in Otolaryngology-Head and Neck Surgery 2008;19:42-58.
5. Asselin A, Hattar S, Oboeuf M, et al. The modulation of tissue-specific gene expression in rat nasal chondrocyte cultures by bioactive glasses. Biomaterials 2004;25:5621-30.
6. Jones JR. Review of bioactive glass: from Hench to hybrids. Acta Biomater 2013;9:4457-86.
7. Nava MM, Draghi L, Giordano C, et al. The effect of scaffold pore size in cartilage tissue engineering. J Appl Biomater Funct Mater 2016;14:e223-9.
8. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71.
9. Pieper D, Antoine SL, Mathes T, et al. Systematic review finds overlapping reviews were not mentioned in every other overview. J Clin Epidemiol 2014;67:368-75.
10. Higgins JP, Thomas J, Chandler J, et al. Cochrane handbook for systematic reviews of interventions. John Wiley & Sons, 2019.
11. Alhussain OH, Alhussain GO, Rajkhan AA, et al. Efficacy of autologous vs. homologous costal cartilage grafts in dorsal augmentation rhinoplasty: a systematic review and meta-analysis. Annals of Medical and Health Sciences Research 2020;10.
12. Wee JH, Park MH, Oh S, et al. Complications associated with autologous rib cartilage use in rhinoplasty: a meta-analysis. JAMA Facial Plast Surg 2015;17:49-55.
13. Varadharajan K, Sethukumar P, Anwar M, et al. Complications Associated With the Use of Autologous Costal Cartilage in Rhinoplasty: A Systematic Review. Aesthet Surg J 2015;35:644-52.
14. Hudise JY, Aldhabaan SA, Aldosari BF. Complications of the nasal dorsum reconstruction using autologous or alloplastic grafts: evidence from systematic review and meta-analysis. Braz J Otorhinolaryngol 2022;88:406-20.
15. Vila PM, Jeanpierre LM, Rizzi CJ, et al. Comparison of Autologous vs Homologous Costal Cartilage Grafts in Dorsal Augmentation Rhinoplasty: A Systematic Review and Meta-analysis. JAMA Otolaryngol Head Neck Surg 2020;146:347-54.
16. Hoang HLT, Januszyk M, Boyd JB. A systematic review of complications associated with nasal augmentation implants:

expanded polytetrafluoroethylene (Gore-Tex) versus silicone. Journal of Cosmetic Medicine 2018;2:27-31.
17. Peled ZM, Warren AG, Johnston P, et al. The use of alloplastic materials in rhinoplasty surgery: a meta-analysis. Plast Reconstr Surg 2008;121:85e-92e.
18. Whiting P, Savović J, Higgins JP, et al. ROBIS: A new tool to assess risk of bias in systematic reviews was developed. J Clin Epidemiol 2016;69:225-34.
19. Wee JH, Mun SJ, Na WS, et al. Autologous vs Irradiated Homologous Costal Cartilage as Graft Material in Rhinoplasty. JAMA Facial Plast Surg 2017;19:183-8.
20. Griffin MF, Premakumar Y, Seifalian AM, et al. Biomechanical characterisation of the human nasal cartilages; implications for tissue engineering. J Mater Sci Mater Med 2016;27:11.
21. Mella J, Christophel J, Park S. Are Alloplastic Implants Safe in Rhinoplasty? Laryngoscope 2020;130:1854-6.
22. Shirakabe Y, Suzuki Y, Lam SM. A systematic approach to rhinoplasty of the Japanese nose: a thirty-year experience. Aesthetic Plast Surg 2003;27:221-31.
23. Glenny AM, Altman DG, Song F, et al. Indirect comparisons of competing interventions. Health Technol Assess 2005;9:1-134, iii-iv.
24. Kim MS, Kim HK, Kim DW. Cartilage tissue engineering for craniofacial reconstruction. Arch Plast Surg 2020;47:392-403.
25. Pleumeekers MM, Nimeskern L, Koevoet WLM, et al. Cartilage Regeneration in the Head and Neck Area: Combination of Ear or Nasal Chondrocytes and Mesenchymal Stem Cells Improves Cartilage Production. Plast Reconstr Surg 2015;136:762e-74e.
26. Kushnaryov A, Yamaguchi T, Briggs KK, et al. Evaluation of Autogenous Engineered Septal Cartilage Grafts in Rabbits- A Minimally Invasive Preclinical Model. Adv Otolaryngol 2014;2014:415821.
27. Oseni AO, Butler PE, Seifalian AM. Optimization of chondrocyte isolation and characterization for large-scale cartilage tissue engineering. J Surg Res 2013;181:41-8.
28. Phull AR, Eo SH, Abbas Q, et al. Applications of Chondrocyte-Based Cartilage Engineering: An Overview. Biomed Res Int 2016;2016:1879837.
29. Lee WY, Wang B. Cartilage repair by mesenchymal stem cells: Clinical trial update and perspectives. J Orthop Translat 2017;9:76-88.
30. Tan AR, Hung CT. Concise Review: Mesenchymal Stem Cells for Functional Cartilage Tissue Engineering: Taking Cues from Chondrocyte-Based Constructs. Stem Cells Transl Med 2017;6:1295-303.
31. Caron MM, Emans PJ, Coolsen MM, et al. Redifferentiation of dedifferentiated human articular chondrocytes: comparison of 2D and 3D cultures. Osteoarthritis Cartilage 2012;20:1170-8.
32. Alexander TH, Sage AB, Chen AC, et al. Insulin-like growth factor-I and growth differentiation factor-5 promote the formation of tissue-engineered human nasal septal cartilage. Tissue Eng Part C Methods 2010;16:1213-21.
33. Zhang YS, Yue K, Aleman J, et al. 3D Bioprinting for Tissue and Organ Fabrication. Ann Biomed Eng 2017;45:148-63.
34. Di Gesù R, Acharya AP, Jacobs I, et al. 3D printing for tissue engineering in otolaryngology. Connect Tissue Res 2020;61:117-36.
35. Ceccarelli G, Gentile P, Marcarelli M, et al. In Vitro and In Vivo Studies of Alar-Nasal Cartilage Using Autologous Micro-Grafts: The Use of the Rigenera® Protocol in the Treatment of an Osteochondral Lesion of the Nose. Pharmaceuticals (Basel) 2017;10:53.
36. Fulco I, Miot S, Haug MD, et al. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. Lancet 2014;384:337-46.
37. Gentile P, Scioli MG, Bielli A, et al. Reconstruction of Alar Nasal Cartilage Defects Using a Tissue Engineering Technique Based on a Combined Use of Autologous Chondrocyte Micrografts and Platelet-rich Plasma: Preliminary Clinical and Instrumental Evaluation. Plast Reconstr Surg Glob Open 2016;4:e1027.
38. Hoshi K, Fujihara Y, Saijo H, et al. Implant-type Tissue-engineered Cartilage for Secondary Correction of Cleft Lip-nose Patients: An Exploratory First-in-human Trial. Journal of Clinical Trials 2017;7:1-9.
39. Y Yanaga H, Imai K, Yanaga K. Generative surgery of cultured autologous auricular chondrocytes for nasal augmentation. Aesthetic Plast Surg 2009;33:795-802.
40. Deliormanlı AM, Atmaca H. Biological Response of Osteoblastic and Chondrogenic Cells to Graphene-Containing PCL/Bioactive Glass Bilayered Scaffolds for Osteochondral Tissue Engineering Applications. Appl Biochem Biotechnol 2018;186:972-89.
41. Wu J, Xue K, Li H, et al. Improvement of PHBV scaffolds with bioglass for cartilage tissue engineering. PLoS One 2013;8:e71563.
42. Yao Q, Nooeaid P, Detsch R, et al. Bioglass®/chitosan-

polycaprolactone bilayered composite scaffolds intended for osteochondral tissue engineering. J Biomed Mater Res A 2014;102:4510-8.
43. Siclari A, Mascaro G, Kaps C, et al. A 5-year follow-up after cartilage repair in the knee using a platelet-rich plasma-immersed polymer-based implant. Open Orthop J 2014;8:346-54.
44. Wasyłeczko M, Sikorska W, Chwojnowski A. Review of Synthetic and Hybrid Scaffolds in Cartilage Tissue Engineering. Membranes (Basel) 2020;10:348.

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