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Corresponding Author: Dr. Kyriacos A Athanasiou, PhD, PE

Corresponding Author's Institution: University of California Irvine

First Author: Laura Lavernia, BS

Order of Authors: Laura Lavernia, BS; Wendy E Brown, PhD; Brian J Wong, MD, PhD; Jerry C Hu, PhD; Kyriacos A Athanasiou, PhD, PE

Abstract: Nasal cartilage pathologies are common; for example, deviated nasal septum conditions afflict up to 80% of people. Because cartilage provides the supportive framework of the nose, afflicted patients suffer low quality of life. To correct pathologies, graft cartilage is often required. Grafts are currently sourced from the patient's septum, ear, or rib. However, their use yields donor site morbidity and is limited by tissue quantity and quality. Additionally, rhinoplasty revision rates exceed 15%, exacerbating the shortage of graft cartilage. Alternative grafts, such as irradiated allogeneic rib cartilage, are associated with complications. Tissue-engineered neocartilage holds promise to address the limitations of current grafts. The engineering design process may be used to create suitable graft tissues. This process begins by identifying the surgeon's needs. Second, nasal cartilages properties must be understood to define engineering design criteria. Limited investigations have examined nasal cartilage properties; numerous additional studies need to be performed to examine topographical variations, for example. Third, tissue-engineering processes must be applied to achieve the engineering design criteria. Within the recent past, strategies have frequently utilized human septal chondrocytes. As autologous and allogeneic rib graft cartilage is used, its suitability as a cell source should also be examined. Fourth, quantitative verification of engineered neocartilage is critical to check for successful achievement of the engineering design criteria. Finally, following the FDA paradigm, engineered neocartilage must be orthotopically validated in animals. Together, these steps delineate a path to engineer functional nasal neocartilages that may, ultimately, be used to treat human patients.

***Statement of Significance**

Nasal cartilage pathologies affect up to 80% of people and lead to diminished quality of life. The ability to correct pathologies is limited by cartilage graft quality and quantity, as well as donor site morbidity and surgical complications, such as infection and resorption. Despite the significance of nasal cartilage pathologies and high surgical revision rates (15%), little characterization and tissue-engineering work has been performed compared to other cartilages, such as articular cartilage. Furthermore, literature is published in clinical journals, with little in biomedical engineering. Therefore, this review summarizes current literature, discusses the current understanding of nasal cartilage properties, makes recommendations regarding tissue-engineering strategies, and aims to motivate innovation and progress toward engineering functional neocartilage grafts to address the current limitations.

Toward Tissue-engineering of Nasal Cartilages

Laura Lavernia[a*], Wendy E. Brown[b*], Brian J. F. Wong[c], Jerry C. Hu[d], Kyriacos A. Athanasiou[e]

[a]Department of Biomedical Engineering, University of California Irvine, 3120 Natural Sciences II Irvine, CA 92697-2715, USA. Email: laura.lavernia@gmail.com.
[b]Department of Biomedical Engineering, University of California Irvine, 3120 Natural Sciences II Irvine, CA 92697-2715, USA. Email: wendy.brown@uci.edu.
[c]Division of Facial Plastic Surgery, Department of Otolaryngology-Head and Neck Surgery, University of California Irvine, 1002 Health Sciences Road, Irvine, CA 92617, USA; Department of Biomedical Engineering, University of California Irvine, 1002 Health Sciences Road, Irvine, CA 92617, USA. Email: bjwong@uci.edu.
[d]Department of Biomedical Engineering, University of California Irvine, 3120 Natural Sciences II Irvine, CA 92697-2715, USA. Email: jerry.hu@uci.edu.
[e]Department of Biomedical Engineering, University of California Irvine, 3120 Natural Sciences II Irvine, CA 92697-2715, USA. Email: athens@uci.edu.

*These authors contributed equally to this work.

Correspondence and reprint requests should be addressed to:
Athanasiou, KA
Department of Biomedical Engineering, University of California Irvine, 3120 Natural Sciences II Irvine, CA 92697-2715 USA
Tel.: (949) 432-2248
Fax: (949) 824-1727
Email: athens@uci.edu

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Nasal cartilage pathologies are common; for example, deviated nasal septum conditions afflict up to 80% of people. Because cartilage provides the supportive framework of the nose, afflicted patients suffer low quality of life. To correct pathologies, graft cartilage is often required. Grafts are currently sourced from the patient's septum, ear, or rib. However, their use yields donor site morbidity and is limited by tissue quantity and quality. Additionally, rhinoplasty revision rates exceed 15%, exacerbating the shortage of graft cartilage. Alternative grafts, such as irradiated allogeneic rib cartilage, are associated with complications. Tissue-engineered neocartilage holds promise to address the limitations of current grafts. The engineering design process may be used to create suitable graft tissues. This process begins by identifying the surgeon's needs. Second, nasal cartilages properties must be understood to define engineering design criteria. Limited investigations have examined nasal cartilage properties; numerous additional studies need to be performed to examine topographical variations, for example. Third, tissue-engineering processes must be applied to achieve the engineering design criteria. Within the recent past, strategies have frequently utilized human septal chondrocytes. As autologous and allogeneic rib graft cartilage is used, its suitability as a cell source should also be examined. Fourth, quantitative verification of engineered neocartilage is critical to check for successful achievement of the engineering design criteria. Finally, following the FDA paradigm, engineered neocartilage must be orthotopically validated in animals. Together, these steps delineate a path to engineer functional nasal neocartilages that may, ultimately, be used to treat human patients.

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1: Introduction

Damage or malformation of the cartilage structures of nose may lead to compromise in nasal airway function or distortion of shape (cosmesis), and may occur as a consequence of trauma, surgery, or congenital malformation. Trauma to the nasal cartilages is prevalent among both civilians and military personnel. The most common nasal deformation is the deviated nasal septum, which has been observed in up to 80% of the general population, and is a major contributor to airway obstruction [1]. Surgery to correct nasal airflow includes fracture repair, septoplasty, and functional rhinoplasty, all of which require the modification of native nasal cartilages, and in many cases, the use of cartilage grafts. Nasal reconstruction to replace missing components, for example, due to injury and cancer, also requires the use of cartilage grafts to reconstruct the framework provided by the nasal cartilages. The nose is the most common site for skin cancer on the face (~36%), and resection of associated tumors may require removal of nasal cartilage to establish clear margins [2]. This may lead to profound disfigurement that also compromises the airway and often requires additional cartilage to reestablish nasal stability during reconstruction. The nose is frequently injured by burns; up to 70% of patients at civilian burn centers have facial burns [3]. Burns to the nasal area, and subsequent infection, scarring, and contracture may lead to significant deformities of nasal cartilage structures [3]. Within the military, blast or burn injuries to the head, neck, and upper airway, including those to the face, account for 28.1% of the combat wounds veterans sustained in Iraq and Afghanistan between 2005 and 2009 [4]. Of the total number of military personnel evacuated from combat zones during Operation Enduring Freedom and Operation Iraqi Freedom, 42% were due to craniomaxillofacial injuries [5]. Fracture to the midface region,

including the nasal area, via explosive mechanisms were the most common, accounting for 44% of those with injures evacuated out of combat zones [5]. Additionally, concomitant facial burns were present in 10% of patients [5]. The prevalence and complexity of nasal trauma, damage, and pathology underscore the need for corrective nasal surgery and the availability of appropriate cartilage graft material.

While nasal surgery is rarely performed in the context of a life-or-death situation, lack of a functioning nasal airway has significant negative effects on patient health, as well as social and psychological consequences. Nasal airway obstruction can lead to recurrent sinus infections, nosebleeds, headaches, insomnia, and sleep apnea, all of which greatly affect patient quality of life [1,6]. Obstruction of breathing may lead to a chronic hypoxic state, which is detrimental to cognitive function [6]. Normal social function in public and in one's personal life is largely dictated by the aesthetics and utility of the nose [7]. Surveys have shown significant negative effects of facial deformities on perceived employability, honesty, trustworthiness, and effectiveness, as well as the resulting impediment to interpersonal development [7]. Perception in society is a large factor of self-esteem, and unsightliness of the nose, whether in the individual or others' opinion, adversely affects self-image and self-value [8]. Effective rhinoplasty procedures are paramount to improving the quality of life for those with nasal dysfunctions and deformities.

Craniomaxillofacial operations are amongst the most common reconstructive procedures performed in the United States, with the number of these procedures increasing each year [9]. Rhinoplasty is amongst the most common structural operation performed on the face. Specifically, over $1.1 billion was spent on rhinoplasty operations in the United States in

2016 [9]. However, it was recently shown that rates of complication, patient dissatisfaction, and subsequent surgical revision associated with rhinoplasty were 7.9%, 15.4%, and up to 15.5%, respectively [10,11]. Postoperatively, 13% of patients retained their anatomic nasal deformities [10]. The revision rate associated with rhinoplasty is influenced by this lack of anatomic correction [10] and is, notably, much higher than the revision rate for articular cartilage repair procedures (5.2%) and total knee arthroplasties (0.49%) [12,13]. Despite the evolution of surgical rhinoplasty techniques [14], the frequency of rhinoplasty operations, in combination with their high revision rates, highlights the importance and need for their continued refinement. Furthermore, there is a well-reported scarcity of suitable graft materials that can be employed to correct nasal deformities [15]. These limitations motivate the development of products to address the lack of native graft material, reduce rhinoplasty revision rates, or simplify revision surgery. There is a compelling need for a viable, biocompatible, and biomimetic equivalent to nasal cartilage, and tissue-engineering has the potential to address this need.

This review discusses the role of tissue-engineering in nasal surgery by outlining the engineering design process as it applies to nasal cartilage. Data are presented regarding the structure, function, content, and mechanics of native nasal cartilages, followed by a discussion of the additional data needed to guide the field toward developing gold standards for nasal cartilage engineering. The use and type of grafts used in rhinoplasty are listed to inform the use and requirements of tissue-engineered nasal neocartilages. An up-to-date summary of current nasal cartilage engineering strategies and their successes is also reviewed. The importance of quantitative verification of engineered neocartilage, as well as a functionality index to perform

such verification, are discussed. The use of animal models to validate engineered neocartilage against surgeon and patient needs is also presented. Importantly, this review aims to guide the field by providing recommendations regarding the characterization of nasal cartilages, strategies to engineer biomimetic cartilage, and appropriate animal models.

2: Nose Anatomical Structures, Interfaces, and Functions

2.1: Nasal Cartilage Structures and Functions

The structure and function of nasal cartilages are crucial to inform graft selection for rhinoplasty, as well as in guiding efforts to fabricate functional, tissue-engineered alternatives to native cartilage grafts. All nasal cartilages are hyaline and are categorized into three structures, the septum, the paired upper lateral cartilage (ULC), and paired lower lateral cartilage (LLC) (Figure 1). Structural integrity of the nasal cartilages is crucial for proper respiratory function because the nose is the only means to provide heated, humidified, and filtered air to the lungs [16,17]. The structure of each nasal cartilage and their relationship with one another should inform the shape and size requirements of engineered neocartilage. Additionally, the structure-function relationships of nasal cartilages must be preserved or restored within the nose when clinically utilizing engineered neocartilage.

The septal cartilage, also known as the central septum, cartilaginous septum, and quadrangular cartilage (henceforth referred to as the septum) divides the nose into two nasal cavities, resists deformation, and acts as a beam providing midline structural support for the soft lateral sidewalls [18]. The ULC extends off the septum and interfaces with the nasal bones above and with the maxilla laterally. While the septum and ULC are referred to as distinct

structures, this is not anatomically accurate; the septum and ULC are one contiguous structure of the same embryonic origin [19]. The ULC and septum form the soft lateral sidewall (henceforth referred to as the lateral wall) and medial wall of the nasal vault, respectively. The lateral and medial walls, along with the anterior aspect of the inferior turbinate, form what clinicians refer to as the internal nasal valve [20]. The internal nasal valve maintains normal air flow through the nose and plays a major role in the overall anatomic shape of the nose [21].

Alar cartilage consists of the paired greater alar cartilage, more commonly known as the lower lateral cartilage (LLC), and the sometimes-present minor or lesser alar cartilage, also known as sesamoid cartilage. The LLC consists of three regions: the lateral, intermediary, and medial crura. If the minor alar cartilage is present, it may be connected to the lateral crus of the LLC via a cartilaginous bridge, or it can be a distinct structure completely engulfed by fibro-fatty tissue [22]. The medial crura of the left and right LLC are joined by fibrous connections and support from surrounding connective tissue [23]. The triangle formed by the LLC, columella (the skin and distal portion of the septum that divides the nostrils), and the nasal spine (the anterior portion of the maxillary crest) (Figure 1) constitutes the external nasal valve [18]. Additional pieces of cartilage can exist between the ULC and LLC and are referred to as accessory cartilage. The accessory cartilage can reside freely or on the cephalic edge of the LLC. The region between the ULC and LLC is called the scroll region and is important because it is transected in many endonasal rhinoplasty and airway surgery operations. In this region, the junction between the ULC and LLC has a variable geometry: the caudal ends of the ULC and the cephalic ends of the LLC's lateral crura can be simply overlapped, positioned end-to-end, hooked around one

another, or not interface directly at all. Additionally, the configuration of the right ULC and LLC interface does not necessarily mimic that of the left.

2.2: Cartilage Interfaces and Surrounding Tissues

The septum and ULC interface with several facial bones via osseocartilaginous junctions, or bone-cartilage interfaces. There are four osseocartilaginous junctions of the septum. These interfaces, moving in an anterior caudal to posterior cephalic manner, involve 1) the nasal spine, 2) the vomer bone which lies on top of the palatine process of the maxilla, 3) the perpendicular plate of the ethmoid bone, and 4) the nasal bones at the keystone region [24] (Figure 1). The keystone region is named as such because of its important role in structural stability of the nose [24]. The septum thickens at its anterior end and at its osseocartilaginous interfaces to reinforce these regions [24,25]. The interface of the ULC with the nasal bones provides mechanical strength and stability to the nose [24,26]. While osseocartilaginous junctions are crucial for stability within the nose, little is known about the nature of these interfaces and whether they mirror the osteochondral interfaces present with other hyaline cartilages, such as between the articular cartilage and subchondral bone of articulating joints. As will be discussed in Section 3.1, currently used grafts in rhinoplasty are largely cartilaginous and generally do not require surgical attachment to bony structures. Thus, toward tissue-engineering of nasal cartilages, there does not appear to be a need to form osteochondral implants.

In addition to nasal cartilages and bone, other tissues, divided into anatomical layers, also play a role in the functionality of the nose. Between the skin and osseocartilaginous structures lie the superficial fatty panniculus, the fibromuscular layer, the deep fatty layer, and

the periosteum or mucoperichondrium [27]. The superficial fatty layer panniculus is a subcutaneous layer of fat responsible for the flare of the nostrils and contributes to the flexibility of the nose [28]. The fibromuscular layer, also called the nasal superficial musculoaponeurotic system (SMAS), contains muscles, ligaments, and vasculature [23,28]. The deep fatty layer is a sub-SMAS layer of fibro-fatty tissue which makes direct contact with the cartilage and bone structures in the nose [28]. The mucoperichondrium is a general term given to the four layers of tissue which line the interior of the nasal vault and serve to protect the internal structures of the nose: The outermost layer is exposed to airflow and is composed of stratified, goblet, and basal cells and functions as a protective layer. The basal layer is composed of mostly of collagen fibers. Within the lamina propria reside the tubuloalveolar glands, capillary vessels, and venous plexus. The innermost layer, the perichondrium, is a connective tissue layer that runs parallel and adjacent to the cartilage [29]. During septoplasty, surgeons must elevate the mucoperichondrium from the septum, and failure to elevate all layers can result in complications such as perforations or tears [30]. The tissues surrounding the nasal cartilages help define the shape and stability of the nose, as well as ensure proper nasal function. Therefore, great care must be taken to preserve the relationship between the nasal cartilages and their surrounding tissues when integrating new cartilage or altering native cartilages during rhinoplasties. Strategies to engineer the tissues surrounding nasal cartilage are under development and reviewed elsewhere [31,32].

2.4: Pathologies of Nasal Cartilages

The etiology of nasal cartilage pathology is multifactorial and dependent on the affected structures. Septal deviations may be congenital or acquired [1]. Congenital septal defects may

occur due to compressive pressure across the maxilla during pregnancy or birth, hereditary factors, irregular growth or development of the maxilla and maxillary sinuses, such as cleft palate deformities and choanal atresia, eruption of permanent incisor teeth, or thumb-sucking behavior [1]. The most common cause of acquired septal deformity is trauma [1]. Fractures in the septum tend to occur above or anterior to the osseocartilaginous junctions [24]. Septal fractures commonly dislocate the septum off the maxillary crest and anterior nasal spine, making the keystone osseocartilaginous interface the critical interface with remaining structural integrity [24]. As the central structural support of the nose, septal deviation can obstruct airway patency and, in extreme circumstances, disarticulation can lead to full nasal collapse and the saddle nose deformation [33]. Avulsion of the ULC may occur from trauma, as well as excessive resection of the ULC or surgical treatment of adjacent structures during rhinoplasty [21,34]. Iatrogenic failure of the ULC is common and is due to lack of dorsal stabilization, leading to nasal airway encroachment, nasal collapse, and subsequent obstruction to the normal passage of air through the nose [21]. Together, the ULC and the surrounding skin are a deformable structure that may collapse upon excessive transmural pressure [35]. While fracture of the LLC is rare, deformation from trauma and excessive resection during rhinoplasty are common [36,37]. Deformation or over-resection of the LLC often results collapse, stenosis, external valve dysfunction, and airway impairment [37]. Structural integrity of all cartilaginous nasal structures is key in proper nasal function and airway patency.

3: Rhinoplasty and Septoplasty

Rhinoplasty and septoplasty are the most common procedures performed on the nose. In rhinoplasty surgery, graft cartilage is used to alter the nasal framework or augment regions for reconstructive, functional, or cosmetic objectives. In reconstructive cases, rhinoplasty restores the form and function of a nose that has absent or severely compromised nasal cartilages following trauma, congenital defects, or medical procedures, such as tumor resections. Functional rhinoplasties are performed to alter the structural framework of the nose to restore or improve airflow [38]. Cosmetic rhinoplasty procedures aim to reconfigure the nose to meet an aesthetic goal. The delineation between these categories of rhinoplasty is not strict and may overlap; many cases of structural deformities or insufficiencies that require correction or reconstruction manifest in a patient's cosmetic appearance and vice versa [39]. Septoplasty procedures are as common as functional rhinoplasty procedures. In septoplasty, deformed and obstructive nasal septa are either reshaped *in situ,* or more commonly, cartilage from the central area of the septum is removed. Generally, additional cartilage graft tissue is not required for this procedure. However, the need for graft cartilage tissue varies with each clinical application and patient.

3.1: Graft Use

In addition to reshaping or reconfiguring the nasal cartilages, surgeons utilize cartilage grafts to augment or to mechanically bolster the nasal framework. In general, grafts are sutured to already-present cartilage structures in the nose to add strength or to change their size and shape, known collectively as form factor. Non-load bearing grafts, such as diced-cartilage fascia

grafts (discussed further in Section 3.2), exist to play a largely cosmetic role and to simply occupy volume. However, most grafts serve a mechanical function and must resist both static (gravity, wound healing contracture) and dynamic (cyclical nasal valve deformation, muscle contraction) forces. For example, septal extension grafts are pieces of cartilage sutured to the caudal portion of the septum to extend its length and/or projection (Figure 2). The LLC is often sutured to this graft to change the shape and support of the nasal tip [40]. Therefore, the extension graft must exhibit high mechanical strength to resist the forces imposed upon it, and ideally be perfectly straight. Many surgical operations are designed to reduce ULC and LLC deformation either by stiffening the LLC and ULC or by increasing the cross-sectional area in the internal nasal valve, and, thus, reducing the transmural pressure difference. Alar batten grafts are placed at the site of maximum lateral wall deformation during inspiration to support that area and to prevent valve collapse. These grafts may extend cephalic or caudal to the lateral crus of the LLC to support the ULC or LLC, respectively [40]. Lateral crural strut grafts provide support to the LLC, counteract LLC retraction and collapse, and/or alter LLC shape or positioning. These grafts are placed beneath the lateral aspect of the LLC, forming a laminate structure that provides resistance to flexure. These grafts must reduce distal nasal sidewall flexure to improve the patency of the external nasal valve.

Grafts may also require unique structural qualities, such as topographical changes in thickness, straightness, and resistance to flexure. When there is native curvature in grafts, their geometry may be exploited to counteract the abnormal curvature of native cartilage or abnormal mechanical forces in the recipient site. An extensive review on grafts currently used in rhinoplasty can be found elsewhere [40]. Based on how grafts are used in rhinoplasty,

stiffness, straightness, thickness, and uniformity are parameters that must be accounted for in cartilage tissue-engineering strategies. Additionally, the properties of currently used graft materials should not be used as benchmarks for nasal cartilage tissue-engineering because their they are not always used orthotopically, and, thus, their biomimicry, long-term efficacy, and suitability remain to be studied. With the exception of cases that involve large external forces, the properties of healthy, functional, native nasal cartilages should be used to inform tissue-engineering strategies toward creating biomimetic engineered neocartilage. In specialized cases, such as complete nasal reconstructions in which grafts undergo large wound contraction forces, additional criteria, such as super-native stiffness or resistance to flexure, must also be accounted for.

3.2: Autologous Grafts

The use of autologous grafts is the gold standard for grafting in rhinoplasty due their lack of immunogenicity [41]. Septal cartilage is the preferred source of graft cartilage because it is stiff, straight, and accessible. However, the limited amount of septal cartilage that may be removed without compromising the structural integrity of the nose often creates a shortage of graft material in all types of rhinoplasty procedures [15]. This limitation is exacerbated by the body's inability to regenerate hyaline cartilage, resulting in progressively less graft tissue available at each successive procedure. In revision surgery, septal cartilage is almost always exhausted. In all cases, harvesting a patient's cartilage for grafting creates donor-site morbidity and may cause unnecessary weakness to the nasal framework, especially for patients who do not also require septoplasty.

In patients with depleted amounts of septal cartilage, or in those with acutely bent, weak, or thin septa, autologous ear (auricular) or rib (costal) cartilage is used [42]. While obtaining auricular cartilage for grafting is simpler than harvesting costal cartilage, the fact that auricular cartilage is curved, relatively thin, and may not be as stiff as septal cartilage can present difficulties when straight grafts are required [43]. However, in cases that require thin, curved pieces of cartilage, auricular cartilage may be desirable [40]. Despite its versatility and ease of harvest, the use of auricular cartilage for grafting is limited due to its size. Alternatively, a cartilaginous or an osseocartilaginous region can be isolated from a rib (typically 5^{th} to the 12^{th} ribs) [44–50]. Costal cartilage is favorably used for grafts that require large or mechanically robust pieces of cartilage for dorsal augmentations [40] or for total nasal reconstructions, such as in cases of rhinectomy due to the presence of cancer [51]. Harvesting costal cartilage requires a small chest incision (1-8 cm, depending upon habitus) and can cause complications such as pneumothorax (collapsed lung; 0.9% of patients) [52], pleural tears and seromas (each 0.6% of patients) [16], breast implant rupture, and infection at the recipient site (2.5% of patients) [16]. Other potential complications include scarring at the donor site and prolonged post-operative pain [52]. The most common difficulty associated with the use of costal cartilage is warping of the graft tissue which can greatly affect the structures of the nose if experienced post-implantation [53]. Costal cartilage graft warping has been observed up to 24 hours after graft isolation [54]. Composite grafts combining septal and auricular cartilage have also been used [55]. While autologous auricular and costal cartilages provide alternative graft sources to alleviate the scarcity of septal cartilage, neither tissue presents an ideal solution to providing the needed graft cartilage for nasal surgery. Limitations, such as graft tissue availability, tissue

form factor, and donor site morbidity still exist. Potential solutions to the limitations associated with the use of autologous graft tissues may be alleviated with allogeneic approaches, particularly allogeneic tissue-engineering approaches that use passaged cells.

In addition to cartilage grafts, non-cartilaginous tissue and composite grafts containing cartilage and non-cartilaginous tissue are also used. Auricular cartilage and adherent skin (chondrocutaneous grafts) [56,57], as well as skin, fascia, or adipose tissue alone [57,58] have been used in rhinoplasty. For simple dorsal augmentation, finely diced cartilage wrapped in autologous fascia [59,60] or congealed in fibrin glue is used as an alternative to monoblock graft augmentation [61]. Surprisingly, diced cartilage has been reported to fuse into a semi-rigid graft over time [62]. Bone, typically isolated from the bony septum (vomer and ethmoid bones), is used as a batten to straighten moderately or severely deviated caudal septa [63,64], or in some cases, to form a rigid strut in total septal reconstruction (extracorporeal septoplasty) [65]. The variety of graft materials currently used in rhinoplasty bring to light the necessity of establishing distinct engineering design criteria for each graft type.

3.3: Allogeneic Grafts

Allografts, also known as homologous grafts, are used in rhinoplasty when sufficient amounts of autologous tissue are not available. An allogeneic approach is advantageous because there is only one surgical site. Cartilage, in general, exhibits low antigenicity due to the isolation of chondrocytes within lacunae [66]. Articular cartilage, for example, is considered to be immunoprivileged, and allografts are frequently used to repair focal defects in the knee [67,68]. However, the immunogenicity of an allogeneic approach in the nose is unstudied. These grafts also have potential to transmit disease, to become infected, and to resorb [41,69–73].

Irradiated homologous costal cartilage (IHCC) grafts or irradiated homologous rib grafts (IHRGs) are the principal allografts used in rhinoplasty. After isolation from the donor, these grafts are gamma-irradiated to kill the resident cells in an effort to eliminate the graft's potential to stimulate an immune response in the recipient. However, it has been suggested that decellularization of tissues, including cartilage, does not remove all antigenic materials [74,75]. While the use of IHRGs is considered safe, the incidence of IHRG resorption (31%) has been reported to be significantly greater than that of autologous costal cartilage (3%) [73,76]. This resorption, however, may be due to the decellularization processing of the graft tissue or the absence of viable cells within the treated graft tissue and subsequent inability to remodel *in vivo*. Additional research should be conducted to determine the factors which may affect its variable success.

Ultimately, it is unclear whether the cartilages of the nose also possess any degree of immunoprivilege akin to articular cartilage. Allogeneic approaches to tissue grafting in rhinoplasty should be further studied because they have the potential to greatly alleviate graft tissue shortage, mismatched tissue form factor, and donor site morbidity associated with autologous grafting. Furthermore, the use of allogeneic grafting motivates allogeneic tissue-engineering strategies to further overcome graft limitations.

3.4: Synthetic Grafts

Synthetic or alloplastic nasal implants are commercially available as an alternative to tissue grafts. The most common implants are composed of silicone, porous high-density polyethylene (MedPor), or expanded polytetrafluoroethylene (Gore-Tex) [77,78]. Synthetic implants are readily available, making multiple procedures unnecessary, have ideal form factor or are easily

carved, and decrease surgical time and costs [77]. Despite these perceived advantages of using synthetic implants, they are frequently associated with complications including inflammation, infection, resorption, dislocation, and extrusion [77,79]. Infection rates associated with silicone, MedPor, and Gore-Tex implants have been reported at 3.9%, 20%, and 5.3%, respectively [77,80]. Silicone implants have an extrusion rate of 2.9% and have also led to capsular contracture in 71% of patients [77,81]. Extrusion has been reported in 12% of patients who received an alloplastic implant [80]. Despite containing diced autologous cartilage, the "Turkish delight" graft has also led to clinical failure because of its use of Surgicel, and is therefore no longer used [59,82]. Regardless of their convenience and availability, the complications and unpredictability associated with synthetic implants for rhinoplasty cause most surgeons in the United States and Europe to shy away from their use. Furthermore, the properties of these synthetic materials have not been quantitatively compared against those of native cartilage, and, therefore, the applicability of these materials and their long-term effectiveness and safety in replacing native tissue has not been shown.

Synthetic fixture materials, such as resorbable polydioxanone (PDS) plates and foil, resorbable and non-resorbable sutures, and Kirschner wires (K-wires) are also used as support materials in rhinoplasty. While many consider PDS plates and synthetic suture materials to be safe, they are also not without complications [83–85]. Some have speculated that the enthusiasm over PDS plates and foil may lead to their "cavalier overuse" and subsequent complications including extrusion, septal cartilage loss, and prolonged postoperative edema and inflammation [86]. PDS sutures are amongst the most commonly used sutures in rhinoplasty and septoplasty, and they are thought to fully dissolve by the time wound healing

has stabilized. However, PDS sutures have also been reported to cause inflammation, hyperemia, and extrusion [85]. K-wires have been used as percutaneous pins to secure grafts, stabilize nasal bones, or form a rigid framework to prevent costal cartilage warping, but are also associated with extrusion and rarely used [59]. The complications associated with synthetic fixture materials are common, further underscoring the risk associated with alloplastic materials in rhinoplasty.

Despite the obvious importance of rhinoplasty and septoplasty procedures to improve patients' quality of life, the success of these procedures is limited by the availability of suitable graft tissue. Autologous, orthotopic sources of cartilage are the most desirable, but also the most limited in quantity. While heterotopic autologous cartilage, allogeneic cartilage, and synthetic materials have been used as alternatives to grafts of septal cartilage, their limitations are significant. Allogeneic sources, such as IHRGs, are more available, however, they are more susceptible to resorption and infection [73,76]. The use of synthetic materials is associated with many complications [83–85]. Ideally, graft tissue would have functional properties that match those of the recipient tissue or be able to withstand external forces in reconstructive circumstances, be biocompatible and bioactive to promote integration and healing, and be readily available without creating donor site morbidity. Graft tissues must also meet the needs of surgeons; for example, they must be easy to carve, shape, form, and secure at the recipient site. Tissue-engineering has the potential to address many of the challenges regarding graft sourcing for rhinoplasty, the morbidity associated with their isolation, and the currently nebulous properties of graft material. However, as evidenced by the wide variety of grafts in use, the quantitative requirements of graft tissue, and thus, the engineering design criteria for

nasal implants are unclear. Tissue-engineering via the engineering design process holds promise to create large amounts of mechanically robust, non-immunogenic, neocartilage in desired shapes and thicknesses from small amounts of heterotopic donor cartilage, but native nasal cartilage properties and quantitative engineering design criteria must first be developed.

4: The Role of Tissue-engineering in Rhinoplasty and Septoplasty

As with any engineering challenge, the engineering design process should be followed to yield suitable solutions. Adapting this approach to cartilage tissue-engineering, the process consists of 1) defining the needs of the users, e.g. surgeons, 2) using native cartilage properties and interpreting user needs to create quantitative engineering criteria, 3) applying tissue-engineering processes to fabricate cartilage, 4) verifying engineered neocartilage against the engineering design criteria, and 5) validating engineered neocartilage against the original needs of the users (Figure 3). Critical review of progress during each step is important to ensure that the engineering criteria, and ultimately the needs of the users, are addressed. It is also important that the criteria are not adjusted to fit potential solutions. Using the engineering design process allows complex tissue-engineering challenges, such as creating engineered septal neocartilage, to be approached methodically and quantitatively, while keeping clinical needs in mind.

4.1: Surgeon Needs

While the ideal parameters of tissue-engineered graft neocartilage vary depending on the surgical indication, flat, mechanically robust cartilage is required in almost all cases. Septal cartilage is considered the ideal material and gold standard for grafting in rhinoplasty because

of its stiffness and straightness. Most grafts used in primary rhinoplasty are less than 25 mm in length, 15 mm in width, and are approximately 1.5 mm thick. Alternative sources of cartilage, such as ear or rib, are used when septal cartilage is not available or sufficient. Therefore, based on current surgical practices, the ideal tissue-engineered graft neocartilage for primary rhinoplasty would have similar thickness and mechanical properties to native septal cartilage. However, in revision rhinoplasty, the sizes and mechanics of grafts needed are more diverse. For example, grafts as long as 40 mm in length are often used. In complex reconstructions, graft cartilage is required to reestablish the entire nasal cartilage framework and is subjected to large forces from wound contracture or non-anatomic tissue transfers, such as a pedicle skin flap from the forehead or cheek. Therefore, tissue-engineered graft neocartilage must have adequate stiffness, perhaps above native values, to withstand these forces. While straight and stiff graft cartilage is universally required, the diversity of graft sizes used motivates the fabrication of large tissue-engineered neocartilage constructs of varying thicknesses so that surgeons may select and customize grafts for each case.

4.2: Nasal Cartilage Engineering Design Criteria

In cartilage tissue-engineering, the goal is almost always to achieve the properties of healthy native cartilage tissue that is to be repaired or replaced, known as biomimicry. Distinct interfaces or differences in properties between implanted neocartilage and native cartilage should not persist after implantation because abrupt changes in properties may lead to mechanical breakdown of the interface and surrounding tissues. Several aspects of native nasal cartilages, such as microstructure, biochemical content, and mechanics, have been studied,

which contribute to the establishment of engineering criteria for biomimetic nasal cartilage tissue-engineering.

4.2.1: Microstructure

Nasal cartilage has superficial and central zones, with the distinction between these zones being more evident in the LLC [87,88]. Chondrocytes in the superficial zone exhibit an elongated fibroblastic morphology and are oriented parallel with the cartilage surface [87]. Cells gradually become larger, more rounded, and less frequent in the central zone [87]. The superficial zone shows more intense collagen staining than the central zone, which is more evident in septal cartilage than LLC [87]. Septal cartilage contains thick sheets of highly organized collagen and exhibits anisotropic collagen arrangement [18]. Collagen fibers in close proximity to the maxillary crest are oriented perpendicularly to the interface, while fibers in the central area of the septum lack a definitive orientation [29]. In contrast, LLC has a looser, less organized arrangement of collagen with a more heterogeneous mixture of fiber thicknesses [18]. Information regarding ULC microstructure is unknown. While the septum and ULC constitute a contiguous piece of cartilage, the microstructural properties may vary and should be studied. Microstructure is reflected in the functionality of these tissues and should therefore also be reflected in the engineering criteria to preserve performance.

4.2.2: Biochemical Content

Hyaline cartilage is characterized by its significant collagen II content. As a hyaline cartilage, septal cartilage expectedly shows abundant immunohistochemical staining of collagen II, particularly in the central zone compared to the superficial zone, and little staining for collagen

I [87]. Septal cartilage also contains small amounts of collagen IX, X, and XI [29,87,89,90]. LLC also shows abundant immunohistochemical staining for collagen II and little staining for collagen I [87]. Identification of collagen types within ULC, as well as examining the presence of minor collagens in all nasal cartilages should be conducted.

Unexpectedly, biochemical characterization of septal cartilage has yielded large ranges of collagen content, glycosaminoglycan (GAG) content, and cellularity, as well as inconsistent GAG-to-collagen ratios (Table 1). Topographical study of the septum indicates that there are no significant variations in GAG content, collagen content, or cellularity across six regions of the septum [91]. This is in contrast to human articular cartilage, also a hyaline cartilage, which is well studied and has a water content ranging from 70 - 80%, 10 - 15% collagen per wet weight, and 3-9% GAG per wet weight [92]. For human articular cartilage, collagen per dry weight ranges from 50 - 75% and GAG per dry weight ranges from 15 - 30% [92]. Information regarding the biochemical content of LLC or ULC is not available and, therefore, should be measured. Care should be taken to test for differences in biochemical contents of nasal cartilage across factors, such as age and sex. Because of the importance of biochemical data to serve as quantitative engineering criteria for nasal cartilage engineering efforts, additional studies should be performed to topographically characterize the biochemical content of LLC and ULC, as well as to rectifying discrepancies in reported values for septal cartilage.

Table 1: Summary of human septal biochemical properties. Hydration, cell density per wet weight (WW), glycosaminoglycan (GAG) content per WW and dry weight (DW), and collagen

(Col) content per WW and dry weight DW and are listed. GAG:Col ratios were calculated based on available data.

Hydration (%)	Cells/WW (E6/g)	GAG/WW (%)	GAG/DW (%)	Col/WW (%)	Col/DW (%)	GAG:Col
77.65 [93]	27.75	2.91	13.02 (calc)	8.72	39.02 (calc)	0.33 (calc)
n/a [94]	n/a	4.3	n/a	1.99	n/a	2.16 (calc)
n/a [91]	24.9	1.71	n/a	7.39	n/a	0.23 (calc)

4.2.3: Mechanical Properties

In terms of mechanical properties, the L-strut portion of the septum has been the most extensively characterized. The L-strut, named in reference to its shape, is the remaining intact region of the septum after submucous resection during septoplasty or graft harvesting (Figure 1). To avoid nasal collapse, it is widely accepted that the minimum widths of the caudal and dorsal arms of the L-strut need to be 10 mm [95]. However, it has been shown that thickness of the L-strut has a greater impact on L-strut yield strength [25,95]. Despite its importance, thickness is rarely taken into account in L-strut mechanical strength modeling due to the complexity of accounting for both dimensions during rhinoplasty. When modeled as separate dorsal and caudal cantilevered beams, the compressive Young's modulus of the overall L-strut ranges from 0.38 to 5.91 MPa [25]. In contrast to the understanding of L-strut mechanics, inadequate characterization of the material properties of nasal cartilages has been performed. The compressive elastic modulus values of the septum, ULC, and LLC are 2.72 ± 0.62, 0.98 ± 0.29, and 2.09 ± 0.81 MPa, respectively [96]. Compressive stiffness of the septum ranges from 0.41 ± 0.21 to 19.30 ± 6.80 MPa (at 50% strain/min) [97]. The tensile equilibrium modulus, dynamic modulus, and strength of human septum was 3.01 ± 0.39 MPa, 4.99 ± 0.49 MPa, 1.90 ±

0.24 MPa, respectively [98]. Importantly, the tensile failure strain of septal cartilage was reported to be 35%. This exceeds the mathematical assumptions of infinitesimal deformation and suggests that septal cartilage exhibits hyperelastic behavior. The flexure modulus based on three-point bending tests of septal cartilage is 1.97 ± 1.25 MPa [99]. The variety of published research results and inconsistency in format of presentation suggests the need to establish standardized testing methods for nasal cartilage.

Nasal cartilage is predominantly modeled as a homogeneous, elastic material, but the mechanical testing methods for this tissue are not standardized. In contrast, modeling of both native and engineered articular neocartilage using mixture theories and gathering mechanical data using creep indentation or stress-relaxation are widely accepted [92]. Because both nasal and articular cartilages are hyaline with similar biochemical compositions, and because native-like hyaline cartilages have been engineered by numerous groups, albeit not for nasal applications, it would be instructive to apply viscoelastic or biphasic theories to nasal cartilages. Due to its wide applicability, creep indentation testing should be used. Nasal cartilages experience tensile forces, for example under flexure, thus, measuring tensile properties is necessary to yield another quantitative means by which to compare engineered neocartilage to native cartilage. Care should be taken to test for differences in material properties of nasal cartilages across parameters, such as age, sex, and ethnicity. Due to the hyaline nature of nasal cartilages, their material properties should be obtained using testing methods common to other hyaline cartilages so that they may serve as engineering design criteria for nasal cartilage engineering efforts.

4.3: Nasal Cartilage Tissue-engineering

In many ways, nasal cartilage engineering efforts are similar to the strategies applied to engineer other cartilages in that the goal is to produce biomimetic cartilage to repair, replace, or regenerate damaged native cartilage (Figure 4). The traditional tissue-engineering paradigm, consists of cells, scaffolds, and stimuli, is frequently inspired by native tissue development and maturation. Within recent years, a new paradigm has emerged in cartilage tissue-engineering, stating that in many cases, the only components required to form functional neocartilage are cells and stimuli. Stimuli, such as mechanical forces or growth factors, may be applied to improve neocartilage functional properties and organization [100]. It should be noted that both nasal and articular cartilages are hyaline and result from endochondral ossification [101]. Because of the developmental and tissue-level similarities of nasal cartilage and articular cartilage, tissue-engineering approaches should be shared between these fields.

Nasal chondrocytes from ovine, rabbit, bovine, and human sources have been investigated for nasal cartilage engineering [102–104]. While cartilage engineering efforts typically use animal sources of cells because of availability and cost, human cells have been, surprisingly, the most frequently investigated for nasal cartilage engineering in recent literature. This usage is likely due to the availability of septal remnants from rhinoplasty surgeries. Low-passage (up to P3) human nasal septal chondrocytes have shown the capability to produce neocartilage containing GAG and type II collagen [89,90,99,105–107]. It has also been suggested that the superficial zone of septal cartilage contains a promising population of nasoseptal progenitor cells (NSPs) [108]. These cells are migratory and express surface markers, such as CD29, CD105, CD106, CD90, and CCD44, suggesting a state of differentiation between

mesenchymal stem cells (MSCs) and chondrocytes [109]. NSPs have been shown to differentiate to chondrogenic and osteogenic, but not adipogenic lineages [88] and show a greater proliferation potential than bone marrow- and adipose-derived MSCs [110]. The surface markers and differentiation potential of NSPs have been reported to remain unchanged after 10 passages [108]. The use of both septal chondrocytes and NSPs are limited greatly by donor site morbidity and tissue availability. Alternatively, costal cartilage may serve as an abundant source of chondrocytes whose isolation does not create further pathology or weakness in the cartilage structures of the nose. Passaged costal chondrocytes have shown the ability to form neocartilage which is capable of remodeling *in vivo* to promote healing [111]. While costal cartilage is used clinically as graft material, it often warps when cut into grafts. However, when costal cartilage is used as a cell source, particularly for an allogeneic approach, issues of warping and donor site morbidity are eliminated. Costal cartilage represents a promising and unexplored source of cells for nasal cartilage engineering, the use of which overcomes current limitations with cell sourcing.

Regarding the use of scaffolds, nasal cartilage engineering strategies again bear many similarities to those used for articular cartilage. The use of scaffolds, such as type I and III collagens, polycaprolactone, polylactic-co-glycolic acid (PLGA), and decellularized native cartilage have been explored [112–116]. However, similar to their prominence in articular cartilage engineering, scaffold-free techniques are the most commonly used tissue formation strategy for nasal cartilage. Passaged chondrocytes frequently undergo culture in alginate, dissociation, and seeding into Transwell plates to form neocartilage [72,90,99,106,107,117]. Scaffold-free nasal neocartilage engineered from human septal chondrocytes has been

reported to exhibit different properties depending on the study. For example, it has been reported to exhibit a flexure stiffness of 0.014 N/mm [99], a flexure modulus of 0.32 MPa [99], a compressive modulus of 5.6 kPa [106], an equilibrium modulus of 0.2 MPa [107], a tensile strength of 0.27 MPa [117], GAG per wet weight content of 1.07% [107], and collagen per DNA content of 273 μg/μg [117]. Other techniques, such as pellet cultures and multilayered cell sheets are also used [88,118].

The choice of stimulus is equally as important as the cell type used. Culture supplementation with GDF-5 and IGF-I, as well as bFGF and TGF-β2 was shown to increase the histological staining intensity of GAG and type II collagen content in scaffold-free human nasal chondrocyte-derived neocartilage [89,106,117]. The use of IGF-I and GDF-5 increased the thickness of engineered neocartilage 12-fold over the untreated control [106] in scaffold-free nasal neocartilage engineered from human septal chondrocytes. Other stimulation regimens which increase the content of desirable matrix components and mechanical properties include the use of TGF-β3, BMP-14, platelet rich plasma, and subcutaneous implantation [90,114,115,118,119]. Culture of multilayered P1 human septal chondrocytes in a rotating bioreactor increased construct cellularity and GAG per wet weight content by 200%, as well as bulk modulus by 32.5-fold and elastic modulus by 22-fold of engineered constructs compared to static culture controls [105]. Notably, the bulk and elastic moduli reached 65% and 66% of those of native, human, pediatric septal cartilage [105]. Continuous flow bioreactors and other rotary bioreactors have also been used [89,90]. It would be instructive to examine other culture conditions and stimuli which have been successful in articular cartilage engineering, such as the

self-assembling process and the application lysyl oxidase, hydrostatic pressure, and tensile stimulation.

4.4: Verification of Engineered Nasal Cartilages

Quantitatively verifying the properties of engineered neocartilage against the engineering design criteria is crucial. The same quantitative assays used to characterize native cartilage must be used to evaluate engineered neocartilage and should be normalized in the same way to facilitate direct comparison of engineered to native cartilages. Common biochemical and mechanical assays used to evaluate cartilage include the dimethyl methylene blue assay for GAG content, chloramine-T hydroxyproline assay for collagen content, dye-binding assays for DNA content, creep indentation for compressive modulus, and uniaxial tension for tensile modulus [92]. In addition to using quantitative assays which are standard to the evaluation of both engineered and native cartilages, a functionality index (FI) may be used to yield a single quantitative measure of the overall quality of engineered neocartilage. The FI [120–122] is a powerful method to determine if, and to what degree, biomimicry and the engineering criteria have been satisfied (Eq 1). The FI is a value between 0 and 1 that represents the average difference between engineered (eng) and native cartilages (nat). An FI of 0 equates to no similarity between the tissues, and 1 indicates complete biomimicry. The basic FI accounts for GAG content (GAG), collagen content (Col), compressive aggregate modulus (E^C), and tensile modulus (E^T). However, parameters, such as flexure modulus, anisotropy, or geometry, may be included, excluded, or weighted differently to customize the FI and account for unique requirements and characteristics of different tissues or to reflect user needs [121]. By using standardized and quantitative procedures to evaluate the properties of both native nasal

cartilage and engineered neocartilage, direct comparisons may be made to verify the engineered neocartilage meets the engineering criteria set forth.

Equation 1 [120]

$$FI(eng|nat) = \frac{1}{4}\left[\begin{array}{c}\left(1 - \left|\frac{GAG_{nat} - GAG_{eng}}{GAG_{nat}}\right|\right) + \left(1 - \left|\frac{Col_{nat} - Col_{eng}}{Col_{eng}}\right|\right) \\ + \left(1 - \left|\frac{E^C_{nat} - E^C_{eng}}{E^C_{nat}}\right|\right) + \left(1 - \left|\frac{E^T_{nat} - E^T_{eng}}{E^T_{nat}}\right|\right)\end{array}\right]$$

While the goal of engineering replacement cartilage is to achieve the functional properties of healthy native cartilage, complete biomimicry may not be necessary. For example, engineered temporomandibular joint (TMJ) disc implants with an FI of 0.42, or 42% of the native tissue's biochemical and mechanical properties, successfully resulted in healing of a native TMJ discs in an *in vivo* mini-pig model [111]. These results suggest that replacement tissues may not need to completely recapitulate the properties of native tissue to elicit regeneration. The degree of biomimicry necessary is likely dependent on the native mechanical loading environment, tissue geometry, and manner in which the tissue is used, e.g., as a replacement, mechanical strut, or filler with minimal loading requirements. While parameters such as mechanical loading are often reflected in the mechanical properties of the native tissue, and are, thus, accounted for in the FI, the engineering criteria should be refined to include other factors, such as role of the implanted tissue, to determine what degree of biomimicry is necessary.

4.5: Validation of Engineered Nasal Cartilages

Engineered tissues need to be orthotopically validated in animal models to determine if the needs of the users are met. Several animal models are used in rhinoplasty and nose cartilage tissue-engineering studies. Rabbit models are frequently used due to their affordability and availability, and surgical procedures for rabbits are well-defined [123,124]. However, leporine nasal cartilage is significantly less cellular and has different shapes and thicknesses than human cartilage [125]. Unlike human cartilage, rabbit hyaline cartilage also has the ability to regenerate hyaline cartilage post-injury or resection [126,127]. Rabbit nasal septal cartilage is also completely covered by nasal bones which must be removed for. The presence of these large nasal bones renders surgery time-consuming and labor-intensive [123].

Porcine models have been investigated *in vitro* due to the availability of porcine septum at a low cost [125]. While the porcine model shows promise in terms of the dimensions of the LLC [128], its septum exhibits different cellularity and form factor than the human septum [93,125]. Additionally, the porcine septum, like the rabbit, is protected by large thick nasal bones which may impede surgical access [125]. Alternatively, the ovine model has been suggested as a large animal model due to its more human-like form factor and for the comparative ease of surgical accessibility of the nasal cartilages [129]. Additionally, the ovine model is an FDA-recommended large animal model to generate pre-clinical data for knee articular cartilage [130]. Therefore, using an ovine animal model may accelerate the clinical translation of engineered nasal neocartilages. While promising, the ovine model is not well-studied and requires full characterization of structural, biochemical, and mechanical properties.

A standardized animal model must be established to design consistent and informative *in vivo* studies. The variety of deformities requiring rhinoplasty in humans affects all cartilage structures of the nose. Therefore, the selected animal model would ideally have equivalent nasal structures for potential treatment. Or perhaps, different animal models may need to be established for each cartilage structure. The ability of animal studies to validate engineered nasal neocartilage for translation into human use greatly depends on the ability to extrapolate data from the animal model to the human. Therefore, it is of utmost importance to select an animal model that most closely resembles the human in terms of nasal cartilage structure, function, and properties.

5: Perspectives and Future Directions

This review has provided strong motivation for the role of cartilage tissue-engineering in nasal surgery, the engineering design process toward achieving biomimetic nasal cartilages, as well as a discussion of the current knowledge regarding nasal cartilages, what remains to be studied, and suggestions for future work. Following the engineering design process, further fundamental research must be performed to quantitatively characterize human nasal cartilages and fully define engineering design criteria for tissue-engineering. A wide range of pathologies affecting different cartilage structures occur in patients from diverse ages and ethnic backgrounds. Topographical examinations of the nasal cartilages and studies with respect to factors like gender, age, and ethnicity should be performed toward tissue-engineering biomimetic cartilages to match the diversity of rhinoplasty patients and procedures. It is crucial these studies be performed via quantitative methods best suited for cartilage and the mechanical

forces the nose experiences. For example, biphasic theory and the principles of hyperelasticity should be applied to model the mechanics of nasal cartilage. Bending and buckling mechanics of nasal cartilages should also be studied because these cartilages experience these forces. The complete and quantitative characterization of nasal cartilages is crucial to lay the groundwork and set the objectives for cartilage tissue-engineering.

The great potential to overcome many of the limitations of grafting in reconstructive, functional, and aesthetic nasal surgery exists with an allogeneic engineering approach. Therefore, the degree of antigenicity of allografts and the potential for immunoprivilege must be well-researched. The purpose of irradiating allogeneic rib cartilage is to kill the resident chondrocytes in an effort to reduce the immune potential of the grafts. However, there is evidence that, despite cell death, antigen-containing cell remnants remain and are difficult to remove due to the density of the cartilaginous ECM [74,75]. While chondrocytes express major histocompatibility antigens (MHCs) types I and II which have the potential to trigger an immune response, chondrocytes reside in lacunae where they do not easily encounter immune cells. It has also been shown that antigens associated with the extracellular matrix may also provoke an immune response [74]. Despite the possible remaining antigens in IHRGs, these grafts are commonly used and considered to be well-tolerated. This strongly motivates the investigation of an allogeneic approach for tissue-engineered neocartilage.

Toward fabricating mechanically robust neocartilage to be used as nasal cartilage grafts, functional tissue engineering must be applied. A scaffold-free tissue-engineering approach will provide easily accessible, abundant, and robust cartilage, eliminating the need for uncharacterized grafts with known complications. The self-assembling process, innovatively,

using costal chondrocytes, has successfully generated neocartilage with functional properties on par with native cartilage. Albeit the application of self-assembling cartilage has been for articular, cartilage, knee meniscus, and temporomandibular joint disc applications, its success in engineering cartilages of multiple types motivates its investigation for nasal cartilage tissue-engineering. Additionally, it would be instructive incorporate use of bioactive agents and biomechanical stimuli known to enhance functional properties and matrix organization because nasal cartilages are structures with mechanical roles. Chemical and bioactive stimuli, such as chondroitinase-ABC and lysyl oxidase-like 2, or biomechanical stimuli, such as direct compression, continuous tension (CoTense) [131], and fluid flow induced shear should be investigated. The self-assembling process and stimuli may also be used to engineer large, off-the-shelf grafts and implants to replace the need for synthetic implants, potentially reducing the complexity of or eliminate difficult surgical maneuvers, leading to more consistent and better outcomes [132]. Furthermore, these methods may be integrated with osteochondral strategies [133] to form large implants and replacement tissues to treat large trauma or pathologies.

While the goal of tissue-engineering is to create biomimetic tissues, recent work has suggested that complete biomimicry may not be necessary [111]. The degree of biomimicry required for implanted cartilage likely depends on its location, mechanical role, and the graft function within the larger tissue. For example, some grafts in nasal surgery have no load bearing function and function as spacers to expand the airway or onlays to change soft tissue contour. Immature engineered neocartilage with a lower FI may be more amenable to integration into native cartilage. In contrast, mature neocartilage with a greater FI can

withstand mechanical loading upon implantation but may not be amenable to integration. Recently, it was shown that neocartilage with and FI of 0.42 implanted into a partial thickness defect in a minipig temporomandibular joint disc elicited regeneration and halted osteoarthritic changes compared to empty defect controls [111]. In this case, complete biomimicry was not required for healing. There is likely a balance between neocartilage maturity and bioactivity that must be found to implant neotissues that are mechanically robust enough to immediately handle loading but also maintain their ability to integrate and regenerate injuries. This balance, and subsequent target FI also likely depends on the target tissue. Therefore, additional work must be performed to determine the necessary degree of biomimicry, and thus, the engineering criteria for engineered nasal cartilage grafts.

Achieving a high degree of customization in tissue-engineered neocartilage grafts may be desirable. Bespoke fabricated grafts with identical or improved form factor to a patient's native structures would greatly improve reconstruction efforts, especially with over-resected LLC, for example. Additionally, in patients with native cartilage failure, custom shaped grafts with enhanced stiffness would be valuable to correct airway patency. While large, customized replacement cartilage is greatly needed for patients with autoimmune diseases which affect nasal cartilage and the surrounding tissues, these diseases will affect the performance of tissue-engineered grafts. For example, relapsing polychondritis is a rare and potentially fatal autoimmune disease resulting in the destruction of nasal cartilage, amongst other tissues [134]. Therefore, tissue-engineered grafts which can withstand heightened immune environments or those with immunomodulatory properties should be considered in the future.

Steps necessary to facilitate the translation of engineered neocartilage to clinical products for rhinoplasty include the standardization of an animal model and creation of FDA guidance documents. The standardization of animal models for nasal reconstructions is crucial so that surgical techniques and outcomes can be compared across studies. The animal model selected should closely mimic human anatomy and accommodate the range of pathologies and treatments seen in human patients, or different animal models best suited to each nasal structure or pathology should be identified. Currently, no FDA guidance documents exist for nasal cartilage. Guidance documents provide investigators with recommendations for information that should be submitted to the FDA when submitting Investigational Device Exemption (IDE) or Investigational New Drug (IND) applications, such as mechanical properties of engineered neocartilage and animal toxicology, as well as make recommend large animal models for *in vivo* studies. The FDA recommends recommend the use of sheep, goats, and horses, for *in vivo* repair studies for replacement knee cartilage. Therefore, these models may be used as a starting point to determine suitable, FDA-accepted animal models for nasal cartilage repair.

Finally, collaborations across disciplines and between surgeons and researchers are crucial to fully understanding surgeon needs, and thus, developing appropriate engineering design criteria for nasal neocartilage. It is important for rhinoplasty surgeons to publish their approaches, challenges, and successes in peer-reviewed, interdisciplinary journals to inform engineers. Additionally, attending conferences or mini-courses in rhinoplasty is a key way for engineers to learn first-hand and create collaborations. Interdisciplinary communication and collaboration is the foundational key to creating engineered nasal neocartilages that overcome

the limitations of current cartilage grafting strategies and that provide long-term, easy-to-implement solutions for patients.

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Graphical Abstract

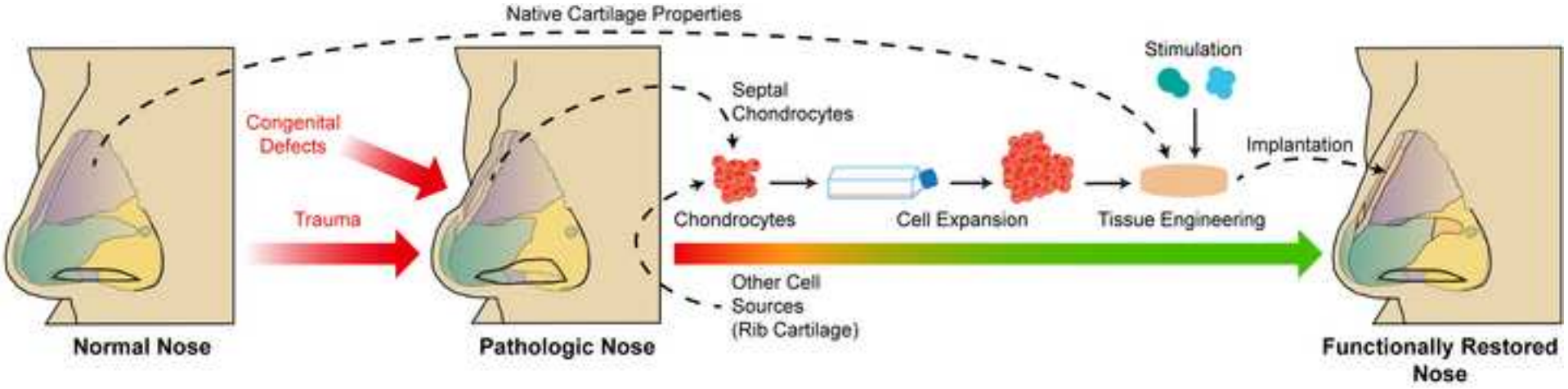

Figure 1

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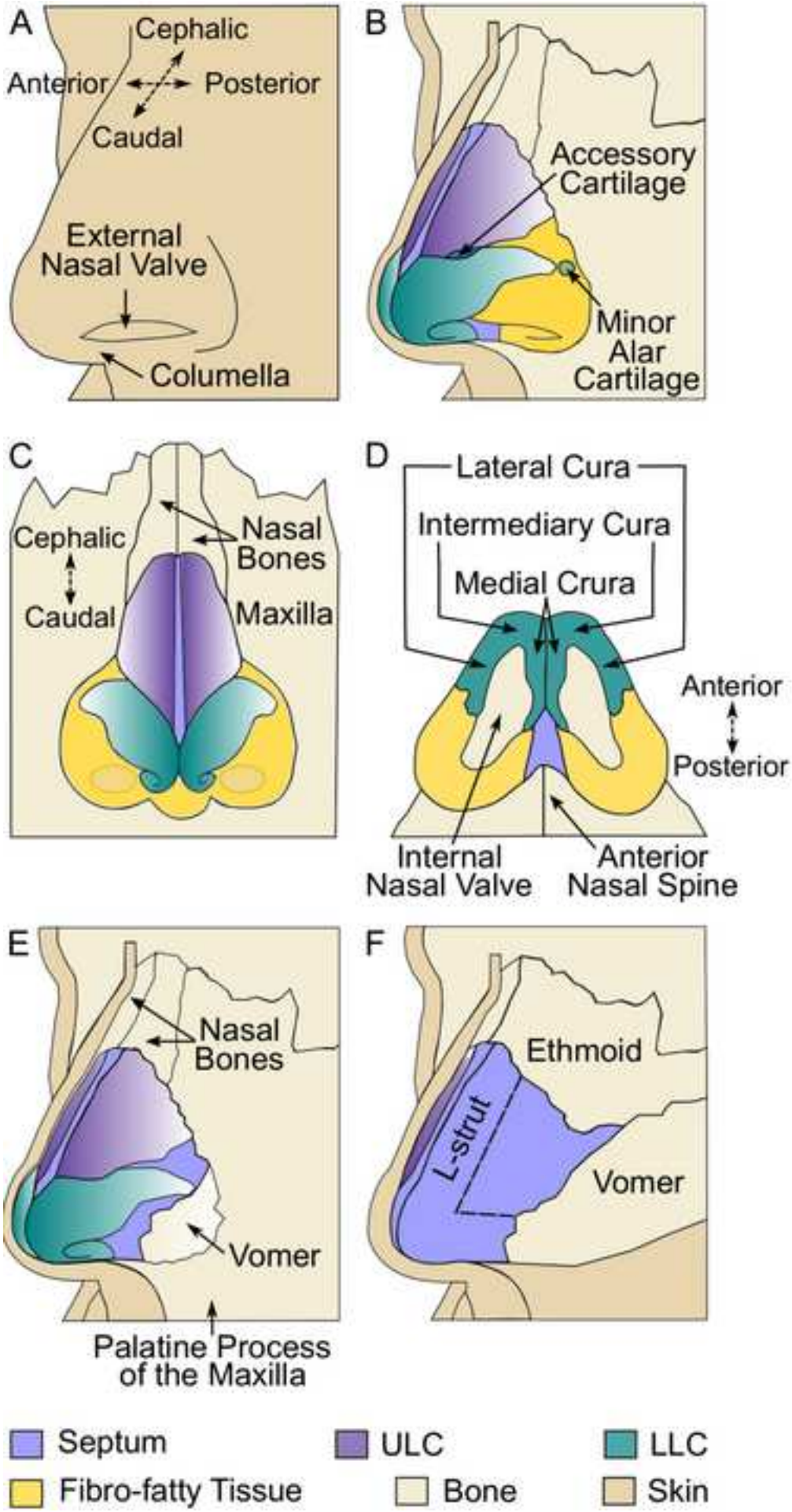

Figure 2

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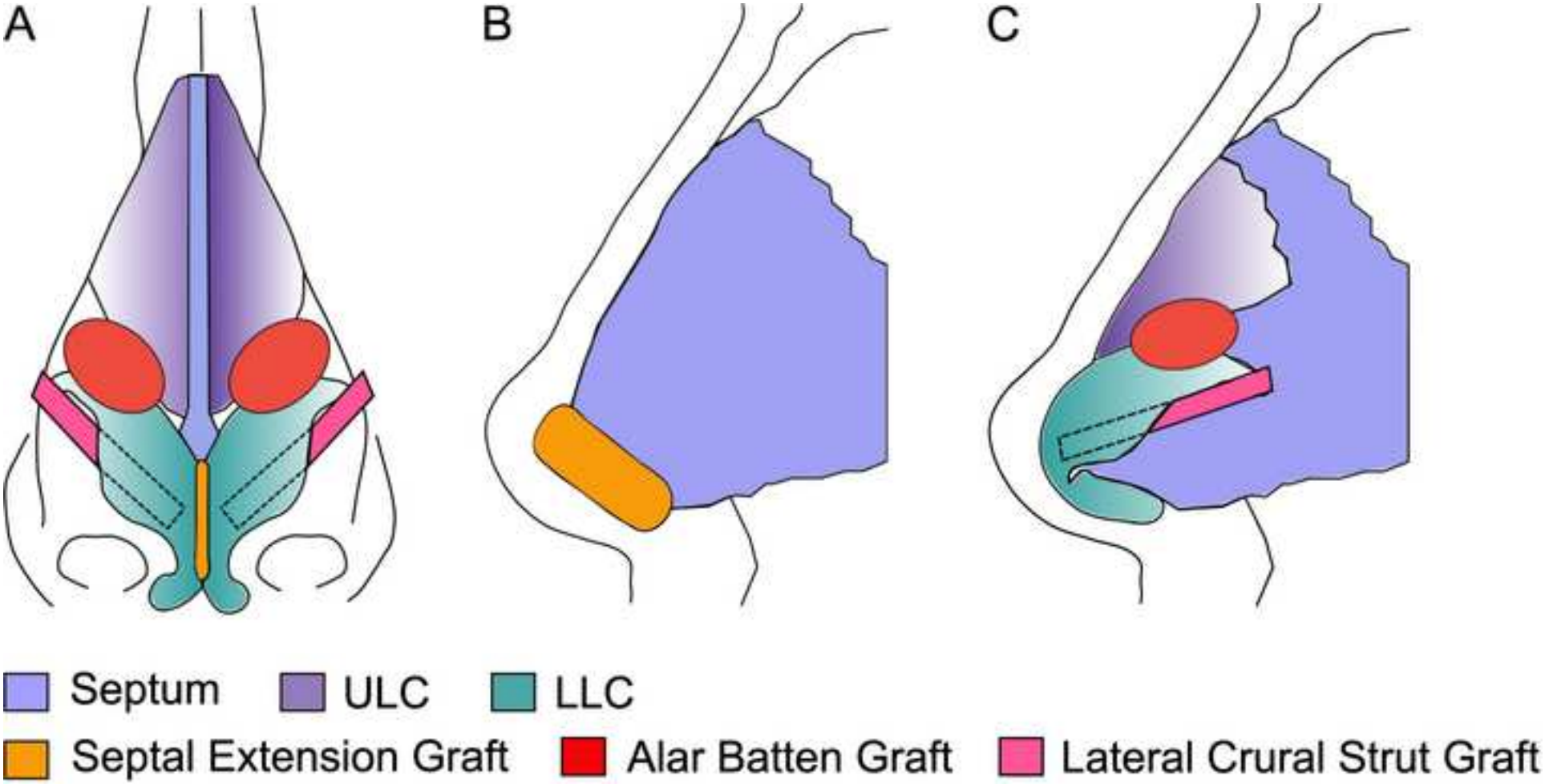

Figure 3

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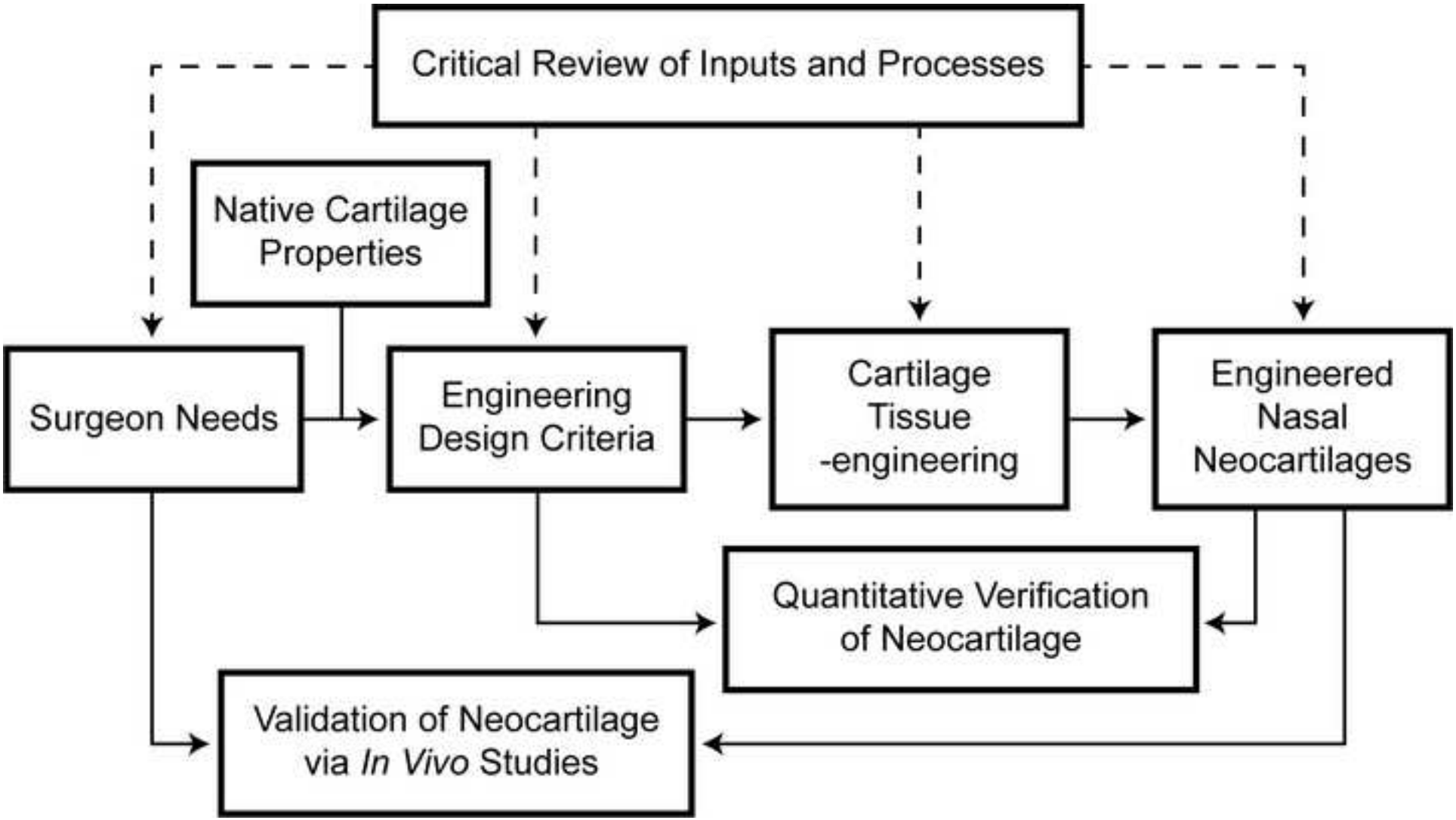

Figure 4

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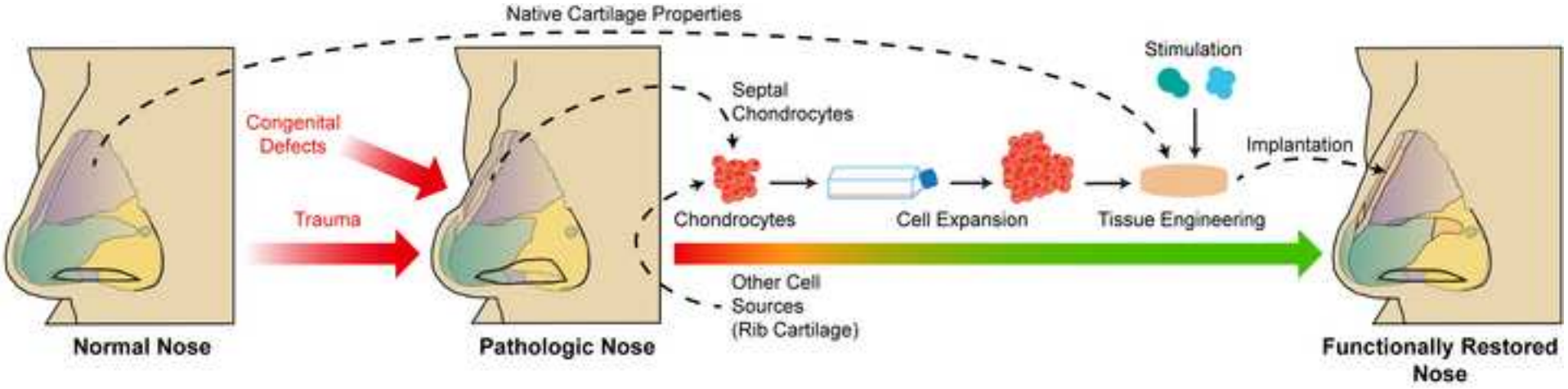

Figure/Table Caption (s)

Figure 1: Anatomy of human nasal cartilages and surrounding tissues. A) Angled frontal view of the intact nose, B) angled frontal view with the skin removed to show the septum, upper lateral cartilage (ULC), lower lateral cartilage (LLC), minor alar cartilage, accessory cartilage, fibro-fatty tissue, and facial bones, C) frontal view with the skin removed to show the septum, ULC, LLC, fibro-fatty tissue, and facial bones, D) worm's-eye view of the septum and LLC, E) angled frontal view with the skin and fibro-fatty tissue removed to show the septum, ULC, LLC and facial bones, F) angled frontal view of only the septal cartilage and bone-cartilage interfaces.

Figure 2: Cartilage grafts commonly used in rhinoplasty procedures. A) Frontal view of the nose showing the anatomic placement of a septal extension graft, alar batten grafts, and lateral crural strut grafts, B) side view showing the anatomic placement of a septal extension graft with respect to the septum, C) side view showing the anatomic placement of an alar batten graft and lateral crural strut graft with respect to the upper lateral cartilage (ULC) and lower lateral cartilage (LLC).

Figure 3: The engineering design process toward creating functional engineered cartilage. The first step of this process is to define the needs of the users, i.e., the rhinoplasty/septoplasty surgeons. The second step is to translate those needs to quantitative requirements, and incorporate native cartilage functional properties, such as structural anisotropy, biochemical contents, and mechanical properties to create engineering design criteria. In the third step, cartilage engineering processes should be applied with the objective of achieving the engineering design criteria. In step four, engineered neocartilage tissue should be quantitatively verified against the engineering design criteria. Once the engineering criteria are satisfied, the engineered neocartilage should be validated to ensure it addresses the needs of the users using relevant animal models (step 5). Throughout this system, critical review of inputs and processes must be performed.

Figure 4: Overview of nasal tissue-engineering. Chondrocytes sourced from the nasal septum or rib cartilage may be expanded and used to fabricate engineered neocartilage. Engineered neocartilage may then be implanted into a patient to correct nasal cartilage pathologies and restore functionality. To strive for biomimicry, the properties of native nasal cartilages should be used as gold standards when applying engineering strategies.