J Oral Res
research in oral sciences

Perspective

nanoBONE: re-visiting Osseo-Reconstruction and -Repair … with a nanoTwist.

nanoBONE: revisando la reconstrucción y reparación ósea… con un nanoGiro.

Ziyad S. Haidar.[1-4*]

Affiliations:

[1]BioMAT'X, Universidad de los Andes, Santiago, Chile.

[2]Centro de Investigación e Innovación Biomédica (CiiB), Universidad de los Andes, Santiago, Chile.

[3]Programa de Doctorado en BioMedicina, Facultad de Medicina, Universidad de los Andes, Santiago, Chile.

[4]Facultad de Odontología, Universidad de los Andes, Santiago, Chile.

Corresponding author: Ziyad S. Haidar. Department for Research, Development and Innovation, Universidad de los Andes, Avenida Monseñor Álvaro del Portillo 12.455, Las Condes, Santiago, Chile. **Phone:** (56-2) 26181372. **E-mail:** zhaidar@uandes.cl

Cite as:

Haidar ZS.

nanoBONE: re-visiting Osseo-Reconstruction and -Repair … with a nanoTwist.

J Oral Res 2021; 10(4):1-6.

Doi:10.17126/joralres.2021.047

As we commenced a new decade, amidst the challenging COVID-19 pandemic, dentists, in general and oral and cranio-maxillo-facial surgeons, in particular, are invited to critically exploit a multi-disciplinary mentality and approach to our rapidly evolving field via opening-up to biologists, chemists, material scientists, mathematicians, pharmaceutical scientists, tissue bio-engineers and new surgical device designers and innovators, as well as to technology transfer offices. Recent developments in the application of nanotechnology have opened doors wide for unprecedented advances in the fields of biomaterials, drug delivery, tissue engineering and regenerative medicine.

Today, besides techniques and methods, such innovations are also rapidly translated from laboratory bench-tops to clinical chair-sides, and to operating rooms. Indeed, nanotechnology, at the fundamental level, allows or facilitates the "controlled" manipulation of distinct atoms and molecules for the design and production of novel structures and materials with unique, improved and highly-desirable physico-chemical, biological and mechanical characteristics and properties. Release-controlled stem cells or growth factor-loaded nano-capsules incorporated into distinct platelet concentrates, to serve as bio-active, localized and accelerated soft tissue regenerative and osseo-augmentative multi-dimensional matrices is perhaps a fine example of an innovative, combinatorial and multi-disciplinary technology. Hence, to embark on such a fast-moving and evolving field train, we should harness inter-/intra-disciplinary communication and partnership via an understanding of cell-material interactions, signaling pathways and the potential role/benefit of "smart" nano-biomaterials in designing, formulating, optimizing and translating modern, user-friendly, controlled, safe, effective, efficacious and superiorly predictable clinical tools and outcomes, for an overall improved quality of life of our patients.

The goal of mandibular reconstruction is to restore mechanical stability, facial form and function; often necessary for repairing defects due to trauma, inflammatory disease, or post-oncologic resection of benign or malignant tumors. Hence, methods of reconstruction and repair imply the restoration of mandibular continuity and muscle attachments (with precise bone replacement, and osteointegration at the contact points between the native and reconstructed mandible).[1,2] Indeed, mastication, speech and facial aesthetics are often severely compromised without reconstruction.

Thereby, surgeons are trained to determine, design and execute methods of mandibular reconstruction based on the location and size of the mandibular defect. Further, we need to consider adequate room for oro-dental implant positioning and insertion, essential for the prosthetic rehabilitation of joint articulation and dental occlusion, whilst restoring the function of the inferior alveolar nerve to assure our patients an adequate sensitivity of their lips and facial skin.[1-3] Hence, this also implies attention to the biological component and action besides the mechanical restoration.[2-5]

While mandibular reconstruction principles, strategies, techniques and tools have intensely advanced over the years, refinements and new biomaterials continue to improve techniques, outcomes (healing, esthetics and function) and overall patient quality of life.[2-6] The use of computer-aided design/computer-aided manufacturing (CAD/CAM), when compared to traditional free-hand techniques, results in improved function, morphology, and precise accuracy regarding bone replacement for complex segmental mandibular reconstruction. Bone is one of the organs with the most spontaneous regenerative capabilities in the human body. Principally, a dynamic connective tissue formed as a direct result of the self assembly and mineralization of the extracellular matrix produced primarily by bone-forming cells or osteoblasts, in a remarkable orchestra-like interaction with two other cell types (at least to-date); osteocytes and osteoclasts maintaining and resorbing bone, respectively.[3,4] Yet, it continues to be the second most transplanted tissue after blood with millions of bone grafts performed annually. Indeed, an osseous defect can cause serious functional abnormalities and aesthetic deformities - thereby impacting overall quality of life, as mentioned earlier. Such substantial, severe and complex defects may result from congenital pathologies or abnormalities, ablative surgery or traumatic avulsion, to name a few (Figure 1).

Luckily, small bone defects tend to heal efficiently via the afore-mentioned physiological regenerative processes.[1,2,4] On the other hand, healing of bone fractures and reconstruction of critical-sized bone defects continue to present a significant challenge for orthopedists, traumatologists and maxillofacial surgeons, alike, as well as their patients and care-givers. Indeed, the reconstruction of critical-sized defects and defects ≥6 cm, where the fibula-free flap approach allows the possibility of using vascularized bone with or without skin for restoring the defect, in terms of form, innate biological activity and thereby function.[3,8,9]

Figure 1. Mandibular Osseous-Defects. **(A, C):** CT-volumetric rendering scan of a cystic lesion/cortical defect in the body of the mandible displayed in axial and coronal views. **(D):** Panoramic radiograph of an ameloblastoma in the right mandibular body. **(E,F):** Representative/Illustrative Histological Assessment of Bone Regeneration by a nano-biomaterial (nano-based polymeric drug delivery system) in a Rat Mandibular Defect Model. Micrograph. **(E):** control defect at week 1. **(F):** treated defect with localized nanoparticle (●) at week 3; **CT:** connective tissue. **HB:** Host Bone. **NB:** Newly-formed bone. **H&E:** Staining, magnification=10X.

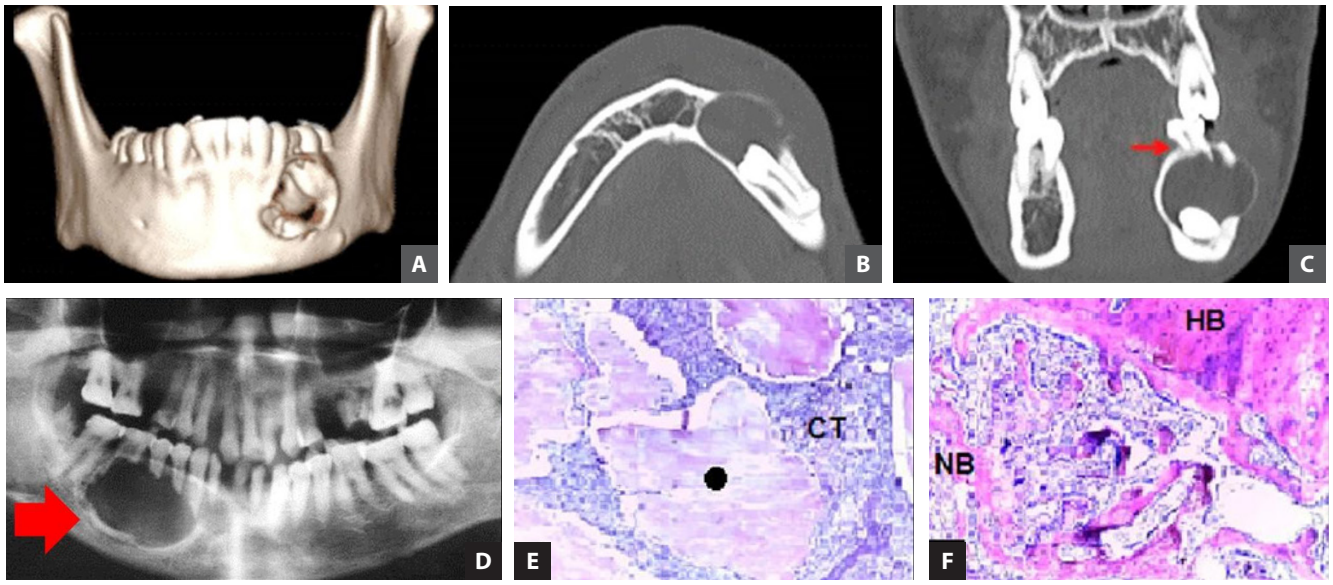


Figure 2. (A): Bone tissue engineering approaches according to complexity/difficulty of osseous defects. **(B):** The diamond ◊ concept (introducing stability to the earlier Δ Triad) for osseo regeneration leading to a functional 'engineered' vascularized tissue equivalent (ideally, indistinguishable from the native bone).

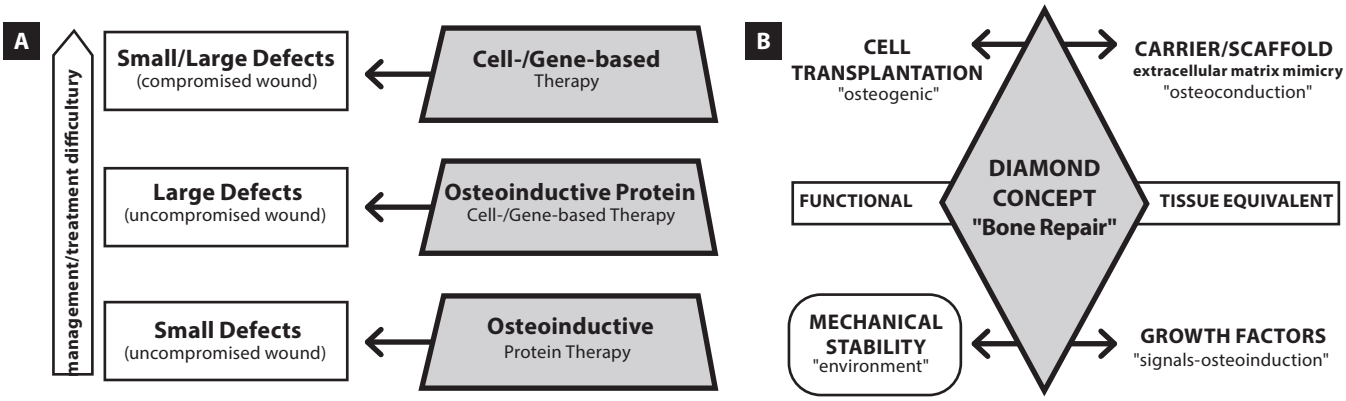
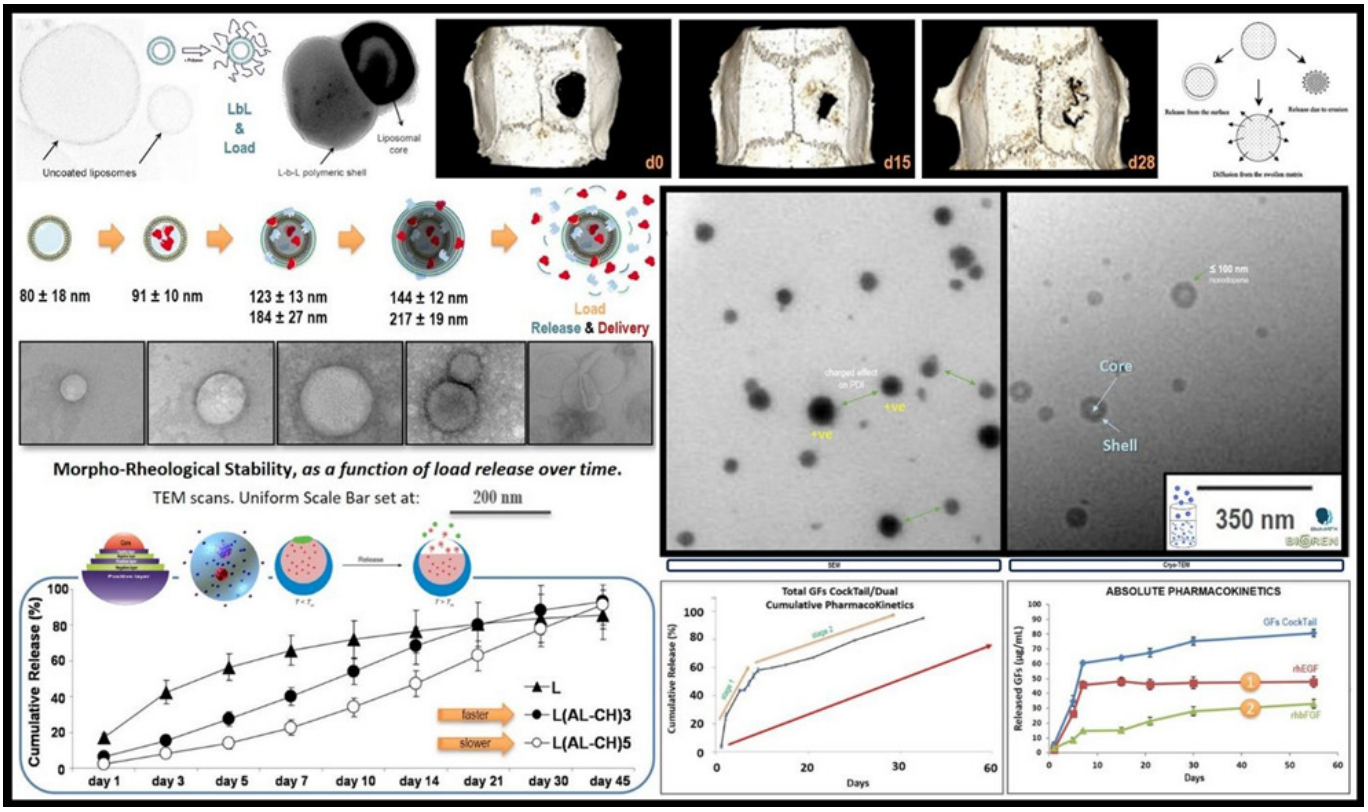


Figure 3. Illustration of our injectable and locally-acting/localized pharmaco-kinetic (load/drug release-controlled) nanocapsules suitable for use in bone regeneration and repair indications, and beyond. Load or drug(s) release is controlled via encapsulation within the multi-compartments created in the polymeric shell (formulated layer-by-layer via stepwise adsorption) surrounding a lipid-base core (uni-/multilamellar liposomes or solid lipid nanoparticles). Nano-particles/-capsules loaded with rhBMPs, rhEGF and rhbFGF/rhGF-2. Controlled and/or timed release of pharmaco-kinetics (single vs dual, bolus vs gradual or ordered, and slow vs rapid) modulated via diffusion and erosion, sustained (continuous release) over a prolonged duration up to 60 days (pre-plateau). Localized qualitative and quantitative de novo osseo-regeneration demonstrated and evidently accelerated (evaluated in a murine cranial critical-sized defect model, over a 28-days period, monitored by µCT scans and bone densitometry for bone-fill, by volume). The BioMAT'X pharmaceutical delivery platform can be customized, personalized and fine-tuned for a range of loads (cells, drugs, growth factors, metals, therapeutic agents) and translational clinical applications.



ISSN Print 0719-2460 - **ISSN Online** 0719-2479.

For reconstruction and osseo regeneration to yield proper healing, mechanical stability in the defect site, osteogenic cells, and osteoinductive growth factors in combination with a suitable carrier or delivery system, conceptualized over a decade ago as the "Diamond Concept" are undeniably necessary.[2-4,6,8,9](Figure 2)

Yet, the long-standing autologous bone grafts continue to be routinely employed despite the well-documented deficiencies such as limited graft accessibility, donor site morbidity and increased costs.[2,4,8-10]

With the progressive introduction of new biomaterials, biomimetics and nano-biomaterials in particular to the clinic, surgeons are required to pay more attention to tissue engineering and regenerative medicine principles and frontiers.[2,6,8-10] This basically implies an understanding of the underlying tissue biology, intrinsic or physiological reparative responses and mechanisms at the tissue-specific cellular and molecular levels, and the effect on stimulated or accelerated and improved healing – and predictable or in other terms, controlled clinical outcomes.[2,4,6,9-12] This becomes most evident in large and complex defects, where when modern surgical techniques are combined synergistically with utilization of innovative nano-biomaterials, including nanoparticles, nanocapsules, polymer scaffolds, matrices and pharmaceutical agents (*i.e.* stem cell, protein/growth factor) delivery systems, surgical outcomes are dramatically superior when compared to traditional outcomes.

The exogenous application of potent osteoinductive recombinant human (rh) cytokines as signaling molecules, such as the commercially-available rhBMP-2 (bone morphogenetic protein-2; InductOs®, Infuse®) and rhBMP-7 (bone morphogenetic protein-7, also known as rhOP-1 or osteogenic protein-1; Osigraft®), to stimulate osteogenesis, is a fine example.[8,9,11,13]

When introduced, without a carrier, typically in supra-physiological dosages, life-threatening adverse effects including soft tissue swelling, adipogenesis, ectopic bone formation, elevated bone resorption (from osteoclast over-activation), and even a high risk of inducing cancer (especially in children and young adults), were reported.[8] However, when locally-acting rhBMPs were loaded or incorporated into pharmaco-kinetic nanocapsules and scaffolds (load/drug release-controlled). (Figure 3)

Functional innervation, vascularization and de novo osteogenesis in the regenerate jaw were optimized, thereby providing the surgeon with more outcome predictability (spatially- and temporally-controlled; maintaining a critical threshold concentration of exogenous cytokines locally at the defect site, callus or de novo regenerate for the necessary period of time is crucial).[3,9,11] Collectively, timing of protein release is important and therefore, novel nano-biomaterials and controlled-release carriers need to contain the cytokines or drugs, prevent ectopic/heterotopic bone formation and utilize safe and cost-effective dosages.[11,13] They should have the ability to provoke optimal localized inflammatory responses, be biocompatible and are often required to be biodegradable/resorbable.[11,13,14]

Indeed, we have explored modified versions of such technological formulations, incorporating a cocktail load, for the early prevention of irradiation-induced salivary gland damage and dysfunction in head and neck cancer patients, demonstrating positive results in diminishing hypo-salivation and xerostomia, following a single direct injection 24 hrs pre-radiotherapy, thereby significantly enhancing the quality of life and cancer survival indices of patients.[2,9,10] Herein, the localized, concentrated (dose-responsive), dual- and tri-load release pharmacokinetics are crucial for the sought-after clinical outcome; early predictive prevention and late regenerative effect of irradiated head and neck complex.

In another example, nanotechnology and pharmaceutical drug delivery advances were introduced to and combined with platelet concentrates (PCs), a family of autologous blood extracts, where product results from the simple centrifugation of collected whole blood samples of the patient, immediately pre-surgery, chair-side; platelet-rich fibrin (PRF), a sub-family of platelet concentrates, was utilized. Briefly, both, pure PRF in general, and L-PRF (leukocyte and platelet-rich fibrin) in specific, can be collectively defined as a three-dimensional autogenous biomaterials obtained chair-side, without the use of anti-coagulants, bovine thrombin, additives, or any gelifying agents during the centrifugation process. PCs, P-PRF and L-PRF today continue to enjoy the most attention, essentially due to their simplicity, rapidness, user-friendliness and cost-effectiveness.[2,4,15] Whether used as the sole bioactive additive material or combined with bone substitutes, the revolutionary second-generation PRFs have been very often associated with promising clinical results.[4,15]

Herein, we are investigating the incorporation of

monodisperse, spherical and release-controlled rhGF(s)-loaded nanocapsules (amongst other loads) into fresh as well as lyophilized L-PRF matrices, for application in maxillary sinus floor lift, graft and surgical augmentation procedures. Processing conditions need to prevent protein aggregation or denaturation. Preserving cellular and biological cues, optimal porosity and mechanical properties is critical. Also, such novel systems, strategies or clinical alternative solutions, overall have to be easily, cost-effectively and sterile-manufactured for scaled production and use. Indeed, proper storage, stability, handling and sterilization conditions are favored as well.[10,15] All such progressions will, typically, also need to serve and facilitate the sought-after approval by operators and regulatory agencies; for a smooth technology transfer and translational process.[2,4,10, 15]

Henceforward, novel nano-biomaterials will provide the bio-mechanical/-physical stability and chemical stimuli, critical for a fully-integrated accelerated and functional tissue regenerate and optimal natural contour restoration of the jaw, face and head; examples of a few projects currently undergoing research, development and innovation (R&D&I) at our BioMAT'X-UAndes, in collaboration with our national/international research and bio-clinical network.

Conflict of interests: The author declare that they have no conflict of interests.

Acknowledgements: This work was supported by operating grants provided to BioMAT'X (Laboratorio de Biomateriales, Farmacéuticos y Bioingeniería de Tejidos Cráneo Máxilo-Facial), member of CiiB (Centro de Investigación e Innovación Biomédica), through the Faculty of Dentistry and I+D+i (FAIN-II 2020) Universidad de los Andes, Santiago-Chile, CONICYT-FONDEF # 16I10366 and CORFO # 18COTE-89695.

ISSN Print 0719-2460 - **ISSN Online** 0719-2479.

REFERENCES.

1. Kumar BP, Venkatesh V, Kumar KA, Yadav BY, Mohan SR. Mandibular reconstruction: overview. J Maxillofac Oral Surg. 2016; 15:425–41.
2. Haidar ZS, Di-Silvio L, Noujeim ZEF, Davies JE, Cuisinier F, Banerjee A. Engineering Solutions for Cranio-Maxillo-Facial Rehabilitation and Oro-Dental Healthcare. J Healthc Eng. 2019;2019:5387305.
3. Olate SM, Haidar ZS. Growth Factor-assisted Distraction Osteogenesis and Histiogenesis. J Oral Res.2017; 6(5):112-114.
4. Zumarán CC, Parra MV, Olate SA, Fernández EG, Muñoz FT, Haidar ZS. The 3 R's for Platelet-Rich Fibrin: A "Super" Tri-Dimensional Biomaterial for Contemporary Naturally-Guided Oro-Maxillo-Facial Soft and Hard Tissue Repair, Reconstruction and Regeneration. Materials (Basel). 2018; 11(8):1293.
5. Tee BC, Sun Z. Mandibular distraction osteogenesis assisted by cell-based tissue engineering: a systematic review. Orthod Craniofac Res. 2015;18(Suppl 1):39–49.
6. Haidar ZS. NanoDentistry: Perspectives on the Role of NanoBio-technology in Biomaterials, Pharmaceutics and BioDental Tissue Engineering. EC Dental Science 3.2. 2015: 506-7.
7. Tarsitano A, Battaglia S, Ricotta F, Bortolani B, Cercenelli L, Marcelli E, et al. Accuracy of CAD/CAM mandibular reconstruction: a three-dimensional, fully virtual outcome evaluation method. J Craniomaxillofac Surg. (2018) 46:1121–5. doi: 10.1016/j.jcms.2018.05.010.
8. Haidar ZS, Hamdy RC, Tabrizian M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part A: Current challenges in BMP delivery. Biotechnol Lett. 2009;31(12):1817–24.
9. Haidar ZS, Hamdy RC, Tabrizian M. Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair. Part B: Delivery systems for BMPs in orthopaedic and craniofacial tissue engineering. Biotechnol Lett.2009;31(12):1825–35.
10. Haidar ZS. Bio-Inspired/-Functional Colloidal Core-Shell Polymeric-Based NanoSystems: Technology Promise in Tissue Engineering, Bioimaging and NanoMedicine. Polymers.2010;2(3):323–52.
11. Haidar ZS, Tabrizian M, Hamdy RC. A hybrid rhOP-1 delivery system enhances new bone regeneration and consolidation in a rabbit model of distraction osteogenesis. Growth Factors. 2010;28(1):44–55.
12. Olate SM, Haidar ZS. NanoBioTe-chnology-guided Distraction Osteogenesis and Histiogenesis. J Oral Res.2017; 6(6):142-4.
13. Haidar ZS, Azari F, Hamdy RC, Tabrizian M. Modulated release of OP-1 and enhanced preosteoblast differentiation using a core-shell nano-particulate system. J Biomed Mater Res A. 2009;91(3):919–28.
14. Haidar ZS, Hamdy RC, Tabrizian M. Biocompatibility and safety of a hybrid core-shell nanoparticulate OP-1 delivery system intramuscularly administered in rats. Biomaterials. 2010;31(10):2746–54.
15. Damsaz M, Castagnoli CZ, Eshghpour M, Alamdari DH, Alamdari AH, Noujeim ZEF, Haidar ZS. Evidence-Based Clinical Efficacy of Leukocyte and Platelet-Rich Fibrin in Maxillary Sinus Floor Lift, Graft and Surgical Augmentation Procedures. Front Surg. 2020;7:537138.