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Research Article

Potential Use of Adipose-Derived Mesenchymal Stromal Cells and Bone Graft for Dental Implant: A Systematic Review

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Abstract

Introduction: Over the past 30 years, the number of dental implant procedures has increased worldwide, reaching about one million dental implants per year. In Brazil, in recent decades, there has been a very rapid evolution in implant dentistry with high success rates.

Objective: It was performed a systematic review of the main findings and potential use of adipose-derived stem cell and bone graft for a dental implant. Methods: A total of 55 clinical studies and reviews that were submitted for eligibility analysis were checked, and after that, 38 studies were selected, following the rules of systematic review-PRISMA. The search strategy was performed in PubMed, Embase, Ovid and Cochrane Library, Web of Science, ScienceDirect Journals (Elsevier), Scopus (Elsevier), OneFile (Gale).

Major Findings and Conclusion: Tissue engineering is a tool that allows by means of a suitable biological niche for any construction and regeneration of tissues and organs. Another advantage is minimally invasive surgery or allows the use of surgical techniques that cause less risk to the patient. This condition is maintained because the cells involved in the biological niche secret growth factors for cell proliferation and differentiation and supramolecular structures that ensure the functional organization of tissue generated by means of stereochemistry and its systemic integration ADSCs can potentiate osteogenesis and reduce the possibility of developing bone necrosis. Moreover, the ADSC, when cultured in the presence of bone, releases cytokines osteopoiesis undergo a process that involves the proliferation and maturation of primitive precursor cells to the formation of functional osteoblasts and bone cells originated ADSC this phenomenon is committed osteoprogenitor cells and pre-osteoblasts osteoblasts and osteocytes. Therefore, the lack of bone in the alveolar ridges has been a major problem in functional aesthetic recovery in patients who have suffered dentoalveolar trauma, traumatic tooth extractions, congenitally missing teeth, maxillary and mandibular pathologies. For the filling of large bone defects, the development of bone regeneration improves epithelial barriers to bone graft, favoring dental implantation.

Keywords: Adipose-derived stem cell. Stromal vascular fraction. Platelet-rich plasma. Bone loss. Dental implant.

Introduction

Over the past 30 years, the number of dental implant procedures has increased worldwide, reaching about one million dental implants per year [1]. In Brazil, in recent decades, there has been a very rapid evolution in implant dentistry with high success rates [2]. The development of biomaterials for use in dental clinics in recent years has been a powerful therapeutic tool in the correction of bone defects [3]. However, despite the proven benefits, its use requires that the professionals take care of clinical and ethical criteria in the analysis of risks and benefits that each biomaterial may present.

The lack of bone in the alveolar ridges has been a major problem in functional aesthetic recovery in patients who have suffered dentoalveolar trauma, traumatic tooth extractions, congenital missing teeth, maxillary and mandibular pathologies, as well as infections due to the emotional and the possibility of deformity. economic impact on public health [4]. In addition, maxillofacial trauma can be considered one of the most devastating aggressions encountered in traumatology and oncology due to the emotional consequences and the possibility of deformity as well as the economic impact [4,5].

In this scenario, tissue engineering is a tool that enables, through the appropriate biological niche, the construction and regeneration of many tissues and organs. For this, xenografts, autografts, and allografts are used, with and without the use of cells [6]. Thus, bioengineering and cell therapy work together for regenerative medicine, favoring and improving biological conditions to accelerate tissue repair and regeneration and thus maintain tissue homeostasis [6].

Normal bone formation and tissue restoration involve coordinated interaction between bone-forming cells and biological signals. The main predictors in this process are osteoblasts and their precursors [7]. Osteoblasts may produce new bones together with biomaterials and may initiate the release of biological signals that guide the bone formation and remodeling [8].

In clinical studies, Adipose-Derived Stem Cell (ADSC) obtained from Vascular Stromal Fraction (SVF) as the regeneration of periodontal tissues has been shown and ADSC showed that when associated with platelet-rich plasma can regenerate alveolar bone, cementum, and periodontal ligament eight weeks after implantation [10]. In addition, there is a combined study of bone graft with fibrin glue, biodegradable biomaterial and ADSC for the reconstruction of large bone defects in the skull of a victim of seven years of trauma. In a few cases, the ADSC complex allowed the regeneration of wounds, improving the resulting radiation fibrosis. Other studies involve research on the safety and efficacy of ADSC for treating fistulae in patients with Crohn's disease, critical limb ischemia in diabetic patients, developing bone engineering and treatment of myocardial failure [17].

Therefore, the present study aimed to make a systematic review of the main findings and the potential use of adipose-derived stem cell and bone graft for the dental implant.

Methods

Study design

A total of 55 clinical studies and reviews that were submitted to the eligibility analysis were checked, and after that, 38 studies were selected, following the rules of systematic review-PRISMA (Transparent reporting of systematic reviews and meta-analyzes-http: // www.prisma-statement.org/).

Search strategy and information sources

The search strategy was performed in PubMed, Embase, Ovid and Cochrane Library, Web of Science, ScienceDirect Journals (Elsevier), Scopus (Elsevier), OneFile (Gale) followed the following steps: - search for MeSH Terms: Adipose-derived stem cell. Stromal vascular fraction. Platelet rich plasma. Bone loss. Dental implant, and - use of boolean "and" between mesh terms and "or" among historical findings.

Risk of bias

According to the Cochrane model for risk of bias in the present study, the overall assessment resulted in 4 studies with high bias risk and 4studies with uncertain risk. In addition, there was an absence of funding source in 7 studies and four studies did not disclose the information on the declaration of conflict of interest (Figure 1).

Development

Bone tissue engineering

Tissue engineering is a tool that allows by means of a suitable biological niche for any construction and regeneration of tissues and organs [5]. Tissue engineering offers numerous benefits that meet the needs of injured tissue or to the regeneration process organ. Therefore, understanding the chemical, physical and biological processes of both organic materials as the biological niche in the host is necessary. The crossing of compatible information between microenvironments allows cascades of cell recognition and signaling to neovascularization [5].

Another advantage is minimally invasive surgery or allows the use of surgical techniques that cause less risk to the patient. So biomaterial Bio Oss® (Geistlich), being biodegradable, biocompatible, non-toxic, and low immunogenicity, can act in the regeneration of bone tissue, they establish with mesenchymal stem cells from adipose tissue appropriate biological niche (microenvironment favorable) to bone growth in both animal studies and human studies beings [5].

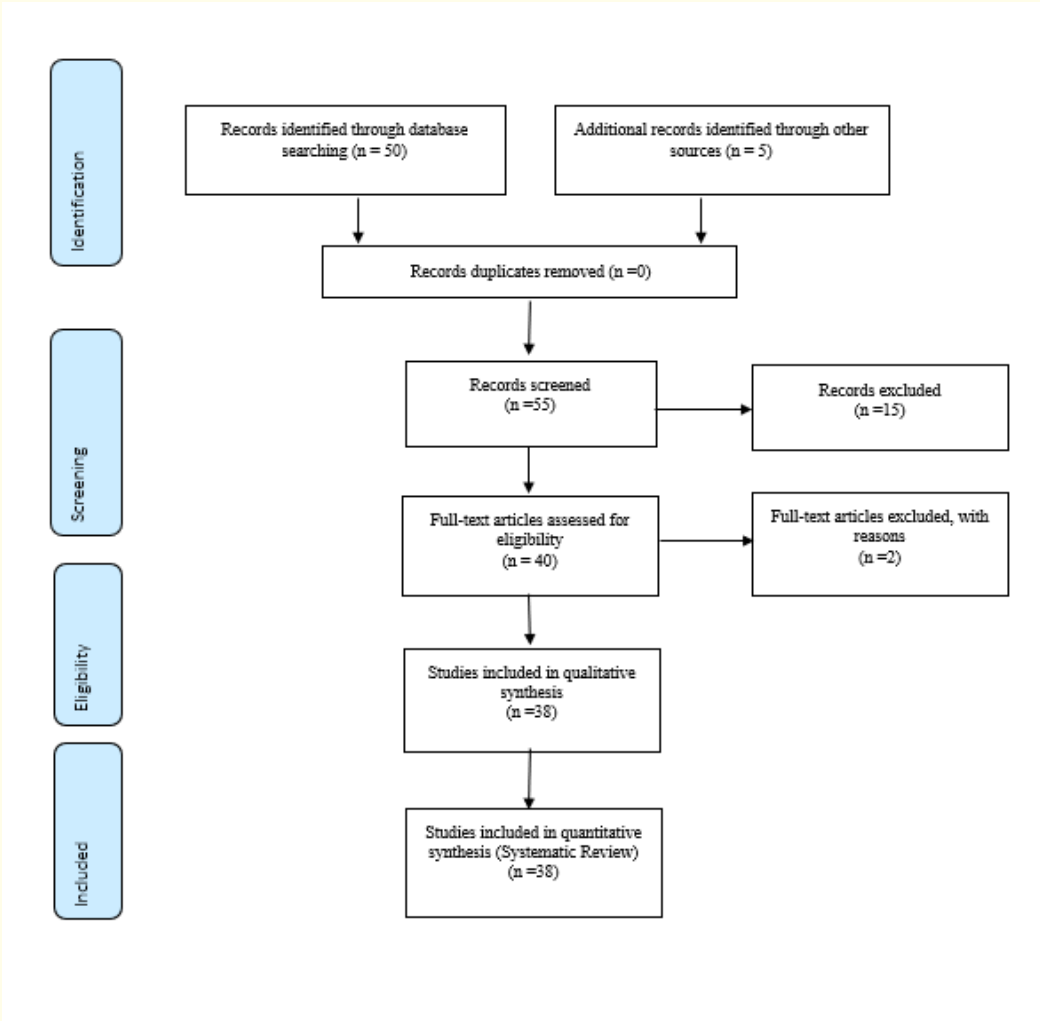

Figure 1

The maxillary sinus augmentation procedure has been well publicated and the long-term clinical survival (> 5 years) of implants placed, regardless of graft materials used, compares favorably to implants placed conventionally as reported in other systematic reviews [1]. Studies that met the inclusion criteria seemed to be comparable and yielded favorable results in supporting dental implants. However, alveolar ridge augmentation techniques do not have detailed documentation or long-term follow-up studies [1,2]. The alveolar ridge augmentation procedures may be more technique and operator experience sensitive, and implant survival may be a function of residual bone supporting the dental implant rather than grafted bone. More in-depth, multicenter studies are required to provide further insight into augmentation procedures to support dental implant survival [6].

Another clinical study, forty-eight maxillary sinuses were treated in 37 patients. Lateral sinus augmentation was used with grafting using either Bio-Oss® (ABB) (control group; 23 sinuses) or Biphasic Calcium Phosphate (BCP) (test group; 25 sinuses). Histology showed close contact between new bone and graft particles for both groups, with no significant differences in the amount of mineralized bone, the bone-to-graft contact, remaining percentage of graft substitute material and more soft tissue components [5].

Bone graft and bone remodeling action mechanism with ADSC

The challenge is to understand the science of multidisciplinary biomaterials and its application requires adjustments to its processing, sterilization and structural changes to promote interaction with the tissue of interest [5]. Thus, the bioengineered and cell therapy act together to regenerative medicine, facilitating and improving the biological conditions to accelerate tissue repair and regeneration, and thereby maintain tissue homeostasis course [5].

This condition is maintained because of the cells involved in the biological niche secret growth factors for cell proliferation and differentiation and supramolecular structures that ensure the functional organization of tissue generated by means of stereochemistry and its systemic integration [6].

Normal bone formation and tissue restoration involve coordinated interaction between bone-forming cells and biological signals [13]. The main force in this process are osteoblasts and their precursors. Osteoblasts may produce new bones together with biomaterials and may initiate the release of biological signals that guide the bone formation and remodeling [14,15].

These biological signals attract bone-forming cells to the receptor site. Growth factors and other proteins are some biological signs that may be involved in bone neoformation and tissue remodeling. In addition, through chemotaxis, bone-forming cells are migrated to the application area because stimulation of cell migration occurs in response to chemical stimuli [16].

Monocytes, macrophages and endothelial cells contribute to bone remodeling, either by contact with osteogenic cells or by releasing soluble factors such as cytokines and GF [8]. In the skeletal system, TNF-α stimulates bone and cartilaginous resorption and inhibits collagen and proteoglycan synthesis. IL-1 induces expression of a wide variety of cytokines. LIF and IL-6 are two such molecules that are known to stimulate differentiation of mesenchymal progenitor cells in the osteoblastic lineage, are also potent osteoblast anti-apoptotic agents. In bone, the main sources of IL-6 are osteoblasts rather than osteoclasts. Prostaglandin E2 (PGE2) is also directly related to IL-6 cytokine expression [8].

The microscopic bone structure consists of osteoprogenitor cells, support cells (osteoblasts and osteocytes), remodeling cells - osteoclasts - and a non-mineralized extracellular matrix called osteoid, composed of collagen type I and non-collagen proteins such as osteonectin, osteocalcin, bone morphogenetic protein (BMP), glycosaminoglycans and bone sialoproteins [7]. Osteoprogenitor cells are small spindle cells found on all non-resorbable bone surfaces, derived from primitive mesenchymal cells and form a population and precursor cells that can differentiate into more specialized cells such as osteoblasts and osteocytes [7].

In this sense, the osteoinduction process is influenced by several factors, requiring the presence of inducers, which include β-glycerolphosphate, ascorbic acid, and dexamethasone [8]. In the presence of these substances, mesenchymal cells acquire morphology and osteoblastic membrane components and express alkaline phosphatase to deposit calcium-rich extracellular matrix and certain proteins such as osteopontin and osteocalcin [8].

In addition, bone morphogenetic proteins (BMP) function as growth factors with a specific role in the proliferation and differentiation of mesenchymal stem cells present in the lesion niche [9]. In particular, BMP-2 is involved in the early stages of osteogenesis; In addition, it has been shown that differentiation of human mesenchymal stem cells into the osteogenic lineage requires the presence of BMP-2 in the first days of culture and that these cells after 21 days express osteogenic lineage-specific proteins such as osteonectin, osteocalcin, and osteopontin [9].

In this context, facial bone defects are found in clinical practice. Thus, bone grafts, flaps, and alloplastic materials are used in their treatment. In this regard, an animal model study examined whether adipose-derived stromal vascular fraction (SVF) has an osteogenic effect on the critically sized membranous bone defect of the zygomatic bone. Twenty male Wistar Albino rats were used [8]. The bilateral zygomatic arches were opened with lateral incisions. A standard 3 mm bone defect was created bilaterally in the zygomatic arches of the rats. On the side of the experiment, stem cell-rich SVF, which was obtained by applying the centrifugal process to adipose tissue-derived from the inguinal adipose layer, was injected into the site of the right zygomatic arch bone defect. On the control side, the left zygomatic arch was left for secondary bone healing without any treatment after the creation of a critical bone defect of 3 mm. At 10th (n = 5) and 20 weeks (n = 13) postoperatively, bone defect healing areas were evaluated by three-dimensional tomography and then rats were sacrificed and bone healing was examined histologically. There were no statistically significant differences in the 10th-week results. At week 20, the new amount of bone for-

mation calculated from the results of three-dimensional computed tomography was significantly higher on the side of the experiment ($p = 0.033$). Histological examination at week 20 showed significantly more callus formation on the side of the experiment ($p = 0.0112$). Thus, stem cells can increase the rate of bone healing by differentiating into certain tissues. SVF derived from mesenchymal stem cell-rich adipose tissue is expected to increase bone healing in facial bone defects, and this application may replace the use of bone grafts and flaps in clinical practice. Therefore, ADSC can potentiate osteogenesis and reduce the possibility of developing bone extremity necrosis [8].

In this sense, the ADSC have several advantages such as low cost, availability, and low immunogenicity compared to acellular fillers such as collagen, hyaluronic acid, chitosan and others [16]. When added to biomaterials as Bio-Oss® its site of improved and more permanent implant survival, reaching several mechanisms including increased revascularization, reduction of apoptosis and promotion of differentiation of osteocytes for the regeneration of alveolar bone, increasing thickness of jawbone [16-18]. Moreover, the ADSC when cultured in the presence of bone releases cytokines osteopoiesis undergoing a process that involves the proliferation and maturation of primitive precursor cells to the formation of functional osteoblasts and bone cells originated ADSC this phenomenon is committed osteoprogenitor cells and pre-osteoblasts osteoblasts and osteocytes [22-24].

Unlike osteoconductive materials, osteoinductive substances promote bone formation in extra-skeletal sites. Also members of the TGF-β family. TGF-β belongs to a family of multifunctional growth proves to be one of the mediators of normal cellular physiology, embryogenic tissue involved in a number of responses associated with inflammation and tissue repair factors [23]. Its main source is the extracellular bone matrix and platelet reservoir for the second polypeptide. TGF-β has an excellent performance in cellular activity, including control of proliferation and expression of different phenotypes of various types of specific cells in the skeleton, especially the precursor of mesenchymal stem cells to chondrocytes, osteoblasts, and osteoclasts [23].

The ADSC contains several types of cells, such as stem cells from adipose tissue endothelial cells and smooth muscle cells and their progenitors for preadipocytes [16]. The adipose stem cells can secrete VEGF, HGF, and IGF-1, which are pro-angiogenic, and pro- adipogenic effects antipoptóticos. Furthermore, due to the abundance of progenitor cells in the vessels of ADSC also suggests that these cells are highly capable of enhancing neovascularization, such studies have shown that there is a significant relationship between fat cells and vascular system. These cells represent a heterogeneous population of microvascular endothelial cells, are a convenient source of multipotent cells and are not restrictive [22].

The ADSC has the advantages of self-renewal, immunomodulatory character multipotentiality, ease of isolation, purification, expansion "in vitro" as well as cryopreservation [9,24]. Thus, ADSC must provide the minimum requirements adherent cells and the proliferation, differentiation into at least three cell lines (adipocytes, chondrocytes, and osteocytes) and display panel with the surface markers typical of MSCs so that they can study be used according to the recommendations of the International Society for Cellular Therapy. In culture conditions, the adipose stem cells grow easily in monolayers, retain multipotentiality normally until the 10th passage and feature fibroblastoid morphology (elongated) [9,27].

For immunophenotyping of ADSC series of markers (antibodies) positive CD9, CD10, CD13, CD29, CD44, CD49, CD54, CD55, CD59, CD73, CD90, CD105, CD106, CD144, CD146, CD166 and HLA-1 are necessary and one negative number for CD11, CD14, CD19, CD31, CD34, CD45, CD79 alpha, CD80, CD117, CD133, CD144, HLA-DR, and Stro-1 [9]. The ADSC secrete a cascade of cytokines and growth factors with paracrine, autocrine and endocrine activities such as colony-stimulating factor macrophage (M-CSF), colony-stimulating factor granulocyte-macrophage (GM-CSF), macrophages inflammatory protein (MIP-1α / CCL3). These factors, when combined, may produce a series of local immune responses by stimulating angiogenesis and the induction of proliferation and differentiation of mesenchymal stem cells to the desired tissue. Furthermore, the ADSC induces expression of proteins junction and increase microvessel integrity and nitric oxide (NO) by macrophages [27].

Dental implants and biomaterials

In the context of successful dental implant practice, osseointegration is essential. However, it is a complex process with many factors interfering with the formation and maintenance of bone tissue around the implant, such as topography and surface roughness, biocompatibility and loading conditions [19,20]. In addition, a healthy and compatible bone layer of the host is required, allowing primary stability [28].

Dental implants are increasingly being used due to high success rates [30,31]. However, a large proportion of patients do not have sufficient bone conditions for implant placement, thus requiring prior reconstructive bone surgery. It is essential that the dentist master the knowledge in the healing process of post-extraction alveoli in order to provide correct planning of cases [31].

In this sense, after extraction the repair process occurs in the internal region of the alveolus along with the formation of cell-rich clot and growth factors, promoting neoformation, bone remodeling and soft tissue epithelialization [32]. During this process, the alveolar ridge undergoes relevant changes, both in height and thickness, which influence the possibility of implant placement. Thus, the optimized implantology and biomaterials processes allow the implantation of implants in areas of low thickness and bone width, with simpler surgeries and greater patient success and comfort [32].

The lack of bone in the alveolar ridges has been a major problem in functional aesthetic recovery in patients who have suffered dentoalveolar trauma, traumatic tooth extractions, congenital missing teeth, maxillary and mandibular pathologies [33,34]. For the filling of large bone defects, the development of bone regeneration improves epithelial barriers to bone graft, favoring greater predictability in alveolar and peri-implant reconstructions and have a good prognosis [35-37]. In this sense, the filling biomaterials can be fibrin-rich plasma (PRF), Bio-Oss®, hydroxyapatite, lyophilized and ground demineralized bone marrow, autogenous bone, which is considered the gold standard, among others [38].

Conflicts of Interest

There are no conflicts of interest.

Conclusion

Therefore, the lack of bone in the alveolar ridges has been a major problem in functional aesthetic recovery in patients who have suffered dentoalveolar trauma, traumatic tooth extractions, congenital missing teeth, maxillary and mandibular pathologies. For the filling of large bone defects, the development of bone regeneration improves epithelial barriers to bone graft, favoring dental implantation.

Bibliography

1. Zhou Z., *et al*. "Two-stage closed sinus lift for severe bone deficiency in the posterior maxilla improves long-term clinical outcomes". *Nan Fang Yi Ke Da Xue Xue Bao* 39.6 (2019): 731-735.
2. IBGE- Instituto Brasileiro de Geografia e Estatística (2019).
3. Abdel-kader MA., *et al*. "Oral rehabilitation of a case with regional odontodysplasia using a regenerative approach-A case report and a review of literature". *Special Care in Dentistry* 39.3 (2019): 330-339.
4. Momen-Heravi F., *et al*. "Acellular Dermal Matrix as a Barrier for Guided Bone Regeneration of Dehiscence Defects Around Dental Implants: A Clinical and Histological Report". *Implant Dentistry* 27.4 (2018): 521-524.
5. Moreira AC., *et al*. "Application of Bio-Oss in tissue regenerative treatment prior to implant installation: literature review". *Brazilian Dental Science* 22.2 (2019).
6. Starch-Jensen T., *et al*. "A systematic review and meta-analysis of long-term studies (five or more years) assessing maxillary sinus floor augmentation". *International Journal of Oral and Maxillofacial Surgery* 47.1 (2018): 103-116.
7. Wu IH., *et al*. "Retrospective Analysis of the Outcome of Ridge Preservation with Anorganic Bovine Bone Mineral: Marginal Bone Level at Implants Placed Following Healing of Grafted Extraction Sockets". *International Journal of Periodontics and Restorative Dentistry* 39.1 (2019): 131-140.
8. Toplu G., *et al*. "Adipose Tissue Derived Stromal Vascular Fraction Increases Osteogenesis in an Experimental Design Zygomatic Bone Defect Model". *Journal of Craniofacial Surgery* 28.8 (2017): 2179-2182.
9. Strauss FJ., *et al*. "The use of platelet-rich fibrin to enhance the outcomes of implant therapy: A systematic review". *Clinical Oral Implants Research* 18 (2018): 6-19.
10. Zuk PA., *et al*. "Multilineage cells from human adipose tissue: implications for cell-based therapies". *Tissue Engineering* 7.2 (2001): 211-228.
11. Pye AD., *et al*. "A review of dental implants and infection". *Journal of Hospital Infection* 72 (2009): 104-110.
12. Branemark PI., *et al*. "Osseointegrated implants in the treatment of edentulous jaw. Experience from a 10-year period". *Scandinavian Journal of Plastic and Reconstructive Surgery* 16 (1977): 1-192.
13. Bugarin Júnior JG and GarrafaII V. "Bioethics and biosafety: the use of biomaterials in dental practice". *Revista de Saúde Pública* 41.2 (2007):223-228.

14. Saghiri MA., *et al*. "The role of angiogenesis in implant dentistry part II: The effect of bone-grafting and barrier membrane materials on angiogenesis". *Med Oral Patol Oral Cir Bucal* (2016).

15. Aubin JE and Liu F. "The osteoblast lineage". In: Bilizekian, J., Raisz, L., and Rodan, G., editors. Principles of Bone Biology. San Diego, CA: Academic Press (1996): 39-50.

16. Chan YL and King NM. "Use of focused íon bean milling for investigating the mechanical properties of biological tissues: A study of human primary molars". *Journal of the Mechanical Behavior of Biomedical Materials* 2.4 (2009): 375-383.

17. Gimble JM., *et al*. "Adipose-Derived Stem Cells for Regenerative Medicine". *Circulation Research* 100 (2013): 1249-1260.

18. Hallman M., *et al*. "A clinical histologic study of bovine hydroxyapatite in combination with autogenous bone and fibrin glue for maxillary sinus floor augmentation. Results after 6 to 8 months of healing". *Clinical Oral Implants Research* 12.2 (2001): 135-143.

19. Hing KA. "Bone repair in the twenty-first century: biology, chemistry or engineering?" *Philosophical Transactions of the Royal Society B: Biological Sciences* 362.1825 (2004): 2821-2850.

20. Langer R and Vacanti JP. "Tissue Engineering". *Science* 260 (1993): 920-926.

21. Lima AF Martorelli. "Enxertos ósseos: características de alguns materiais". *Associação Brasileira de Ouvidores / Ombudsman - ABO Nacional* 16.3 (2008).

22. Liu Y., *et al*. "Hyaluronic acid-gelatin fibrous scaffold produced by electrospinning of their aqueous solution for tissue engineering applications. In Advances in Material Design for Regenerative Medicine". *Drug Delivery and Targeting/ Imaging* 1140 (2010): 131-136.

23. Locke M., *et al*. "Human adipose-derived stem cells: isolation, characterization and applications in surgery". *ANZ Journal of Surgery* 79 (2009): 235-244.

24. Mesimäki K., *et al*. "Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells". *International Journal of Oral and Maxillofacial Surgery* 38 (2009): 201-209.

25. Planat Bernard V., *et al*. "Plasticity of human adipose lineage cells towards endothelial cells: physiological and therapeutic perspectives". *Circulation* 109 (2004): 656 -63.

26. Vacanti JP and Langer R. "Tissue engineering: The design and fabrication of living replacement devices for surgical reconstruction and transplantation". *Lancet* 354 (1999): 32-34.

27. Valentini P and Abensur D. "Maxillary sinus floor elevation for implant placement with demineralized freeze-dried bone and bovine bone (Bio-Oss®): a clinical study of 20 patients". *International Journal of Periodontics and Restorative Dentistry* 17.3 (1997): 232- 241.

28. Zotarelli Filho IJ., *et al*. "Chitosan-collagen scaffolds can regulate the biological activities of adipose mesenchymal stem cells for tissue engineering". *Journal of Regenerative Medicine and Tissue Engineering* 2 (2013): 12.

29. Simonpieri A., *et al*. "Simultaneous sinus-lift and implantation using microthreaded implants and leukocyte- and platelet-rich fibrin as sole grafting material: a six-year experience". *Implant Dentistry* 20.1 (2011): 2-12.

30. Anitua E. "Enhancement of Osseointegration By Generating a Dynamic Implant Surface". *Journal of Oral Implantology* 32 (2006): 72-76.

31. Tejero R., *et al*. "Toward the biomimetic implant surface: Biopolymers on titanium-based implants for bone regeneration". *Journal of Progress in Polimeral Science* 39 (2014): 1406-1447.

32. Paolantonio M., *et al*. "Autogenous Periosteal Barrier Membranes and Bone Grafts in the Treatment of Periodontal Intrabony Defects of Single-Rooted Teeth: A 12-Month Reentry Randomized Controlled". *Clinical Trial Journal of Periodontology* 81.11 (2010):1587-1595.

33. Zhang Y., *et al*. "Effects of Choukroun's platelet-rich fibrin on bone regeneration in combination with deproteinized bovine bone mineral in maxillary sinus augmentation: A histological and histomorphometric study". *Journal of Cranio-Maxillo-Facial Surgery* 40 (2012): 321-328.

34. Tatullo M., *et al*. "Platelet Rich Fibrin (PRF) in Reconstructive Surgery of Atrophied Maxillary Bones: Clinical and Histological Evaluations". *International Journal of Medical Sciences* 9.10 (2012): 872-880.

35. Angelo T., *et al*. "Biomechanical Stability of Dental Implants in Augmented Maxillary Sites: Results of a Randomized Clinical Study with Four Different Biomaterials and PRF and a Biological View on Guided Bone Regeneration". *Hindawi Publishing Corporation BioMed Research International* (2015).

36. Chen Y., *et al*. "Inlay osteotome sinus floor elevation with concentrated growth factor application and simultaneous short implant placement in severely atrophic maxilla". *Scientific Reports* 6 (2016): 27348.

37. Kumar NK., *et al*. "Comparative Study of Alveolar Bone Height and Implant Survival Rate Between Autogenous Bone Mixed with Platelet Rich Plasma Versus Venous Blood for Maxillary Sinus Lift Augmentation Procedure". *Journal of Oral and Maxillofacial Surgery* 14.2 (2015): 417-422.

38. You JS., *et al*. "Effects of Platelet-Derived Material (Platelet-Rich Fibrin) on Bone Regeneration". *Implant Dentistry* (2019).