



Major considerations of the biological mechanisms of bone regeneration through mesenchymal stem cells, exosomes, and microRNAs in the scenario of bucco-maxillo-facial surgery: a systematic review

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Abstract

Introduction: In the context of bucco-maxillo facial surgery, the development of biomaterials for use in clinical dentistry in recent years has represented a powerful therapeutic instrument in the correction of bone defects. Adult tissue stem cells (mesenchymal stem cells) mediate homeostasis and regeneration of tissues and organs. Growing evidence suggests that metabolism during quiescence, activation, and differentiation may vary between tissues, integrating signaling cues and metabolic inputs with the release of exosomes and microRNAs as important metabolic messengers. **Objective:** It was to carry out a systematic review to present the main considerations and scientific evidence of the cellular and molecular mechanisms of bone formation or regeneration through mesenchymal stem cells, exosomes, and microRNAs in the scenario of bucco-maxillo-facial surgery with bone graft or biomaterials. **Methods:** The systematic review rules of the PRISMA Platform were followed. The search was carried out from March to May 2023 in the Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases, with articles dated 2001 (gold standard) through 2022. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed accordingly, according to the Cochrane instrument. **Results and Conclusion:** A total of 145 articles were found, 45 articles were evaluated and 34 were included and developed in this systematic review study. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 30 studies with a high risk of bias

and 15 studies that did not meet GRADE. The greater potential of guided bone regeneration was associated with the graft material due to the higher grade of vital bone and the lower percentage of residual graft particles. Inorganic bovine bone and porcine bone minerals combined with autogenous maxillary cortical bone showed similar biological and radiological characteristics in terms of biomaterial resorption, osteoconduction, and osteogenesis when used for maxillary sinus floor augmentation. In this regard, three fundamental parameters in bone tissue engineering that determine the capacity for osteoinduction were evidenced, such as the presence of soluble osteoinductive signals, the viability of undifferentiated mesenchymal stem cells, having the ability to differentiate into bone-forming cells and production of adequate extracellular matrix. The exosomes that contain proteins, mRNAs, microRNAs, and DNAs stand out. Exosomes change the biochemical characteristics of recipient cells through the delivery of biomolecules and play a role in cell communication. Evidence suggests that exosomes derived from mesenchymal stem cells exhibit functions similar to mesenchymal stem cells with low immunogenicity and without tumorization.

Keywords: Bucco-maxillo-facial surgery. Bone regeneration. Exosomes. MicroRNAs.

Introduction

In the context of bucco-maxillo facial surgery, the development of biomaterials for use in clinical dentistry

in recent years has represented a powerful therapeutic instrument in the correction of bone defects [1-4]. In this sense, guided bone regeneration (GBR) favors the formation of new bone tissue and prevents the gingival tissue from invading the space between the bone and the implant [5,6].

In this regard, the filling materials can be hydroxyapatite, freeze-dried and ground demineralized medullary bone, and autogenous bone, which is considered the gold standard, among others. Together with the filling materials, it is often necessary to use resources to isolate the implant using biological membranes, which are epithelial barriers that guide tissue regeneration, work as a mechanical barrier separating the periodontal tissues from the bone or implant surface, thus promoting bone neoformation, filling material containment and graft stability [6,7].

Therefore, when grafting procedures are necessary, the focus is often on the type of biomaterial to be used and the success and predictability of results do not depend only on the biomaterial. It is also necessary to consider the type of defect to be treated, and its morphology. The morphology will have an impact mainly because the defects have different vascularization capacities, different osteogenic cell recruitment capacities, and different natural graft stabilization capacities, therefore, the characteristics of the biomaterials that we must use, but also the characteristics, must be considered. bed and bone defect for treatment [1].

Also, adult tissue stem cells, mesenchymal stem cells, mediate homeostasis and regeneration of tissues and organs by making decisions about whether to remain quiescent, proliferate, or differentiate into mature cell types. These decisions are directly integrated with the body's energy balance and nutritional status. Metabolic by-products and substrates that regulate epigenetic and signaling pathways are considered to play an instructive rather than an observer role in regulating cell fate decisions [8-10].

Furthermore, the quiescent state of stem cells is characterized by glycolytic metabolism, followed by a transition to favor mitochondrial oxidative phosphorylation during differentiation [10-13]. However, increasing evidence suggests that metabolism during quiescence, activation, and differentiation may vary between tissues, integrating signaling cues and metabolic inputs with the release of exosomes and microRNAs as important metabolic messengers in the organism, this process is strongly regulated by nutrients. [14-17].

Based on this, bioengineering and cell therapy work together with regenerative dentistry, favoring and

improving biological conditions to accelerate tissue repair and regeneration [1]. The condition of tissue homeostasis is maintained because the required cellular elements are provided, the cell proliferation and differentiation factors, and supramolecular structures that guarantee the functional stereochemical organization of the generated tissues and their systemic integration [2,3].

Therefore, the present study carried out a systematic review to present the main considerations and scientific evidence of the cellular and molecular mechanisms of bone formation or regeneration through mesenchymal stem cells, exosomes, and microRNAs in the scenario of bucco-maxillo-facial surgery with bone graft or biomaterials.

Methods

Study Design and Data Sources

This was followed by a systematic literature review model, according to the PRISMA rules. The literary search process was carried out from March to May 2023 and was developed based on Scopus, PubMed, Science Direct, Scielo, and Google Scholar, with articles dated 2001 (gold standard) through 2022, using the descriptors (MeSH Terms): *Bucco-maxillo-facial surgery. Bone regeneration. Exosomes. MicroRNAs*, and using the Booleans "and" between the descriptors (MeSH Terms) and "or" between the historical findings.

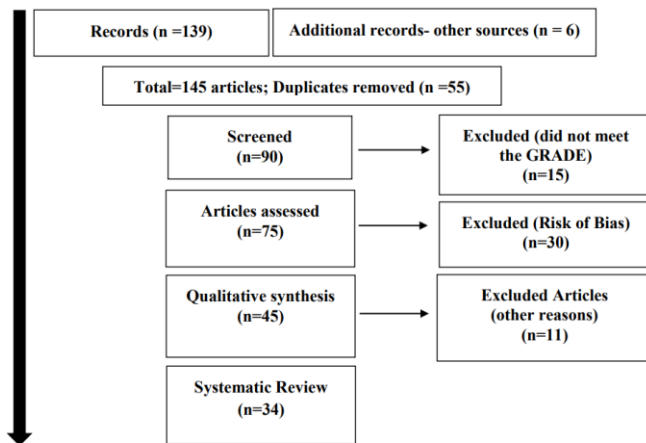
Study Quality and Risk of Bias

The quality of the studies was based on the GRADE instrument, with randomized controlled clinical studies, prospective controlled clinical studies, and studies of systematic review and meta-analysis listed as the studies with the greatest scientific evidence. The risk of bias was analyzed according to the Cochrane instrument.

Results and Development

Summary

A total of 145 articles were found. Initially, duplication of articles was excluded. After this process, the abstracts were evaluated and a new exclusion was performed, removing the articles that did not include the theme of this article, resulting in 75 articles. A total of 45 articles were evaluated and 34 were included and developed in this systematic review study (**Figure 1**). Considering the Cochrane tool for risk of bias, the overall assessment resulted in 30 studies with a high risk of bias and 15 studies that did not meet GRADE.

Figure 1. Selection of studies.

Source: Own authorship.

Clinical Findings - Grafts and Biological Processes

The authors Zampara et al. 2022 [18] clinically evaluated the potential of guided bone regeneration (GBR) of allograft, xenograft, and alloplastic materials in combination with resorbable membranes in extraction sockets. The qualitative and quantitative assessments of this prospective study were performed using histological and histomorphometric analyses. Three experimental groups and one control group for comparison (n = 8) received an allograft (lyophilized human cancellous bone, Deutsches Institut für Zell und Gewebersatz, Berlin, Germany), xenograft (BioOss, Geistlich Pharma AG, Wolhusen, Switzerland), or alloplastic (biphase calcium sulfate, Bondbone, MIS Implants Technologies Ltd., Charlotte, NC). The negative control group did not receive regenerative material. Tissue samples were then evaluated qualitatively and quantitatively for a percentage of vital new bone, graft particle content, soft tissue, and bone marrow over time. All 3 study groups had adequate bone volume for the successful placement of a dental implant. The xenograft group yielded significantly less vital bone compared to the allograft and alloplastic groups. When comparing the percentage of residual graft particles, there were significantly greater amounts associated with the xenograft group as opposed to the allograft and alloplastic groups. Likewise, a significantly increased amount of soft tissue percentage was observed in the xenograft group relative to all other groups. No significant differences were observed in the percentage of residual graft particles between the allograft and alloplastic groups. There were also no significant differences detected in the percentage of vital bone between the allograft, alloplastic, and control groups. When evaluating the percentage of bone marrow, the only significant difference detected was between the xenograft and

alloplastic materials. Overall, no complications (ie, fever, malaise, purulence, or fistula) were observed throughout the clinical trial among all patients. The highest GBR potential was associated with the graft material due to the higher grade of vital bone and the lower percentage of residual graft particles. All bone substitute materials studied resulted in bone apposition for efficient use in alveolar ridge preservation procedures.

Also, a randomized clinical study carried out by the authors Galindo-Moreno et al. 2022 [19] compared the effectiveness of two xenografts for maxillary sinus floor augmentation in terms of clinical, radiographic, histological, and molecular results. A total of 10 consecutive patients in need of two-stage bilateral maxillary sinus floor augmentation were included. Each patient received both biomaterials (porcine bone mineral and inorganic bovine bone), which were randomly assigned to bilateral breast augmentation. Autogenous maxillary bone scraped from the sinus access window was mixed with each xenograft in a 20:80 ratio. After a 6-month healing period, bone biopsies were taken with trephine during implant placement in the regenerated area. The resulting anatomical features were similar between the two groups. After six months of graft healing, graft resorption rates were similar between the two biomaterials. Histological, histomorphometric, and immunohistochemical results did not show statistical differences between groups. Therefore, inorganic bovine bone and porcine bone mineral combined with maxillary autogenous cortical bone showed similar biological and radiological characteristics in terms of biomaterial resorption, osteoconduction, and osteogenesis when used for maxillary sinus floor augmentation.

Added to this, the authors Meschi et al. 2021 [20], through a multicenter controlled clinical trial, evaluated the impact of platelet and leukocyte-rich fibrin (LPRF) in regenerative endodontic procedures (REP) of immature permanent teeth in terms of periapical bone repair (PBR) and subsequent development (SD). Healthy patients aged 625 years with an inflamed or necrotic immature permanent tooth were included and divided into test (= REP + LPRF) and control (= REP-LPRF) groups. After receiving REP ± LPRF, patients were recalled after 3, 6, 12, 24, and 36 months. At each recall session, the teeth were evaluated clinically and radiographically (using a periapical radiograph [PR]). A cone-beam computed tomography (CBCT) scan was performed preoperatively and 2 and 3 years after surgery. PBR and SD were evaluated quantitatively and qualitatively. Twenty-nine teeth with necrotic pulp were included, of which 23 (9 test and 14 control) were analyzed. Three teeth in the test group reacted in the first year after REP. Except for

2, all analyzed teeth survived up to 3 years after REP and, in case of failure, apexification preserved them. Complete PBR was obtained in 91.3% and 87% of cases based on qualitative and quantitative assessments of PR, respectively, with no significant difference between groups from baseline. Quantitative change in PR in SD at the last recall session from baseline was not significant (all p -values >0.05) in either group. The qualitative assessment of the REP healing type was not uniform. In the test group, 55.6% of the teeth did not show SD or apical closure. Only 50% of the 14 teeth evaluated with CBCT showed complete PBR. Concerning volumetric measurements in SD 3 years after REP for change from baseline in root hard tissue volume, mean root hard tissue thickness, and apical area, the control group performed significantly in favor of the SD than the test group ($p= 0.03, 0.003, \text{ and } 0.05$, respectively). For volumetric change 3 years after REP from baseline in root length and maximum root hard tissue thickness, no significant differences ($p=0.72$ and 0.4 , respectively) were found between groups. The correlation between PR and CBCT variables assessing SD was weak (root elongation) to very weak (root thickening). Therefore, REP-LPRF appears to be a viable treatment option to obtain PBR and aid in the SD of necrotic immature permanent teeth.

For a better understanding of this, bone morphogenetic proteins (BMP) function as growth factors with a specific role in the proliferation and differentiation of mesenchymal stem cells from adipose tissue [21]. BMP-4 is involved in the initial stages of osteogenesis, in addition, it was demonstrated that the differentiation of human mesenchymal stem cells into the osteogenic lineage requires the presence of BMP-4 in the first days of culture and that these cells, after 21 days express specific proteins of the osteogenic lineage such as osteonectin, osteocalcin and osteopontin [22]. Three fundamental parameters in bone tissue engineering that will determine the osteoinduction capacity are the presence of soluble osteoinductive signals, the viability of undifferentiated mesenchymal stem cells in responding, the ability to differentiate into bone-forming cells, and the production of extracellular matrix. adequate [22].

Tissue engineering contemplates numerous advantages that meet the needs of the injured tissue or organ for the regeneration process. For this, it is necessary to understand the chemical, physical and biological processes, both biological material and the biological niche of the host. Crossing compatible information between microenvironments enables cell recognition and signaling cascades for neovascularization. Another advantage is the minimally

invasive surgical intervention, that is, it allows the use of faster surgical techniques that cause less risk to the patient [21,22]. Added to this, MSCs induce the expression of junction proteins and increase microvascular integrity and the production of nitric oxide (NO) by macrophages. The stromal vascular fraction (SVF) derived from MSCs is a heterogeneous mixture of cells, including fibroblasts, pericytes, endothelial cells, blood cells, and adipose tissue-derived mesenchymal stem cells (AMSC) [23].

Added to this, exosomes stand out along with AMSC. Exosomes are extracellular vesicles with a size of 40-100 nm in diameter and a density of 1.13-1.19 g/mL, containing proteins, mRNAs, miRNAs, and DNAs. Exosomes change the biochemical characteristics of recipient cells through the delivery of biomolecules and play a role in cell communication. These vesicles are produced from body fluids and different types of cells. Evidence suggests that AMSC-derived exosomes exhibit AMSC-like functions with low immunogenicity and no tumorization [24].

In this sense, the composition of exosomes differs based on their sources. Rabs and Annexin, including Annexin I, II, V, and VI are cytosolic proteins present in exosomes that contribute to exosome docking, membrane fusion, and kinetic regulation of cytoskeletal membranes. Furthermore, adhesion molecules such as intercellular adhesion molecule-1, CD11a, CD11b, CD11c, CD18, CD9 adipose tissue globule, EGFactor VIII (MFG-E8), CD58, CD146, CD166 have also been identified in exosomes. Exosomes also contain heat shock proteins (Hsp70 and Hsp90), which facilitate the loading of peptides into MHC I and II [25-27].

In addition, exosomes contain non-coding microRNAs or fragments. The exosomes contain miR-1, miR-15, miR-16, miR-17, miR-18, miR-181 and miR-375. In addition, various cytokines such as Tumor Necrosis Factor- α (TNF- α), Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interleukin (IL)-2, IL-6, IL-8, IL-10, IL-15, IL-1 β , are expressed in exosomes [28-30].

Based on this, normal bone formation and tissue repair involve coordinated interaction between bone-forming cells and biological signals. The main force in this process is the osteoblasts and their precursors. Osteoblasts can produce new bone along with biomaterials and can initiate the release of biological signals that guide bone formation and remodeling [1].

These biological signals attract bone-forming cells to the receptor site. Growth factors and other proteins are some biological signals that may be involved in new bone formation and tissue remodeling. In addition, through chemotaxis, there is a migration of bone-

forming cells to the area of application, as the stimulation of cell migration occurs in response to chemical stimuli [31].

In this sense, monocytes, macrophages, and endothelial cells contribute to bone remodeling, either through contact with osteogenic cells or through the release of soluble factors such as cytokines and GF. In the skeletal system, TNF- α stimulates bone and cartilaginous resorption and inhibits collagen and proteoglycan synthesis. IL-1 induces the expression of a wide variety of cytokines. LIF and IL-6 are two such molecules that are known to stimulate the differentiation of mesenchymal progenitor cells into the osteoblastic lineage, they are also potent anti-apoptotic agents for osteoblasts. In bone, the main sources of IL-6 are osteoblasts and not osteoclasts. Prostaglandin E2 (PGE2) is also directly related to the expression of the cytokine IL-6 [32,33].

A study by Liang et al. 2022 [34] showed that exosomes derived from mesenchymal stem cells (MSC-Exos) carry out the regulatory function of stem cells transporting proteins, nucleic acids, and lipids. Intervertebral disc degeneration (IDD) is one of the main causes of low back pain and is characterized by a decrease in the number of cells in the nucleus pulposus, decomposition of the extracellular matrix, aging of the annulus fibrosus and calcification of the cartilage endplate. In addition, nutrient transport and structural repair of intervertebral discs depend on bone and cartilage and are closely related to the state of the bone. Trauma, disease, and aging can cause bone damage. The recent fine-tuning of the MSC-Exos has led to significant progress in the treatment of IDD and bone repair and regeneration.

Conclusion

The greater potential of guided bone regeneration was associated with the graft material due to the higher grade of vital bone and the lower percentage of residual graft particles. Inorganic bovine bone and porcine bone minerals combined with autogenous maxillary cortical bone showed similar biological and radiological characteristics in terms of biomaterial resorption, osteoconduction, and osteogenesis when used for maxillary sinus floor augmentation. In this regard, three fundamental parameters in bone tissue engineering that determine the capacity for osteoinduction were evidenced, such as the presence of soluble osteoinductive signals, the viability of undifferentiated mesenchymal stem cells, having the ability to differentiate into bone-forming cells and production of adequate extracellular matrix. The exosomes that contain proteins, mRNAs, microRNAs, and DNAs stand

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Ethical Approval

Not applicable.

Informed consent

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Data sharing statement

No additional data are available.

Conflict of interest

The authors declare no conflict of interest.

Similarity check

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