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CHITOSAN SCAFFOLD, CONCENTRATED GROWTH FACTOR AND GINGIVAL MESENCHYMAL STEM CELLS AS THE OSTEOPOROTIC JAWBONE THERAPY : A REVIEW

Fianza Rezkita[1], Andari Sarasati[1], Fadhilah N. Wijaya[1], Alexander Patera Nugraha[2*], Nike Hendrijantini[3], Rini Devijanti Ridwan[4], Nastiti Faradilla Ramadhani[5], Amaq Fadholly[6], Arif Nur Muhammad Ansori[6] and Raden Joko Kuncoroningrat Susilo[7]

[1] Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
[2]Department of Orthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
[3]Department of Prosthodontic, Faculty of Dental Medicine, UniversitasAirlangga , Surabaya, Indonesia.
[4]Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
[5]Department of Dentomaxillofacial Radiology, Faculty of Dental Medicine, UniversitasAirlangga , Surabaya, Indonesia.
[6]Doctoral Program in Veterinary Science, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.
[7]Doctoral Program in Mathematics and Natural Sciences, Faculty of Science and Tech., Universitas Airlangga, Surabaya, Indonesia.
*Corresponding author e-mail: alexander.patera.nugraha@fkg.unair.ac.id

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ABSTRACT : Osteoporosis affects the oral and maxillofacial parts which can cause an osteoporotic jawbone (OJB). Osteoporosis therapy can be through both surgical and non-surgical approaches. One of the effective and optimal alternative therapies needed for OJB therapy is, for example, the regenerative therapy. The concept of regenerative therapies involving the triad of tissue engineering such as cells, growth factors and biomaterials can be applied in OJB therapy by combining the injectable chitosan scaffold (ICS) as a biomaterial scaffold, concentrated growth factor (CGF) as a growth factor, and gingival mesenchymal stem cells (GMSCs) as medicinal signaling cells. The purpose of this review was to describe the future prospect of ICS, CGF, and GMSCs as regenerative osteoporotic jaw bone therapy. ICS is biocompatible and biodegradable and easy to apply. It has the osteoinductive, osteoconductive, and osteotemplate properties. While, GMSCs are chosen compared to other MSCs because they are easily available and proliferate faster than Bone Marrow-derived Stem Cells (BMSC) and Dental Pulp Stem Cells (DPSCs).Besides, CGF is picked because, it has more fibrin and growth factors (GF) than Platelet Rich Fibrin (PRF) and Platelet Rich Plasma (PRP). The combination of ICS, CGF and GMSC has the potential and promising therapy in treating OJB.

Key words : Medicine, concentrated growth factor, gingival mesenchymal stem cells, osteoporosis.

INTRODUCTION

Osteoporosis is a chronic and progressive degenerative bone disease that affects most of senior citizens, especially in women. The number of elderly populations in the world is increasing in line with the higher increasing life expectancy, it becomes a global health challenge because it can affect one's quality of life. The prevalence of osteoporosis in the world reaches 200 million people, while in Indonesia based on data from the Ministry of Health of the Republic of Indonesia, there is as much as 1 in 4 women at risk of osteoporosis (Sozen *et al*, 2016; Health Ministry of Indonesia, 2015). The etiology of osteoporosis is divided into primary such as estrogen and testosterone deficiency and secondary such as calcium and vitamin D imbalances. The decrease in bone mineral density (BMD) and micro-architecture strength in osteoporosis patients affect all skeletal bones resulting in the bone fragility, thereby, the increasing risk factors for bone fractures (Li *et al*, 2017). Osteoporosis also affects the oral and maxillofacial parts which can cause osteoporotic jaw bone (OJB). OJB can cause alveolar bone resorption and jaw fractures causing morbidity in patients like osteointegration failure, loss of adhesion of dentures and a decreased stomatognathic function. Furthermore, regeneration of bone defects or bone remodeling cannot occur optimally resulting in a decreasing quality of life of the patients. In osteoporosis patients, there are a dysregulation of vascular endothelial growth factor (VEGF) and transforming growth factor-β1 (TGF-β1) which cause the disruption of bone regeneration and healing (Li *et al*, 2017). Osteoporosis therapy can be through both surgical and non-surgical approaches. Surgical therapy uses approaches such as arthroplasty, vertebroplasty, kyphoplasty, while, the non-surgical therapeutic approaches apply anti-bone resorption drugs that still have dangerous and harmful side effects. The use of drugs such as bisphosphonate can cause osteonecrosis of the jaw or bisphosphonate-related osteonecrosis of the jaw (BRONJ), calcitonin therapy is able to lead to malignancy like prostate cancer, and raloxifene can have an impact on increasing the risk of thromboembolism and stroke. These drugs only inhibit bone resorption that has already occurred without healing effects (Pavone *et al*, 2017; Paspaliaris and Kolios, 2019). One of the more effective and optimal alternative therapies needed for OJB therapy is by employingthe regenerative therapy with a tissue engineering approach. The concept of regenerative therapies involving the triad of tissue engineering; such as cells, growth factors and biomaterials; can be adopted in therapy OJB by combining injectable chitosan scaffold (ICS) as a biomaterial scaffold, concentrated growth factor (CGF) as a growth factor and gingival mesenchymal stem cells (GMSCs) as medicinal signaling cells.

Meanwhile, ICS is biocompatible and biodegradable and easy to apply. ICS has the ability of osteoinductive, osteoconductive and osteotemplate and is able to increase attachment, migration, proliferation and differentiation of stromal cells, thus, ICS is the right choice as scaffold (Mekhail and Tabrizian, 2014; Lan and Zhang, 2015). Previous studies using Mesenchymal Stem Cells (MSC) showed a significant increase in BMD in osteoporosis sufferers, while the addition of CGF to MSC, especially GMSCs, can support and accelerate osteogenic proliferation and differentiation (Chen *et al*, 2019). GMSCs are chosen compared to other MSCs because they are easily available and proliferate faster than bone marrow-derived stem cells (BMSC), Adipose Derived Mesenchymal, Stem Cells and dental pulp stem cells (DPSCs), stem Cell from human exfoliated deciduous teeth (SHED), Hair follicle derived MSCs (Chen *et al*, 2019; Narmada *et al*, 2019; Sari *et al*, 2019; Suciadi *et al*, 2019; Prahasanti *et al*, 2020; Rantam *et al*, 2020). Moreover, CGF is chosen because they have more fibrin and growth factors (GF) than platelet rich fibrin (PRF) and platelet rich plasma (PRP). The combination of ICS, CGF and GMSCs may increase the rate of bone remodeling and regeneration in OJB. The purpose of this review was to describe the future prospect of injectable chitosan scaffold, concentrated growth factor, and gingival mesenchymal stem cells as the regenerative osteoporotic jawbone therapy.

Osteoporotic Jaw Bone

Osteoporosis is a degenerative and metabolic disease of bone that causes bone biological changes leading to the decreased bone mass, weakened bone tissue microarchitecture, resulting in the changes in bone biomechanics and an increased risk of fracture (Pouresmaeli *et al*, 2018; Tomasevic-Todorovic*et al*, 2018). According to the World Health Organization (WHO), osteoporosis occurs when a person has a BMD of 2.5 or lower compared to the average in the young adult population or has a calculation of bone density or a T-score of -2.5 or lower 1. Around 200 million people suffer from osteoporosis and around 8.9 million experience pathological fractures due to osteoporosis, which can cause a significant decrease in quality of life through the increased morbidity and mortality due to complications and comorbidity of the disease (Tomasevic-Todorovic *et al*, 2018; Akkawi and Zmerly, 2018).

The main predominant factor for bone loss is the estrogen deficiency resulting in a primary osteoporosis. The pre-menopausal and menopausal phase women will experience a significant decrease in the levels of the hormones estrogen and progesterone, thus, this prevalence makes bone loss occurs more in women than men (Tu *et al*, 2018). Estrogen deficiency causes an increase in the number of receptor activators of nuclear factor kappa-ligand (RANKL), a decrease in osteoprotegerin (OPG) and a decrease in angiogenesis (Portal-Núñez *et al*, 2016). When the bone remodeling process occurs, osteoclasts have a shorter life span compared to osteoblasts, thus, the resorption phase (2-4 weeks) is shorter than the formation phase (4-6 months). The activation frequency of bone remodeling increases 2-3-fold in the elderly and post-menopause. This induces an increase in osteocyte and osteoblast apoptosis characterized by the increased expression of IL33, annexin, caspase-3 and an increase in osteoclastogenesis and osteoblast activity (Okman-Kilic, 2015). The increased osteoclasts activity is characterized by increased expression of nuclear factor associated t-cell 1 (NFATc1), which is a gene of osteoclasts and enzyme secretions bone resorption as cathepsin k (ctsk), tartate resistant acid phosphatase (TRAP) and carbonic anhydrase II (CA II) (Iseme *et al*, 2017; Jeong *et al*, 2019). The secondary osteoporosis is caused by comorbid diseases, medications or malnutrition, such as calcium deficiency, vitamin D and sex hormone disharmony. The secondary osteoporosis is found in people with endocrine disorders (Tu *et al*, 2018). In addition, a significant decrease in the volume of blood vessels in the bone marrow causes a decrease in bone perfusion. This is related to estrogen deficiency which causes bone loss (Filipowska *et al*, 2017).

The main components of bone consist of 60% hydroxyapatite (inorganic), 10% water, and 30% protein (organic), therefore, the reduction in bone mass in patients with osteoporosis can be correlated with the risk of increasing alveolar bone porosity. The effect of decreasing density on the jawbone causes a reduction in the volume of the residual ridge (Owen and Reilly *et al*, 2018). Osteoporosis is related to the structure stability of the jawbone where the alveolar bone serves as a tooth supporting bone. The reduced systemic BMD, bone volume (BV) and bone volume fraction (BV / TV) in osteoporosis can make the jawbone susceptible to increase the alveolar bone resorption which lead to OJB (Schulz *et al*, 2017).

OJB causes depletion of the bone cortex, alveolar bone resorption, mandibular ridge, and bone medulla density. The lower jaw has spongious bone characteristic, whereas, the maxilla is compact so that the lower jaw resorption is easier to occur when vascularization is disrupted and osteoclast activity increases. Alveolar bone resorption due to OJB leads to the failure of various dental treatments, especially in the field of prosthodontics, such as implant retention failure due to the disruption of osseointegration. In addition, OJB can exacerbate and accelerate bone resorption caused by the production of lipopolysaccharide endotoxins due to periodontal pathogenic bacteria infections such as *P. gingivalis* in patients with low oral hygiene levels (Touyz, 2014; Zhang *et al*, 2014). OJB can also have a negative effect on tooth stability and alveolar crest. Prosthodontic treatment in OJB patients is also risky, because OJB bone defects are more susceptible to injury due to the mechanical stress on the residual ridge that has excessive resorption (Bandela *et al*, 2015). In addition, bone loss caused by OJB makes the making of artificial teeth will no longer be possible, which causes the impaired masticatory function, speech disorders and the decreased quality of life of patients (Marya and Dhingra, 2015).

Injectable Chitosan Scaffold

Chitosan is derived from crustacean waste containing polysaccharides after the chitin deacetylation process. According to the data from the Indonesian Ministry of Maritime and Fisheries Affairs, shrimp waste reaches 26,000 tons and is only used by around 30% (Dompeipen, 2016). Abundant shrimp waste should be able to be processed into chitosan as a scaffold. Various studies show chitosan has biocompatible and non-toxic properties because it has glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) components that are compatible with mammalian body tissues (Rodríguez-Vázquez *et al*, 2015; Rezkita *et al*, 2020). GlcN in chitosan can damage bacterial walls so that chitosan has anti-microbial properties. In addition, chitosan has a biodegradable nature, rapid gelation, controlled porosity and the ability to dissolve under slightly acidic conditions, therefore, it can be used as ICS in a medical regenerative therapy. The choice of injection application using the osteoperfusion technique aims to make the ICS filling the bone trabecula properly. Furthermore, ICS has the ability to change form into a gel when it is penetrated into the body through physical stimulation (pH and temperature), chemical stimulation (photo-cross-linking, chemical cross-linking, ionic-cross-linking, and polymer interactions) and biological stimulation (enzymatic cross-linking). Also, ICS is flowable when injected and has the ability to change shape into a gelrapidly in the oral cavity due to physical stimulation of the oral cavity in the form of normal pH (pH 6-7) and normal oral temperature of about 37ºC. In addition, ICS has biological and chemical components similar to the normal body tissues, thus, it can accommodate the proliferation and differentiation of MSC and has osteoinductive, osteoconductive, and osteotemplate properties. Finally, ICS has the ability to increase the retention, accumulation, and penetration of CGF & GMSCs on the hard and damaged tissues (Chen *et al*, 2019; Jain *et al*, 2015; Hu *et al*, 2018).

Concentrated Growth Factor

CGF is an organic matrix rich in fibrin, containing GF, platelets and leukocytes that play the roles in the regeneration process (Chen *et al*, 2018). CGF is known to have higher tensile strength, GF and viscosity than PRF, thus, the compressed CGF can be used as a protective membrane with GF. These protectors induce faster the bone formation and soft tissue healing (Upadhayaya *et al*, 2017).

CGF accelerates the process of osteogenesis. CGF administration in rabbits with calvaria defects had a significantly higher ratio of new bone volume compared to PRP and PRF (Kim *et al*, 2014). CGF secretes various GFs such as platelet-derived growth factor (PDGF), TGF-β, TGF-β2, fibroblast growth factor (FGF), VEGF and insulin-like growth factor (IGF), which stimulate cell proliferation, matrix remodeling and angiogenesis (Yu and Wang, 2014). PDGF-AB, TGF-β1 and IGF-I have the constant kinetic release and reach their maximum on the 3rd and 6th day, respectively. VEGF and bone morphogenetic protein-2 (BMP-2) have the slow kinetic release and reach a maximum on the 8th day. GF plays a role in osteoblast proliferation and differentiation

(Nareswari *et al*, 2019). PDGF-AB increases the secretion of collagen and glycoprotein by osteoblasts to synthesize bone matrix through the action of osteoblasts and participate in calcification of bone matrix (Hu and Olsen, 2016). VEGF stimulates angiogenesis which is an important stage in the process of bone regeneration because the blood supply supports osteogenesis (Wang *et al*, 2019). Angiogenesis and osteogenesis have mutually reinforcing effects on bone regeneration. Angiogenic factors play an important role in healing and regeneration, while VEGF can induce the mobilization, recruitment, proliferation and differentiation of endothelial progenitor cell, and the recruitment and survival of osteoblasts (Zhou *et al*, 2015). CGF promotes neovascularization better than groups without CGF. Neovascularization increases the disturbed angiogenic capacity and facilitates bone healing. Study by Chen *et al* (2018) used wistar rats with calvaria defects and then analyzed histologically in the sixth and twelfth weeks postoperatively to determine the effect of CGF in osteogenesis. The results showed the group given CGF treatment with BMSC revealed the presence of new bone formed in the defect area with a large distribution of collagen fibril matrixin the sixth week postoperatively. The CGF treatment group showed the formation of several new bone tissue and fibrous tissue in the calvaria defect areaonly on the sixth day postoperatively. New bone formed in the CGF and BMSC and CGF treatment groups only appeared in the twelfth week after surgery almost covered half of the defective calvaria bone, in addition, there was neovascularization at the same observation time in both treatment groups, thus, it can be concluded that CGF can induce osteogenic differentiation and angiogenesis (Chen *et al*, 2018).

Gingival Mesenchymal Stem Cells

GMSCs are stem cells that can be isolated from gingival lamina propria (Venkatesh *et al*, 2017; Nugraha *et al*, 2018a). GMSCs can be isolated from free gingiva, attached gingiva, and hyperplastic gingiva but without a history of periodontal disease with aseptic techniques to avoid infection and inflammatory contamination (Jin *et al*, 2015; Du *et al*, 2016) GMSCs express positive MSC markers of Cluster of Differentiation (CD) such as CD73, CD90, CD105, CD44, CD146, CD166, CD271, SSEA-4, STRO-1 and vimentin (Jin *et al*, 2015; Nugraha *et al*, 2018b; Nugraha *et al*, 2018c). GMSCs have an advantage over MSCs that originate from other sources because of their abundant numbers and are easily accessed with minimally invasive cell isolation techniques.

GMSCs can differentiate in osteogenic, adipogenic, chondrogenic, endothelial, and neural (Nugraha *et al*, 2018c; Grawish, 2018; Fawzy *et al*, 2016a). Osteogenic differentiation is evidenced by the formation of calcified deposits through alizarin-red staining or through microscopic transmission electrons that show cellular properties of osteoblasts (Fawzy *et al*, 2016a). Osteogenic differentiation of GMSCs is also evidenced at the mRNA level through the increased expression of runt related transcription factor 2 (RUNX2), which is a marker of the early phase of osteogenic differentiation, alkaline phosphatase (ALP), which plays a role in hard tissue mineralization and osterix (OSX), which is a specific transcription factor for osteoblasts (Sitasari *et al*, 2020). In addition, GMSCs are also known to increase the expression of collagen type 1 (Col-1), collagen type iii (Col-3), osteonectin (OSN) and osteopontin (OPN) (Fawzy *et al*, 2016a; Monterubbianesi *et al*, 2019; Vidoni *et al*, 2019; Nugraha *et al*, 2019a; Nugraha *et al*, 2018d). Chondrogenic differentiation seen in alcian-blue staining and the expression of sox-9, aggrecan and Col-II. Adipogenic differentiation can be seen in oil-red-o staining and expression of gamma peroxisome proliferator-activated receptor (PPARg), fatty acid synthesis and lipoprotein lipase (LPL) (Nugraha *et al*, 2018c; Fawzy *et al*, 2016a; Nugraha *et al*, 2019b).

GMSCs are immunomodulatory through expression of toll-like receptors (TLRs), inhibition of maturation and excessive activation of dendritic cells (DC), increase in anti-inflammatory cytokines interleukin-10 (IL-10), IL-6, granulocyte-macrophage colony-stimulating factors (DC) GM-CSF) and suppression of pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) (Fawzy *et al*, 2016b).

GMSCs are known to express TLRs 1-10, which are pattern recognition receptors (PPRs) that detect pathogen-associated molecular patterns (PAMPs) and specific damage-associated molecular patterns (DAMPs) in the innate immune system (Fawzy *et al*, 2016b; Ito, 2014; Todd and Palmer, 2017; Najar *et al*, 2017). TLR2 activation shows osteogenic, pro-inflammatory and anti-inflammatory responses, while TLR3 activation shows anti-inflammatory response, whereas TLR4 activation enhances osteogenic responses through Wnt3a and Wnt5a signaling and pro-inflammatory responses (Najar *et al*, 2017; Mekhemar *et al*, 2018). GMSCs are known to reduce monocyte CD11b expression by 40% and suppression of T lymphocyte proliferation through the indoleamine signal 2,3-dioxygenase (IDO), which then reduces the levels of Interferon-γ (IFN-γ) and IL-4 (Mekhemar *et al*, 2018; Huang *et al*, 2017). GMSCs also decreases the expression of CD86 as a marker of macrophage pro-inflammatory by 42.4% and increases the expression of CD206 as a marker of anti-inflammatory

M2 macrophages significantly (Zhang *et al*, 2018; Xia *et al*, 2015). GMSCs have better proliferation properties than BMSCs and DPSCs and are morphologically stable and non-teratogenic, although they originate from healthy tissue or hyperplastic or inflammatory tissue. This non-teratogenic nature is caused by the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by GMSCs, which plays a role in apoptosis and necrosis of cancer cells (Todd and Palmer, 2017; Xia *et al*, 2015).

The proliferation of GMSCs is characterized by the expression Oct-4, Nanog and Sox2 which are pluripotent transcription factors of MSCs and also play a role in self-renewal and survival of MSCs (Pavone *et al*, 2017; Tomasevic-Todorovic, 2015). GMSCs express Oct-4 at 98.23% and Nanog at 58.77%. The decreased and inactivated Oct-4, Nanog and Sox2 expressions are found to significantly reduce the ability of MSCs to differentiate into osteoblasts by inhibiting upregulation of RUNX2 (Matic *et al*, 2016; Gentile *et al*, 2019; Malvicini *et al*, 2019). GMSCs also reveal better migration and angiogenic potential compared to DPSCs (Fawzy *et al*, 2016b; Malvicini *et al*, 2019). This was proven by previous study that administration of systemic GMSCs through cell homing can migrate towards mandibular bone defects and increase the bone regeneration. The mechanism of cell homing is known through the expression of CXC chemokine receptor type 4 (CXCR4) by GMSCs which are bound to chemokine stromal cell-derived factor-1 (SDF-1), wherein GMSCs will migrate to the target network with signals from GF such as IGF-1 and PDGF (Angelopoulos *et al*, 2018; Xu *et al*, 2014; Liu *et al*, 2015). GMSCs transplants can form connective tissue-like structures, whereas transplanted DPSCs and periodontal ligament stem cells (PDLSCs) form dentin-like and cementum / periodontal ligament-like structures. This causes GMSCs to be superior to another oral cavity MSCs when applied on extraoral or intraoral. These superior properties show the potential of GMSCs to be the best source of mesenchymal cells in regenerative dentistry.

CONCLUSION

Based on a literature reviews, it can be concluded that regeneration therapy with tissue engineering triad approach using a combination of ICS, CGF and GMSC has the potential for OJB therapy. Further research needs to be done on the relationship between the combination of ICS, CGF and GMSCs, therefore,the innovations in OJB alternative therapy can be implemented clinically and can be used as potential alternative therapies.

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