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Review article

TRIAD TISSUE ENGINEERING : GINGIVAL MESENCHYMAL STEM CELLS, PLATELET RICH FIBRIN AND HYDROXYAPATITE SCAFFOLD TO AMELIORATE RELAPSE POST ORTHODONTIC TREATMENT

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ABSTRACT : Relapse post-orthodontic treatment rate approximately 61.5% and this phenomenon is a problem in the orthodontic field that cannot be solved yet. Efforts to prevent have been carried out such as retention devices, but require a fairly long duration. The need for the latest effective methods to inhibit relapse post-orthodontic treatment is tissue engineering approach. The aim of this review was to summarize tissue engineering approach to ameliorate relapse post orthodontic tooth movement. The tissue engineering approach in inhibiting relapse is by combining biomaterials between Gingival Mesenchymal Stem Cells (GMSCs), scaffold (Hydroxyapatite) and growth factors (Platelet Rich Fibrin). GMSCs have an ability to differentiate into osteogenic lineage, immunoregulator and immunomodulation property. Thus, it has autocrine and paracrine effect that can stimulate endogenous stem cell. PRF is an autologous blood line that contains concentrated platelets consisting of a strong fibrin matrix with mechanical properties to assist remodeling process. It also has abundant growth factor that can facilitate GMSCs to differentiate and proliferate optimally. Efforts are needed to accelerate remodeling alveolar bone post-orthodontic treatment. Hydroxyapatite scaffold is a good choice as a osteogenic template for GMSCs. It can act as an osteoinductive and osteo-conductive that can stimulate GMSCs' osteogenic differentiation. Furthermore, the combination of those biomaterial is expected to ameliorate relapse post orthodontic treatment.

Key words : Gingival mesenchymal stem cells, platelet rich fibrin, bovine hydroxyapatite, relapse, orthodontic treatment.

INTRODUCTION

The prevalence of malocclusion in Indonesia reaches 80%, thus demand for the use of fixed orthodontic treatment is high (Ardani *et al*, 2014). Relapse post-orthodontic treatment rate approximately 61.5% and this phenomenon is a problem in the orthodontic field that cannot be solved yet (Sutijati *et al*, 2017). Relapse can occur because remodeling of the alveolar bone and principal periodontal fibers are rudimentary. Efforts to prevent have been carried out such as retention devices, but require a fairly long duration of at least 6 months and are highly dependent on the patient's level of cooperation, thus other efforts are needed to accelerate remodeling alveolar bone post-orthodontic treatment (Armstrong *et al*, 2017). The need for the latest effective methods to inhibit relapse post-orthodontic treatment is tissue engineering approach.

Previous research has been done by combining Carbonated Hydroxyapatite Scaffold and Platelets Rich Fibrin (PRF) resulting in a decrease in relapse, but relapse is still found (Alhasyimi *et al*, 2018). The tissue engineering approach in inhibiting relapse is by combining biomaterials between Gingival Mesenchymal Stem Cells (GMSCs), scaffold (Hydroxyapatite) and growth factors (Platelet Rich Fibrin) (Nugraha *et al*, 2018; Septiani *et al*, 2019). Bovine Hydoxyapatite hydrogel scaffold is made from minerals bovine hydroxyapatite developed in Dr. RSUD Soetomo Surabaya which has properties zero rejection and good biocompatibility for cells and tissues after transplantation (Saskianti *et al*, 2017). Biohydrox® hydrogel scaffold can be combined with PRF which has many growth factors and has the potential to provide a good niche while GMSCs are cultured (Saskianti *et al*, 2017). GMSCs were chosen because they were easily

isolated by minimally invasive, proliferative ability of multipotent differentiation of mesenchymal lineages, accelerated regeneration of periodontal tissues (Fawzy *et al*, 2016; Iizuka *et al*, 2017; Nugraha *et al*, 2018; Rantam *et al*, 2018). Furthermore, purpose of this review is to disclose the combination of Bovine Hydroxyapatite hydrogel scaffold, PRF and GMSCs to ameliorate relapse post-orthodontic treatment.

Relapse post-orthodontic treatment

Orthodontic treatment cause in structural changes in the alveolar bone and periodontal ligament fibers (Alhasyimi *et al*, 2018). Movement of teeth will produce two zones, namely compressive and tension zone. Compressive zone causes alveolar bone to decrease in density because there is an increase in resorption activity by osteoclasts, whereas in the tension zone there is a remodeling process carried out by osteoblasts and stretching of periodontal ligament fibers (Sutijati *et al*, 2017). Post-orthodontic treatment affect alveolar bone and periodontal ligament fibers in unstable conditions (Maleeh *et al*, 2016; Rajendran *et al*, 2015). It happens because the process of remodeling tissue takes more than 4 months (Littlewood *et al*, 2017). If the condition is ignored, it will cause relapse condition. Relapse is the condition the tooth return to its original position after the release of the orthodontic device and it is a sign of failure of orthodontic treatment. In relapse condition, a material is needed to help accelerate remodeling process both alveolar bone and periodontal ligament, one of which is a combination of Bovine Hyroxyapatite hydrogel with PRF which is seeded with GMSCs (Alhasyimi *et al*, 2018).

Biohydrox®

Biohydrox® is a biomaterial developed by Dr. Soetomo Surabaya. Biohydrox® contains hydroxyapatite made from bovine with a microarchitecture and mineral composition resembling human bone namely. The advantage of using Biohydrox® is it has zero rejection properties in its application to the body along with the process of adhesion and proliferation is higher than carbonate apatite scaffold (Saskianti *et al*, 2017).

Platelet rich fibrin

PRF is an autologous leukocytes rich in fibrin matrix and growth factors (Kim *et al*, 2017). Compared to other platelet concentrations PRF has better healing and regeneration effectiveness because it is obtained from the centrifugation process, without biochemical treatment, and does not cause rejection to the body (Khiste *et al*, 2013). The fibrin content in PRF acts as a scaffold to facilitate the process of osteogenic differentiation and the process of osteogenesis from GMSCs. Growth factors contained in platelets include TGF-β, IGF, VEGF, FGF, and PDGF (Nugraha *et al*, 2019). All of these growth factors have an angiogenic, hemostatic and osteoconductive role. TGF-β plays a role in stimulating osteoblast proliferation, stimulates bone matrix formation, and activates blood vessel formation (Kim *et al*, 2017). IGF plays a role in stimulating osteoblast migration, proliferation and differentiation. VEGF initiates the angiogenesis process to increase tissue oxygenation. FGF acts as a chemotactic factor to help the proliferation process of osteoblasts, while also contributing to increasing OCN expression. PDGF regulates the process of migration, proliferation and survival of MSCs has a mitogenic effect on MSCs and osteoblasts (Borie *et al*, 2017; Uthappa *et al*, 2017).

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are cells with the ability to differentiate into specific cells derived from mesoderm (Nugraha *et al*, 2019). MSCs are known as multipotent cells, are self-renewable, and can be cultured in vitro with good genome stability. The source of efficient MSCs based on research is from the bone marrow (Nugraha *et al*, 2018) cells that show the characteristics of MSCs are isolated from adipose tissue, amniotic water, amniotic membrane, endometrium, menstrual blood, peripheral blood, placenta and fetal membrane, salivary glands and synovial fluid (Ullah *et al*, 2015; Egusa *et al*, 2012; Rantam *et al*, 2018; Septiani *et al*, 2019). The criteria for MSCs are it has plastic adherent properties when in culturing conditions, must express CD105, CD73, CD90, but must not express CD45, CD11b, CD34, CD14, CD79Q, CD19 and HLA-DR molecules, together with MSCs *in vitro* must have the ability to differentiate into osteoblasts, adipocytes and chondroblasts (Nugraha *et al*, 2018; Rantam *et al*, 2018).

Gingival mesenchymal stem cells

The periodontal tissues consist of alveolar bone, periodontal ligament, cementum and gingiva, develop and function as a single unit. The majority of embryonic periodontal tissues originate from the neural crest ectomesenchyme. Gingiva is histologically composed of epithelium and connective tissue. One of the characteristics of gingiva is the ability in wound healing and the regeneration process which quickly reconstructs the tissue structure due to injury or excision with little or no scar tissue (Newman *et al*, 2018).

GMSCs are chosen because they can be easily isolated from the layers of the lamina propria gingiva (Rantam *et al*, 2018). Compared to other stem cells in the oral cavity, the gingival extraction process is easier

to do and does not require invasive process therefore it can minimize the occurrence of discomfort. The healing process of gingival tissue is faster than the wound on the skin and does not leave scar tissue. GMSCs can be isolated from gingival tissue from medical waste post-procedure gingivectomy (Nugraha *et al*, 2019). The most important consideration in the use of GMSCs is the stability of the phenotype and no tumorigenic activity even though it is isolated from healthy or hyperplastic gingiva (Fawzy *et al*, 2016; Angelopoulos *et al*, 2018).

Gingival Mesenchymal Stem Cells (GMSCs) are stem cells that are superior to other stem cells in the field of regenerative medicine. When compared with Bone Marrow Stem Cells (BMSCs), GMSCs are preferred because they only occur minimally invasive at the isolation time (Fawzy *et al*, 2016; Nugraha *et al*, 2018). GMSCs have stable morphology, uniform homogeneity and are able to proliferate rapidly. GMSCs are multipotent *in vitro* so that they have the potential to turn into adipocytes, chondrocytes, osteoblasts, endothelial cells and neuron cells. *In vivo*, GMSCs can turn into connective tissue, bone and cartilage. GMSCs express more than 95% surface markers CD73, CD90 and CD105 and very little on the surface containing CD11b, CD14, CD19, CD34, CD45 and CD79α (only about 2%). GMSC cultures also express Stro-1, Oct-4 and Nanog which are beneficial for transcription factors in stem cells (Fawzy *et al*, 2016; Iizuka *et al*, 2017; Nugraha *et al*, 2018; Rantam *et al*, 2018).

Previous study shown that GMSCs have excellent osteogenic differentiation ability and that this ability has significantly increased with the addition of PRF characterized by an increase in osteogenic differentiation markers such as Bone Alkaline Phosphatase (BALP), Runt-related transcription factor-2 (RUNX2), Osteocalcin (OSC), Osteonectin (OSN) and Osteopontin (OSP) (Nugraha *et al*, 2018; Nugraha *et al*, 2019).

Role of GMSC, PRF and Bovine hydroxyapatite prevent orthodontic relapse

GMSC is a stem cell obtained from the gingiva and its proposed as one of the excellent components to inhibit relapse because it has the ability to (1) renew itself, (2) osteogenic differentiation, (3) anti-inflammatory (Nugraha *et al*, 2018; Rantam *et al*, 2018; Nugraha *et al*, 2019). The ability to renew itself is the basic nature of stem cells that are owned by GMSCs. As a stem cell, GMSCs are able to increase the number by dividing it into two parts of the cell that have the same properties. Both are able to reproduce themselves or differentiate according to the micro environment (Fawzy *et al*, 2016; Iizuka *et al*, 2017). Some studies show that GMSCs have the ability to reproduce better than BMSCs and DPSCs using colony formation tests. Unlike BMSCs, which experience abnormalities when continuously divided, GMSCs show more stable conditions and do not lose traits as MSCs even though they are divided in large numbers (Iizuka *et al*, 2017; Arvidson *et al*, 2011; Hu *et al*, 2018).

GMSC has the ability to differentiate osteogenic into osteoblasts, this has been demonstrated *in vitro* by Du *et al* (2016), which showed a calcified deposit formation in Alizarin-Red staining. Test using electron microscopy shows the nature of osteoblasts, namely the presence of mitochondria with extensive morphology, presence of vacuoles for exocytosis, formation of extracellular matrix, collagen and mineralized areas (Iizuka *et al*, 2017). The differentiation capability of GMSCs is influenced by the conditions of the micro environment such as; growth factors, hormones, and components of the extracellular matrix Differentiation of GMSCs into osteoblasts occurs because of the role of transcription factors (Du *et al*, 2016). It is a factor that helps and initiates the process of cell differentiation by regulating the expression of genes responsible for specific cell types (Fawzy *et al*, 2016; Nugraha *et al*, 2018; Nugraha *et al*, 2019).

RUNX2 and Osterix (OSX) are transcription factors important in differentiating MSCs into osteoblasts (Nugraha *et al*, 2018). RUNX2 is a master regulator of osteoblasts, which plays a role in regulating early osteogenic differentiation from MSC, besides that RUNX2 regulates gene expression related to osteoblasts including Alkaline phosphatase (ALP), OPN, OSX, COL1A1 (type-I collagen), Bone sialoprotein (BSP) and OCN (Nugraha *et al*, 2018; Nugraha *et al*, 2019). The presence of RUNX2 can help differentiate osteoblasts and inhibit adipocyte differentiation from MSCs, in addition MSCs in conditions of RUNX2 deficiency will cause failure of osteoblast differentiation (Zhang *et al*, 2011; Zhang *et al*, 2012; Rutkovskiy *et al*, 2016). RUNX2 as an osteogenic regulator binds to DNA by forming heterodimers containing core binding factor beta (Zhang *et al*, 2011). The second factor transcription is OSX, which is an advanced stage induced by RUNX2, expressed by osteoblasts that play a role in the formation of new bones (Zhang *et al*, 2011; Zhang *et al*, 2012). Moreover, OSX stimulates the final differentiation of pre-osteoblast into adult osteoblasts. RUNX2 and OSX has ability to induce the expression of ALP, BSP, and OCN. The absence of OSX also causes MSCs to not differentiate and form new bones (Zhang *et al*, 2011).

In addition to having the ability to be osteogenic differentiated, GMSCs have similarities as other MSCs,

namely immunomodulatory and anti-inflammatory abilities. Generally, this ability causes MSCs not to stimulate the over activity of immune cells, so that they can be used for allogeneic transplants without taking immunosuppressive action on host (Fawzy *et al*, 2016; Iizuka *et al*, 2017). Immunomodulatory abilities of GMSCs include the adaptive and innate immunity response. In the adaptive immune system GMSCs lowered proliferation and activation of T cells that is dependent on phytohemagglutinin by increasing the production of IL-10 and decrease the secretion of tryptophan mediated by indoleamine2, 3-dioxygenase (IDO) (Iizuka *et al*, 2017). Furthermore, the innate imunity GMSCs affect the work by modulating macrophage polarization proinflammatory macrophages into anti-inflammatory macrophages. This causes a decrease in the number of cytokines that play a role in inflammation (IL-1, TNF-α, IL-6, MMP-9) and increases anti-inflammatory cytokines (IL-10, TGF-â) (Fawzy *et al*, 2016; Caplan, 2017; Caplan and Sorrel, 2015; Caplan and Correa, 2011). Osteogenic differentiation of GMSCs is regulated by transcription factors thus to activate the work of transcription factors its needed stimulation of some growth factors (Rutkovskiy *et al*, 2016; Nugraha *et al*, 2018; Nugraha *et al*, 2019).

In accordance with the concept of tissue engineering for regenerative medicine, to achieve therapeutic success scaffold is needed in addition to stem cells and growth factors (Egusa *et al*, 2012; Kartikasari *et al*, 2016; Alhasyimi *et al*, 2018). Scaffold is a three-dimensional structure that replaces the damaged extracellular matrix, serves to provide a place for interaction between cells and the formation of new extracellular matrix (Saskianti *et al*, 2017). The hydroxyapatite material contained in Biohydrox® is acceptable to the body's biological tissues and provides a place for good cell migration and vascularization (osteoconduction) (Saskianti *et al*, 2017). Osteoconduction ability in hydroxyapatite because it has porosity that is similar to human bone, the porosity possessed forms a micro environment similar to host that of as that it can facilitate growing and differentiating cells (Rushadi and Rantam, 2011; Bano *et al*, 2011; Kattimani *et al*, 2016). Biohydrox® is of the nature zero rejection that has been proven by the MTT test shows no difference in the results of MSCs cultured using Biohydrox® with the control group (Kartikasari *et al*, 2016). Other studies also prove that Biohydrox® has the ability to facilitate adhesion and proliferation stem cell better than carbonate (Saskianti *et al*, 2017).

CONCLUSION

Combination of Biohydrox® scaffold, PRF and GMSC has ability to inhibit post-orthodontic relapse by accelerating the osteogenic process. Further research is needed regarding the combination of Biohydrox® scaffold, PRF and GMSCs. This latest innovation is expected to be implemented in a real way and can inhibit relapse post-orthodontic treatment.

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