Periodontal ligament stem cells,

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PERIODONTAL LIGAMENT STEM CELLS, SOLCOSERYL PASTA INCOPORATED NANO-HYDROXYAPATITE SILICA GEL SCAFFOLD FOR BONE DEFECT REGENERATION IN CHRONIC PERIODONTITIS : A REVIEW

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ABSTRACT : Periodontitis is a periodontal disease with a prevalence of 11.2% in the world. Chronic periodontitis causes progressive loss of alveolar bone around the teeth. Xenograf or allograft implantation procedures are performed in the treatment of bone defects, but it may cause a immune reaction. Therefore, new innovations are needed to regenerate bone defects due to chronic periodontitis. Nano-hydroxyapatite has been widely used for bone regeneration and osseointegration in the field of dentistry because of its osteoconductive and osteoinductive properties. The aim of this review is describe the potential of Periodontal Ligament Stem Cells (PDLSCs) with solcoseryl paste incorporated nano-hydroxyapatite silica gel scaffold for the treatment of bone defects in chronic periodontitis. PDLSCs can differentiate into periodontal ligaments, alveolar bone, cementum, peripheral nerves, and blood vessels. PDLSCs has osteogenic abilities, which are characterized by the expression of bone markers such as type-1 collagen, Runx2, OCN and ALP. Solcoseryl is a dialysate protein-free which acts as a growth factor by activating the transport of oxygen and nutrients into cells, increasing proliferation, stimulating collagen synthesis and formation of granulation tissue. Nano-hydroxyapatite silica gel scaffold can stimulate the proliferation of PDLSCs. Nano-hydroxyapatite seeps into SiO2-sol, forms a porous nano scaffold and connects nano-hydroxyapatite crystallites to produce granules with high porosity and interconnected to stimulate the formation of new bone. PDLSCs with solcoseryl paste incorporated nano-hydroxyapatite silica gel scaffold may potential to regenerate bone defects in chronic periodontitis.

Key words : Periodontal ligament stem cells, solcoseryl paste, nano-hydroxyapatite silica gel scaffold, medicine, chronic periodontitis.

INTRODUCTION

Periodontitis is an inflammation of the periodontal tissue with a prevalence of 11.2% in the world and reaching 60% in Indonesia (Wijaksana, 2019). Periodontitis is a multifactorial infectious disease with a primary cause in the form of bacterial plaque, including *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* (Andriani and Chairunnisa, 2019). Chronic periodontitis is a type of long-term periodontal disease characterized by progressive damage of the tooth-supporting structures, both hard and soft tissue around the teeth (Machado *et al*, 2018; Natto *et al*, 2018). Root resorption is one of the complications that can occur due to periodontitis. The percentage of teeth that have root resorption will increase along with the increasing of periodontal disease severity (Mahajan *et al*, 2017). In addition, periodontal bone loss due to periodontitis can also occur horizontally or vertically, leading to the formation of bone defects. Bone defects bring specific problems in clinical practice (Singh and Kumari, 2017). Alveolar bone loss causes tooth mobility, drifting, flaring, and eventually tooth loss. In advanced cases, this disorder will affect the occlusion function (Könönen *et al*, 2019).

Antibiotics are used as inhibitors or controllers of bacterial infections because bacteria can colonize periodontal tissue, which makes non-surgical mechanical treatments such as scaling and root planing effective. Scaling and root planing is a periodontal tissue treatment that aims to cure tissue inflammation, eliminate pockets, and reduce the number of bacteria and pathogenic

products by using mechanical equipment. However, the effectiveness of both methods is reduced because of the increase in pocket depth. Meanwhile debridement also cannot be done especially on deep pockets and other areas that are difficult to achieve mechanically (Komara *et al*, 2019). Approximately 20%-30% of all cases of chronic periodontitis did not give a positive response to conventional periodontal treatment. Patients with severe chronic periodontitis may need surgical treatment through invasive procedures including open flap debridement and bone grafting (Shaddox, 2010). The main goal of surgical therapy using bone graft is to enhance bone regeneration (Prahasanti *et al*, 2020).

Allograft implantation procedures are performed in the treatment of bone defects, but can lead to immune reaction. Therefore, new innovations are needed to regenerate bone defects due to chronic periodontitis. Hydroxyapatite and Carbonate Apatite has been widely used for bone regeneration and osseointegration in the field of dentistry due to its osteoconductive and osteoinductive properties (Alhasyimi *et al*, 2018; Nugraha *et al*, 2019a). However, chronic inflammation causes mechanical properties and is difficult to absorb. Nano-hydroxyapatite (n-HAp) is a nanometer-sized hydroxyapatite (<100 nm) that can be used as a bone graft material and combined with silica gel silica. The nanostructures of this synthetic material simulate natural hydroxyapatite crystals in bone tissue. In addition, the combination of n-HAp and nanoporous silica gel (SiO_2) is superior because it can be absorbed to very porous structures, and good osteoconductivity (Hamid *et al*, 2018).

Periodontitis

The American Academy of Periodontology (AAP) defines chronic periodontitis as "an infectious disease that causes inflammation in periodontal tissue, progressive attachment loss, and bone loss" (Sistla *et al*, 2108). Periodontitis is one of the main causes of tooth loss (Hardhani, 2014; Nazir, 2017). The main etiology of chronic periodontitis is the accumulation of plaque by microorganisms. Bone attachment and loss are associated with an increase in the proportion of gram-negative organisms in subgingival biofilms, with specific increases in organisms such as *P. gingivalis*, *T. forsythia* and *T. denticola* - also known as "red complexes" (Sistla *et al*, 2108). These bacteria are gram-negative anaerobic bacteria which can grow into pathogens when the environmental conditions and host response support so that periodontitis will be induced (Nagashima *et al*, 2017). Clinical characteristics in patients with untreated periodontitis include symptoms such as the presence of supragingival and subgingival plaques, gingival swelling and redness, changes in gingival margins, the presence of pockets, bleeding on probing, horizontal or vertical alveolar bone involvement, involvement of root furcation and increased tooth mobility (Carranza *et al*, 2018).

Alveolar bone defects in Periodontitis

Periodontal disease begins with inflammation of the gingiva. If the problem is not treated, inflammation spreads to the bone and causes damage to the alveolar bone. Classification of periodontal bone loss was investigated in seven main groups: Horizontal and vertical (angular) defects, craters, furcation involvement, dehiscence, fenestration and combined endodontic periodontal lesions (Ozcan and Sekerci, 2017). The immune system response and the inflammatory response in periodontitis can interfere with bone remodeling which includes the apposition and resorption of bones, triggering alveolar bone defects. Collagenase activity is associated with periodontal damage. Matrix metalloproteinase-8 (MMP-8) enhanced in chronic periodontitis. Studies reveal that collagenase activity is six times greater than gingivitis in gingival cervicular fluid (GCF) in chronic periodontitis (Soud *et al*, 2018). Macrophage precursors such as Tumor Necrosis Factor-α (TNF-α), Interleukin (IL)-1β and Prostaglandin E2 (PGE2) in the inflammatory response can stimulate osteoclast differentiation so that alveolar bone resorption can occur. Proinflammatory cytokines such as IL-6, IL-11, IL-17, TNF-á are mediators that can also trigger alveolar bone resorption in periodontitis (Hienz *et al*, 2015).

TNF-α and IL-β are pro-inflammatory cytokines that are expressed as a result of chronic inflammatory cells such as macrophages and lymphocytes. TNF-α has an important role in the regulation of bone cells and bone resorption. The increase in TNF-α causes osteoclasts to increase in large numbers because TNF-α can express RANKL, so that it can cause alveolar bone resorption in periodontitis (Wisitrasameewong *et al*, 2017; Algate *et al*, 2015). *P. gingivalis* has a fimbriae structure that can bind to C-X-C motif Chemokine Receptor 4 (CXCR4) and inhibit the host's immune system reaction in infected tissue. CXCR4 is one of the receptors which have a role in alveolar bone resorption in the periodontitis process because CXCR4 can express osteoclasts as cells that play an important role in the process of bone resorption. The interaction of *P. gingivalis* fimbriae and CXCR4 that occurs during this periodontitis becomes a pathophysiology of alveolar bone resorption which then becomes an alveolar bone defect (Nagashima *et al*, 2017).

Periodontal Ligament stem cell

Periodontal ligament stem cell (PDLSC) is a stem cell derived from adult periodontal ligaments which has similarity with Mesenchymal Stem Cells (MSCs). PDLSCs is another source of MSCs from oral cavity. MSCs from oral cavity can be isolated or extracted from dental pulp both permanent and deciduous teeth and gingiva (Narmada *et al*, 2019a; Suciadi *et al*, 2019; Nugraha *et al*, 2018a-d). Periodontal ligament is a part of periodontal tissue originated from the neural crest cells during tooth growth (Zhu and Liang, 2015). PDLSC has the excess of self-renewal ability and multipotent capacity to differentiate into osteogenic differentiation, adipocytes, and collagen-forming cells like another MSCs (Sari *et al*, 2019). These cells needed for regeneration and periodontal tissue. PDLSC also possesed MSCs ability to suppressinflammatory reactions and regenerate tissue (Bassir *et al*, 2015; Nugraha *et al*, 2019b). PDLSCs can be used as stem cells in tissue engineering because PDLSCs express a variety of markers from stromal cells such as, Cluster of Differentiation (CD)13, CD29 (integrin â1), CD44, CD73 (ecto-5'-nucleotidase), CD90 (Thy-1), CD105 (endoglin), CD106 (vascular (vascular) cell adhesion molecule; VCAM-1), CD146, and CD166. Furthermore, PDLSCs contain 3% positive STRO-1 cells and also express SSEA4, OCT3/4, SOX2 and specific tendon markers, scleraxis (Song *et al*, 2015).

PDLSCs express several growth factors such as vascular growth factors (VEGF) which can increase osteogenesis and angiogenesis for regenerating periodontal tissue (Keong *et al*, 2019). PDLSCs also secrete special broad bone morphogenic protein (BMP) and BMP-2, both of them plays role in inducing osteoblast differentiation, cartilage foration and new bone formation (Kang *et al*, 2019; Song *et al*, 2015). Fibroblast growth factor (FGF) can inhance PDLSC proliferation and differentiation *in vivo*. The combination of BMP-2 and bFGF can regulate the osteogenic ability of PDLSCs by increasing osteogenesis genes and the activity of alkaline phosphate (ALP), promoting formation of mineral deposition and activating proteins in bone regeneration (Kang *et al*, 2015).

Periodontal ligament stem cells (PDLSCs) was successfully isolated from impacted third molars and these cells could differentiate into periodontal ligaments, cementum, alveolar bone, blood vessels and peripheral nerves (Seo *et al*, 2014; Zhu and Liang, 2015). Periodontal Ligament cells were isolated using enzymatic digestion methods. PDL is divided into 4 mg / mL of dispase II and 3 mg/mL type I collagenase solution for 1 hour at 37°C. These cells then passaged to obtain a single cell suspension and dispersed into a tube. The tube was incubated at 37°C with 5% CO_2 for 2 weeks in complete culture media [CCM: alpha modification of Medium Eagle (á-MEM added with 1% penicillin-streptomycin, along with 15% FBS, and 2% L-glutamine)] with moderate changes every 3 days (Trubiani *et al*, 2019).

Solcoseryl Pasta and Nano-Hydroxyapatite Silica Gel Scaffold

Solcoseryl is a non-antigenic and non-pyrogenic protein dialysate of blood from healthy veal calves. Solcoseryl consists of many organic and inorganic materials with low molecular weight. These materials facilitate wound healing by regulating tissue and metabolic disturbance associated with injury due to stress and hypoxia. Solcoseryl enhances oxygen uptake by cells and glucose transport, stimulates ATP synthesis and collagen formation, also promotes angiogenesis. Furthermore, solcoseryl has activities as well as growth factors and cytoprotective effects that accelerate the recovery of reversibly damaged cells to their normal conditions (Hamid *et al*, 2018).

Nano-hydroxyapatite (n-HAp) silica gel is a material in nanotechnology that has been introduced in dentistry since 2005 as a bone graft material. This bone graft substitute consists of n-HAp in the presence of nanoporous silica (SiO_2) formed by the sol-gel method (Hamid *et al*, 2018). Hydroxyapatite with the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ is the major mineral constituent of human bones and teeth so that nano-hydroxyapatite plays a role in simulating natural hydroxyapatite crystals in hard tissues (Hamid *et al*, 2018; Yang *et al*, 2018). Nano-hydroxyapatite can be used for hard tissue repair, biomedical imaging, and drug delivery. Previous studies involving n-HAp in osteogenesis were mainly focused on scaffold associated with osteogenic differentiation in bone cells. Nano-hydroxyapatite can be used in bone repair and regeneration because of its physicochemical and biological characteristics. Previous study showed the ability of n-Hap to increase the expression of ALP, osteopontin, Runt-related transcription factor 2 (RUNX2) and osteocalcin that was assessed using reverse transcription polymerase chain reaction (RT-PCR) (Yang *et al*, 2018). RUNX2, osteonectin, and osterix were well-known as bone remodelling marker (Sitasari *et al*, 2020; Nugraha *et al*, 2019c).

Osteogenesis

Bone consists of extracellular matrix and bone cells at various stages of differentiation. The process of osteogenesis is influenced by osteoclast and osteoblast activity (Narmada *et al*, 2018b). Proliferation and

differentiation of osteoblasts are promoted by growth factor through neovascularization (Nareswari *et al*, 2019). The cells involved in bone remodeling, namely osteoclasts involved in bone tissue resorption and osteoblasts (the part that eventually differentiates into osteocytes further) process stages are derived from multipotent stem cells from the bone marrow. Osteoblasts and osteoclasts are derived from MSC and hematopoietic stem cells (HSC). These two cellular populations can be distinguished, for example, based on specific surface proteins expressed on their plasma membranes. MSC is characterized by surface expression, for example, CD73, CD90 and CD105, while HSC expresses membrane receptors, such as CD34, CD45 and CD14. In bone remodeling, osteoclasts are multinucleated cells, associated with macrophage-monocyte cells and dendritic lineages. Then, osteoblasts migrate to the resorption area, fill them with new bone matrix and control mineralization (Niedz'wiedzki and Filipowska, 2015; Hisham *et al*, 2019; Rezkita *et al*, 2020).

Role of combination PDLSC and Solcoseryl Paste in n-HAP silica gel scaffold for bone defect therapy chronic periodontitis

Periodontitis causes alveolar bone resorption because of the expansion of inflammation from the gingival margin to the periodontal tissue. In other cases, the damage continues so that the patient loses teeth, despite conventional periodontal treatment. Some bacterial species such as *Actinobacillus actinomycetemcomitans*, *P. gingivalis*, *Prevotella intermedia* and *Bacteroides forsythus* are increasing in number. This bacterial growth is associated with impaired immune system regulatory mechanisms, namely functional defects in polymorphonuclear leukocytes (PMNs), monocytes or both (Könönen *et al*, 2019).

Bone defects can damage both PMN chemotaxis against the area of infection, phagocytic ability and eliminate microorganisms. Defects in PMN, monocytes and genetic factors allow bacterial infections. Therapy for patients with periodontitis is carried out with the aim of (1) to eliminate periodontal lesions, (2) to obtain a form of tissue that allows patients to control plaque and (3) to obtain bone and connective tissue reconstruction to improve support for the teeth. Bone graft material can be grouped into four types, namely: (1) autograft, bone taken from the same individual, (2) allograft, bone taken from other individuals of the same species, (3) xenograft, bone taken from a different species, preserved with ethylenediamine to remove organic and antigenic fractions, (4) alloplast, bone substitutes and synthetic materials such as hydroxyapatite (Singh and Kumari, 2017).

Nano-hydroxyapatite (n-HAp) has osteogenic activity by accelerating osteoblast differentiation in vitro, and enhanced the expression of osteogenesis specific genes such as ALP, COL I, BSP, OSC, BMP2 and RUNX2. nano-HAp affects the rate of absorption by, proliferation, and differentiation of osteoblasts in a dose-dependent manner. ALP is a membrane bound ectoenzyme, has the main function during osteoblast differentiation by regulating phosphate metabolism through hydrolysis of phosphate esters, and is an early marker of osteoblast differentiation (Nugraha *et al*, 2018d). Beside ALP, Aggrecan as early osteogenic differentiation marker of MSCs control the bone remodelling process (Hisham *et al*, 2019; Nugraha *et al*, 2019c). COLIA1 is the main extracellular matrix protein in bone and bone specific markers in osteoblast differentiation. RUNX2 is a transcription factor and a key regulator of osteoblast differentiation in the early stages (Sitasari *et al*, 2020; Nugraha *et al*, 2018b). BMP-2 belongs to the TGF superfamily and plays an important role in osteoblast differentiation and bone formation (Chiquita *et al*, 2020). Bone sialoprotein is important for initiating bone mineralization and bone cell adhesion to the mineralized matrix and osteocalcin is the next marker of bone formation associated with matrix deposition and mineralization (Nugraha *et al*, 2018c-d; Wang *et al*, 2019).

Stem cells are multipotent and play an important role because of their ability to differentiate mesenchymal lineage (Rantam *et al*, 2020). One of MSCs differentiation is osteogenic differentiation (Nugraha *et al*, 2019d). Osteogenesis is part of the focus of bone cells, which is important for bone remodeling. PDLSC is potential for tissue engineering in bone regeneration. PDLSC was isolated and cultured from periodontal tissue in healthy patients. Ligament periodontal were extracted from third molars using the digestive enzyme method (Zhu and Liang, 2015; Chowdhury *et al*, 2016; Yang *et al*, 2018).

Nano-HAp was prepared by the chemical precipitation method through changes in temperature and pH of the reaction solution. Calcium ions and phosphate anions form amorphous phosphate (CaP), which can be turned into hydroxyapatite under appropriate conditions. nano-HAP has an effect on osteogenic differentiation of Human MSCs reflected by increased activity of ALP and bone markers. The smaller nano-HAp particle size can change the micro culture of cell culture so that it can increase osteogenesis and absorb proteins that form neomatrix (Zhu and Liang, 2015).

Solcoseryl has been known to have the ability to

stimulate the formation of ATP, activate the transport of oxygen and nutrients to cells, support the oxygen recovery and consumption and enhance the proliferation of damaged cells, especially in hypoxic conditions, thus accelerating the healing process. In other words, solcoseryl strengthens the transfer of intracellular energy and increases phosphate stock. Solcoseryl stimulates tissue regeneration, promotes revascularization of ischemic tissue, and also creates good conditions to collagen synthesis and granulation tissue formation. The high vascular potential of solcoseryl paste facilitates the growth of periodontal ligament stem cells. Based on previous study, the solcoseryl paste and n-HAp silica gel granules mixture was prepared in 1:1 ratio by volume and mixed by a sterile spatula on a sterile paper pad. The highest percentage of new bone formed was obtained when solcoseryl was combined with nano-hydroxyapatite silica gel, which was 69.9%, followed by the use of nano-hydroxyapatite alone (62.3%), then paste solcoseryl alone (45.2%) and finally the control group at the bottom (21.6%) (Hamid *et al*, 2018).

CONCLUSION

Combination of periodontal ligament stem cells (PDLSCs) with pasta solcoseryl incorporated silica gel nano-hydroxyapatite scaffold may have potential as regenerating bone defect in chronic periodontitis.

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