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THE POTENTIAL COMBINATION OF PLDSCs WITH *ACEMANNAN* IN CHITOSAN-COMPOSITES SCAFFOLD FOR REGENERATION IN DEFECT *AMELOBLASTOMA*

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ABSTRACT : ***Ameloblastoma*** **is a neoplasm that is classified as a benign odontogenous tumor in the mandible.** ***Ameloblastoma*** **can turn into a malignancy. The incidence of benign** ***ameloblastoma*** **about 0.5 per 1,000,000 population annually. While, the incidence of malignant** ***Ameloblastoma*** **about 1.79 per 10,000,000 population annually. As much as 80-93%** ***ameloblastoma*** **occurs in the posterior mandibular part. In eastern Indonesia, studies show that from 2011-2016 there were 84 people with** ***ameloblastoma*****. Handling of** ***ameloblastoma*** **will leave a defect on the rest of the surrounding tissue. Therefore, new therapy is needed to reduce defect in the former of** ***ameloblastoma*****. The purpose of this paper is to know the potential combination of Periodontal Ligament-Derived Stem Cells (PLDSCs) with acemannan on chitosan-composites scaffold for regeneration of** ***ameloblastoma*** **defects.** ***Ameloblastoma*** **is an odontogenic benign tumor derived from an unerupted tooth.** ***Ameloblastoma*** **in the mandible can develop very large and cause facial asymmetry, changes in the position of the tooth and pathological fracture. In** ***ameloblastoma*** **treatment performed is the action of tumor resection. These resections produce defects in surrounding tissues, especially in** ***ameloblastoma*** **malignant. Research shows that PLDSCs can correct defects bone by promoting the formation of mineralized tissue equivalent to Bone Marrow Stem Cells. Acemannan serves as an immunomodulator that can increase the body's defense specifically. Chitosan-composite scaffold as a bone regeneration medium that is widely used to regenerate bones because, it has a natural intrinsic antibacterial, biocompatibility and good biodegradability and high mechanical strength. The combination of PLDSCs with acemannan on chitosan-composites scaffold has the potential to regenerate the** ***ameloblastoma*** **defect.**

Key words : *Ameloblastoma*, *ameloblastoma* defect, PLDSCs, acemannan, chitosan-composites scaffold.

INTRODUCTION

*Ameloblastoma*a is a benign odontogenic tumor in the jawbone and can turn into malignancy (Suma *et al*, 2013). The prevalence of malignant ameloblastoma, metastatic ameloblastoma and ameloblastoma carcinoma shows a number of 1.6-2.2% of all cases of odontogenic tumors that exist. The incidence of malignant ameloblastoma alone reaches 1.79 every 10 million people per year. The incidence is higher in men compared to women (Rizzitelli *et al*, 2015).

In Eastern Indonesia, research shows that from 2011-2016 there was 84 person, who suffered from ameloblastoma. Most cases of ameloblastoma are multicystic ameloblastoma and its location is in the mandible (Ruslin *et al*, 2018). The existing therapy to treat ameloblastoma can only be done conservatively in the form of enucleation and curettage and resection surgery but this therapy still requires a long time of healing and tissue regeneration due to tissue defects from the therapy (Brown, 2015). Research shows that Mesenchymal Stem Cells (MSCs) derived from dental tissue have advantages in differentiating compared to human Bone Marrow Stem Cells (hBMSCs) (Wada *et al*, 2015). Alireza *et al* in their research proved that Periodontal Ligament-Derived Stem Cells (PLDSCs) have the potential to differentiate into osteogenic tissue (van Rensburg *et al*, 2017). Acemannan is a polysaccharide containing β- (1.4) -acetylated polymannose extracted from Aloe vera gel. According to previous research, Acemannan is proven to stimulate bone formation *in vivo* (Zang *et al*, 2017). The chitosan-composite scaffold is a combination that is suitable for repairing bone defects because of its easy to make and manageable nature and has high mechanical strength (Vyas and Kaur, 2017).

The use of PLDSCs as a source of stem cells will induce bone regeneration processes because it has properties as osteoinduction and immunomodulator. Besides adding acemannan to PLDSCs will accelerate

bone regeneration because it can increase the activity of Alkali Phosphatase (ALPase) and induce RUNX2, which acts as a transcription factor in osteogenesis (Chantarawaratit *et al*, 2014). Therefore, the combination of PLDSCs with acemannan in chitosan-composite scaffold for regenerating ameloblastoma defects needs to be investigated to know its potential in accelerating bone regeneration.

LITERATURE REVIEW

Ameloblastoma

Ameloblastoma is a benign odontogenic tumor in the jawbone (Brown, 2015). Tumors originate from the remnants of tooth seed epithelium, stratified squamous epithelium, odontogenic cyst epithelium, and epithelial from organ enamel. About 80% of ameloblastomas occur in the mandible, especially in the region of the third molar and 20% occur in the maxilla. Ameloblastoma clinically appears as an aggressive odontogenic tumor, often without symptoms and growing slowly. Ameloblastoma in the mandible can develop into a large size and cause facial asymmetry, tooth movement, malocclusion, and pathological fractures. Ameloblastoma is classified into solid/multicystic, extraosseous/peripheral, desmoplastic ameloblastoma, and unicystic (Masthan *et al*, 2015). Radiographically, ameloblastoma can appear as unilocular or multilocular cortical radiolucency. Bone septa depict honeycomb or soap bubble appearance, or tennis racket pattern. Also, there can be an expansion of cortical plates and even destroyed (Suma *et al*, 2013).

The literature mentions two therapy strategies namely conservative treatment (enuklease or curettage) and resection surgical procedures (Brown, 2015). Ameloblastoma surgical resection is the treatment of choice. Especially in cases of large and expansive tumors, surgery is an excellent choice for preventing long-term tumor recurrence. Reconstruction of defects with bone graft material enables good functional and aesthetic results (Dandriyal *et al*, 2011).

Periodontal Ligament-derived Stem Cells

Periodontal Ligament-Derived Stem Cells (PLDSCs) are stem cell tissue obtained from periodontal ligaments of teeth (Wada *et al*, 2015). PLDSCs obtained from periodontal ligaments have properties more similar to mesenchymal stem cells (MSC) than neural cells (Xu *et al*, 2017). Cha *et al* (2015) reported that PLDSCs can express osteogenic markers such as RUNX2, OCN and mineralized nodule formation. PLDSCs have immunomodulatory capabilities comparable to bone marrow mesenchymal stem cells (BMMSCs) (Wada *et al*, 2015). PLDSCs have low immunogenicity because they do not have HLA-II DR or T cell costimulatory molecules (CD80 and CD86) (Ding *et al*, 2010). PLDSCs can be found in all teeth, including premolars and 3rd molars. There are several advantages of using PLDSCs derived from premolar and 3rd molars, among others because cells in 3rd molars tend to be young and can be preserved by the cryo-preservation method (Trejo Iriarte *et al*, 2017).

Acemannan

Acemannan is a â-(1-4) -acetylated polymannose polysaccharide compound found in Aloe vera. Previous research suggested that acemannan can accelerate the healing of wounds in the mouth and the formation of reparative dentine and bone formation (Wada *et al*, 2015). Chantarawatit *et al* (2014) in his study also suggested that acemannan can increase bone cell proliferation, mRNA expression, increase endothelial vascular growth factor and differentiation factor 5 which will accelerate the proliferation of bone cells. The research of Boonyagul *et al* (2014) also proved that adding acemannan solution with a concentration of 2 ml/mg in stem cells can increase stem cell proliferation, Vascular Endothelial Growth Factor (VEGF), BMP-2 expression, ALPase activity, bone sialoprotein expression, and osteopontin, and increase deposition minerals.

Chitosan

Chitosan (CS) is a natural copolymer (1 → 4) -2-acetamido-2 deoxy-â-D-glucan (N-acetyl D-glucosamine) and (1 → 4) -2 amino-2-deoxy-â-D-glucan (D-glucosamine) derived from chitin. The reason for using CS as a replacement of bone is because CS has a structure similar to glycosaminoglycans. Glycosaminoglycans are one of the main components connected with collagen fibers in the extracellular matrix (ECM). Previous research also stated that CS showed significant osteoconductivity by inducing osteoblast proliferation and promoting bone formation *in vitro* and *in vivo* (Thein-Han *et al*, 2008). In addition, CS which is able to have a very porous structure makes CS able to mimic the original bone ECM and allow for bone growth to be implanted (Ahmadi *et al*, 2015). In addition, CS has good biocompatibility and biodegradability (Dash *et al*, 2011).

Chitosan/Hydroxyapatite/Montmorillonite Composite Scaffold

Chitosan/Hydroxyapatite/Montmorillonite (CS/HA / MMT) Composite Scaffold is a mixed scaffold of chitosan, hydroxyapatite and montmorillonite. Chitosan tends to have low mechanical properties so that requires the addition of composites to have good mechanical,

biological, and physical and chemical properties. Based on previous research, CS with the addition of HA / MMT composite scaffold experienced a significant increase in mechanical strength, when compared to other scaffolds. Composite scaffolds help cells to proliferate. This happens because the ratio of surface area to volume of material is high so that it gives more room for cells to attach. Between MMT and HA scaffold, CSH has shown better cell viability. High protein adsorption in CSH samples encourages more cell proliferation (Vyas and Kaur, 2017).

DISCUSSION

Ameloblastoma is a benign odontogenic tumor that can turn into malignancy. Enlarged tumors in ameloblastoma cause defects in bone tissue. Ameloblastoma therapy at present includes conservative therapy and surgical resection therapy (Brown, 2015). These surgeries can cause long-term recurrence, muscle disorders and nerve loss that can cause problems in mastication (Oliveira *et al*, 2013). In addition, this surgical therapy also can cause bone defects, which in turn will affect the aesthetics of the face. Healing bone defects can occur in a long time, namely the bone remodeling process that lasts approximately one year. Reconstruction of bone defects using bone graft is necessary to accelerate the healing of defects, restore bone function, and provide good esthetics (Dandriyal *et al*, 2011).

Alveolar bone reconstruction after ameloblastoma surgery can be done by applying stem cells using scaffold media. One of the stem cells that can be used to regenerate bone is Periodontal Ligament-Derived Stem cells (PLDSCs), where these stem cells tend to be easier to obtain related to their location and dental treatments such as impaction (Yalvaç *et al*, 2010). In addition, PLDSCs have abilities as immunomodulators equivalent to BMMSCs (Wada *et al*, 2015). PLDSCs contain osteoprogenitor, which can differentiate into osteoblasts. Furthermore, PLDSCs inhibit allogeneic T cell proliferation through upregulation of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) (Ding *et al*, 2010). PLDSCs suppress the proliferation and differentiation of B cells and T cells through cell contact. Low immunogenicity and immunosuppressive effects on T and B cells facilitate allogeneic PLDSCs in tissue regeneration (Yalvaç *et al*, 2010).

The application of PLDSCs that have been treated with acemannan requires a scaffold so that the PLDScs network does not experience denaturation. Chitosan (CS) scaffold is the material of choice that can support the attachment and proliferation of osteoblast cells and the formation of mineralized bone matrix in vitro. In addition, studies have shown that chitosan is osteoconductive in vivo in surgery that causes bone defects (Levengood *et al*, 2014). However, the CS scaffold tends to have low mechanical strength, so it requires the addition of composites to the CS scaffold. The study of Vyas et al compared several composite materials with chitosan scaffold. The results of the study explained that the mixture of chitosan/hydroxyapatite/Montmorillonite (CS / HA / MMT) became the material of choice for the chitosan scaffold.Some indicators such as cell viability and mechanical strength are very good. Mechanical strength with a large surface area of CS / HA / MMT scaffold causes cells to have a lot of space for cell proliferation so that cell viability is very good and makes this material as the material of choice for bone regeneration (Vyas and Kaur, 2017). MCS cultured on CS scaffold will experience osteogenic differentiation played by gene expression from Runx2, ALP, Col and OCN and ALP activity. Runx2 induces osteogenic differentiation in the early stages and inhibits them in the last stages. ALP is very important in ECM mineralization with an expression that usually indicates the development of osteogenic differentiation. Col1 and OCN are constituents of ECM and their expression is used as a middle and final marker of osteogenic differentiation. ALP activity shows that CS is osteoinductive (Zang *et al*, 2017).

CONCLUSION

The conclusion of this paper is the combination of PDLDSCs with acemannan in the chitosan-composite scaffold has the potential to regenerate defects in ameloblastoma. *In vivo* research needs to be done to see the effectiveness of bone tissue regeneration from PDLDSCs against CS / HA / MMT Composite Scaffold.

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