Molecule Signal Incorporation into Synthetic Coral Scaffold

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Abstract. Introduction: Synthetic coral scaffold as material for tissue graft was developed to regenerate bone tissue. This scaffold resembles a coral design, made from calcium carbonate and bovine gelatin in the form of hydrogel. To make new bone formation effective, molecule signals are needed as a stimulant for cells that live in the scaffold to differentiate among bone cells. Purpose. Exploring the effect of incorporating molecule signals into synthetic coral scaffolds in bone tissue regeneration. Methodology/Approach. These literature review studied the potential of materials containing signaling molecules that could have a role in bone cell differentiation and new bone formation. Findings. Materials containing the right molecule signal could support and accelerate bone regeneration. It happened along with the degradation process that occured in the scaffold. Originality/ Value/ Implication. Synthetic coral scaffold is a new tissue graft material developed for bone tissue regeneration when incorporating the appropriate signaling molecule will produce a complex system for reliable bone regeneration.

Keywords: Molecule signal · Incorporation · Synthetic coral scaffold

1 Introduction

Bone that has been damaged for a long time will experience a natural bone remodeling cycle. This activity is a regular lifelong process to maintain bone integrity and maintain mineral homeostasis [1]. However, if the trauma is quite severe, the tissue will find it difficult to regenerate itself and it require clinical improvement. Clinical improvements are made to help the healing process, one of which can use a substitute material, namely bone graft [2]. A technology to perform bone grafting, tissue engineering is required. Like native biological tissues, tissue engineering systems require good relationships between cells, scaffolds, and signaling molecules or growth factors. These three basic components together make up the Network Engineering Triad [3].

One of the materials being developed is a scaffold using gelatin and calcium carbonate ($CaCO_3$) as the basic ingredients. Artificial coral scaffolds developed with these basic

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materials have properties that resemble coral [4]. Gelatin has physical and biological properties that are suitable to support tissue engineering and are good for cell adhesion [5]. Calcium carbonate was found to have high porosity and a large surface area, making it suitable for the use as a base material for scaffolding [6]. Porosity has a major role as a substitute for bone trabecular structures that function for cell attachment, a place to live and grow [7]. Based on findings from previous studies, artificial coral scaffolds are biocompatible, degradable, and having characteristics that match the needs of tissue regeneration [8].

It is necessary to have a molecule signal to stimulate cells to differentiate into target cells in order to have a well-directed process of cell growth and differentiation on the scaffold. There are several molecule signals, some of which are growth factors. Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF) are materials isolated from blood and are rich in growth factors that can be an alternative as signaling molecules [9].

The three components, namely cells, molecule signals and scaffolds as an important role in the success of tissue engineering. In addition the accuracy of the selection of molecule signal to stimulate target cells is a determining factor for the formation of new tissue to be regenerated. Therefore, the purpose of this literature review is to explore the effect of molecule signal incorporation into synthetic coral scaffolds in bone tissue regeneration.

2 Literature Review

2.1 Synthetic Coral Scaffold

Synthetic coral scaffold is an alloplastic graft material that is developed and designed for mimicking coral, has biocompatible properties and can be degraded well [10]. This scaffold is made of gelatin and $CaCO_3$ as the basic ingredients [6]. The concept of biocompatibility is the ability of a material that is inserted into the body of living things to be able to perform certain functions, does not interfere or trigger changes in organ functions, and is not toxic in local or general actions in the body. Meanwhile, a product is said to be degraded if the product can naturally be eliminated through the body's metabolism [3].

Scaffold made of gelatin has no toxicity and is biocompatible. Gelatin is the result of denaturation of collagen which can be derived from partial acid (gelatin type A) or alkaline hydrolysis (gelatin type B) taken from animals [5]. Through the hydrolysis process, the collagen triple helix is dissolved at a temperature above 40 °C, resulting in single chain of gelatin consisting of small peptides with low molecular weight [11]. The gelatin molecules formed still retain their primary structure. This primary structure provides an RGD (Arginine-Glycine-Aspartic) amino acid sequence, which is a sequence to significantly enhance cell infiltration, adhesion, dispersion, and proliferation of the scaffold. The gelatin scaffold can maintain cells with good affinity and proliferation after 14 days of culture without any signs of biodegradation [12].

In recent years, coral is also often used as a material to regenerate tissue. Coral contains $CaCO_3$ which serves as the main substitute for bone and can be processed into the desired shape and size. $CaCO_3$ is the main component of coral, which has the advantages of high porosity and good osteoconductivity [13]. Porosity, pore size,

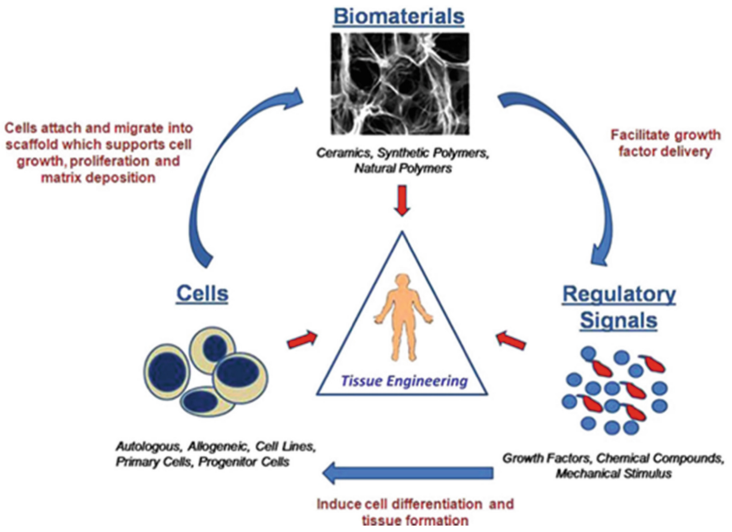

Fig. 1. Network engineering triad. Adapted from [16].

permeability, and interconnectivity between pores serve to channel nutrients and remove residual molecules, so that the scaffold structure as a micro environment can be achieved [14]. Over time, if coral is used in large quantities, it will become a form of exploitation of nature. Therefore, scaffolds made from coral are starting to be replaced with scaffolds made from synthetic materials. The choice of $CaCO_3$ material as an alternative for the manufacture of scaffolds smeared with gelatin is assumed to be able to replace natural coral scaffold [4].

2.2 Biocompatibility

Tissue engineering aims to repair and regenerate damaged tissues and organs and to replace part or all of tissue or organ failure [13]. It is also widely used in dentistry, especially in post-traumatic tooth extraction, successful dental implants, as well as other oral and maxillofacial surgeries to promote bone growth [15].

Tissue engineering is carried out by combining three components, including: (1) a scaffold in which there is a structure and substrate for tissue growth and development, (2) a source of cells to support the needs of tissue formation, (3) growth factors or biophysical stimuli to support tissue formation and to direct cell growth and differentiation in the scaffold. These three things are known as the Network Engineering Triad [16]. Scaffolds trigger cells to attach, grow, and synthesize extracellular matrix and biological molecules [6]. The biomaterial of the scaffold must be designed to be non-inflammatory and biocompatible with the body. Scaffolds are designed and prepared as structural supports of cells for living systems. Therefore, the scaffold has the function of providing a microenvironment for cell attachment, proliferation and tissue regeneration [17] (Fig. 1).

Ideally, the scaffold has biocompatible properties, is capable of being degraded, and has a specific design with high porosity [18]. Tissue engineering requires a sufficiently

large porosity to affect the adhesion of the scaffold to cells. Scaffolds with a porosity between 85–325 um are a good environment for cell attachment. The size of the porosity must be appropriate, not too big and not too small. This will affect cell attachment. Fewer cells will stick if the pore size is too large, otherwise if it is too small it will cause aggregation and cell death [7]. Research from Mahanani et al. [8] states that the porosity of artificial coral scaffolds has a diameter large enough to allow cells to enter the pores. This research was conducted based on observations through an electron microscope and the percentage of porosity with Archimedes' law. The percentage of porosity obtained was 55.85%. If the porosity of the scaffold is more than 50%, it means that it can meet the needs for cell life and the mechanical strength proper of the scaffold.

Ukuran porositas harus sesuai, tidak terlalu besar dan tidak terlalu kecil. Hal ini akan berpengaruh pada perlekatan sel. Sel akan semakin sedikit yang melekat apabila ukran pori terlalu besar, sebaliknya apabila terlalu kecil akan menyebabkan agregasi dan kematian sel.

2.3 Molecule Signal

Growth factors will induce cells to produce extracellular matrix which is important for tissue formation [4]. One of them can come from Platelet Rich Plasma (PRP). Platelet Rich Plasma is defined as the portion of platelets or a concentrated of platelet in a small volume of plasma. The platelet PRP concentration is 3–5 times greater than the physiological concentration of platelets in whole blood (whole blood cells). The normal platelet count in a healthy person ranges between 150,000 and 350,000 cells/μL of blood. The accumulation of platelets in PRP results in an increase in growth factors for a longer period compared to the same volume of blood [19].

PRP is often used in local medicine, aiming to increase cell adhesion, proliferation, and migration. This is due to the high concentration of growth factors in PRP such as platelet-derived angiogenesis factor (PDAF), platelet-derived growth factor (PDGF), endothelium-derived plate. Growth factor (PDEGF), insulin-like growth factor (IGF), transforming growth factor -beta (TGF-β), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). Based on the research conducted by Steller et al. [20], there was an increase in cell viability within 24 h with PRP application. Based on research conducted by Steller et al. [20], there was an increase in cell viability within 24 h with PRP application. Growth factors and bioactive molecules play a local role in proliferation, migration, cell differentiation, and [21].

There are four parameters to test biocompatibility, including cytocompatibility, pathogenicity, immunogenicity, and biodegradation. Cytocompatibility is a measure of qualitative and quantitative aspects related to cell viability. Biodegradability is required to protect patients from possible harm associated with functional assessment and complex interactions between the material and host tissues. The evaluation of the efficiency of decellularization is to avoid rejection and the risk of transmission of infection caused by animals. It is known that materials without growth factors are usually less biocompatible [22].

Platelet Rich Fibrin (PRF) which has been developed by Choukroun is a second generation platelet concentrate. Growth factors found in PRF include insulin-like growth factors (IGF), fibroblast growth factors, epidermal growth factor, platelet-derived growth

factor (PDGFs), vascular endothelial growth factor (VGEF), parathyroid hormone, platelet-derived growth factor. (TGF-β) and bone morphogenic proteins (BMPs). In the firbirn network found in PRF, polymerization occurs gradually, including cytokines, glycanic chains, and glycoprotein structures. PRF significantly induces and continuously stimulates fibroblast and osteoblast proliferation [23].

3 Discussion

Platelets are useful as reservoirs for growth factors and are widely used to help regenerate soft and hard tissues. The regeneration of these tissues is mediated by signaling molecules that are regulated by cytokines. The PRF components that mention before are important factors in supporting the process of cell proliferation and differentiation as well as the formation of neovascularization and collagen synthesis in the process of osteogenesis. Growth factors released by PRF have different roles, namely interleukin-1β as a cytokine that plays a role in the process of cell proliferation, cell differentiation, and apoptosis. Interleukin-4 plays a role in the immune system and is an important factor in hypersensitivity. Transforming growth factor-β (TGF-β) secretes proteins that play a role in the regulation of the proliferation, differentiation, and apoptosis of cell including immune control and stem cell growth. Platelet derived growth factors (PDGFs) play a role in regulating cell division, cell growth, formation of blood vessels (angiogenesis) and in controlling cells to differentiate into osteoblasts and fibroblasts. Insulin-like growth factor (IGF) is a growth hormone. Vascular endothelial growth factor (VEGF) plays a role in stimulating angiogenesis or the growth of blood vessels as new tissue. Bone morphogenic protein (BMP) plays an important role in bone and cartilage formation, and bone morphogenic protein-2 (BMP-2) helps induce osteoblast differentiation in the formation of bone [24–26].

A three-dimensional cross-linked fibrin matrix that binds platelets and growth factors produced by fibrin polymerization. The presence of growth factors in high concentrations at the site of the injured tissue will encourage PRF to regenerate tissue in the area by mimicking the wound repair [27].

As a molecule signal, PRP contains many bioactive molecules, such as growth factors, namely insulin like growth factor (IGF I, IGF II), transforming growth factor – 1 (TGF – 1), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF). Growth factors predispose chemotaxis, differentiation, proliferation, and bone cells synthetic activity, thereby regulating physiological remodeling and fracture healing [28]. Growth factors that are in PRP will be released from the platelet granules. When platelets are activated by collagen, it will stimulate growth thereby accelerating bone regeneration, because collagen is a protein that is naturally involved in the process of releasing growth factors from pellet granules in the human body [29]. Macrophages, monocyte mediators, and a variety of interleukins (IL – 1, IL – 6) are also present in PRP as inflammatory mediators [30]. In addition, PRP is also known to contain cell adhesion molecules such as fibrin, fibronectin, vitronectin, which participate in the process of osteoconduction and epithelial cell migration [31]. The dose required for PRP to effectively contribute to regeneration is four to five times higher than the normal platelet content [23].

The bioactive molecules contained in PRP have their respective roles and functions, including [30]:

PDGF: Stimulates cell replication, promotes angiogenesis, promotes epithelialization, regulates bone cell metabolism (osteogenic differentiation, osteoblast proliferation), promotes extracellular matrix ossification.

TGF –: Increases the production of collagen and mineral matrix

TGF – 1: Stimulate osteogenic proliferation and differentiation

VEGF: Promotes angiogenesis and endochondral ossification

EGF: stimulates cell differentiation and re-epithelialization, angiogenesis, and collagenase activity

FGF: influences the proliferation of osteoblasts, endothelial cells, and fibroblasts. Stimulation of angiogenesis

IGF-1: promotes bone formation through cellular proliferation, differentiation, synthesis of type I collagen IL – 1, IL – 6

TNF: early response in bone repair, endochondral bone formation, and bone remodeling

Fibronectin and vitronectin promotes focal adhesion formation by osteoblasts and osteoblast migration.

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