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Review Article

Tissue engineered bone as an alternative for repairing bone defects

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ABSTRACT

Background: *Dentist especially oral surgeon, are frequently faced with defect in bone resulting from disease or trauma. If the defect is small, it will frequently has a good healing, however, if the defect is larger, incomplete regeneration often occurs and a fibrous scar results. Transplantation of autogenous bone has been one of the most frequent procedures of reconstructive oral and maxillofacial surgery because it has shown excellent clinical success; however, autogenous bone grafting is often related to disadvantages like limited availability, and donor morbidity.* ***Purpose:*** *The purpose of this review is to explain the basic principles of tissue engineering, background of regeneration process, also advantages and disadvantages of tissue engineered bone compared to autogenous bone graft.* ***Review:*** *Recently, tissue engineered bone provides a promising strategic innovation and becomes a new alternative for bone regeneration process. Tissue engineering is a term originally used to describe tissue produced in isolation and culture by cells seeded in various porous absorbable matrices. Tissue engineering generally combines three key elements (Tissue Engineering Triad) i.e: scaffolds (matrices), signaling molecules (growth factors), and cells (osteoblast, fibroblast, etc).* ***Conclusion:*** *Tissue engineering will facilitate initial bone healing in order to accomplish tissue regeneration process.*

Key words: *Tissue engineering, autogenous bone graft, bone defects*

ABSTRAK

Latar belakang: *Seorang dokter gigi khususnya dokter gigi bedah mulut, seringkali dihadapkan dengan keadaan defek tulang akibat dari suatu penyakit atau trauma. Jika defeknya kecil mungkin dapat sembuh dengan baik, tetapi bila defeknya besar, kemungkinan regenerasi tulang tidak sempurna dan menghasilkan scar/ jaringan parut. Transplantasi dengan menggunakan autogenous bone graft meskipun sampai saat ini masih banyak digunakan untuk operasi rekonstruksi di bidang bedah mulut dan maksilofasial karena telah menunjukkan keberhasilan klinik yang cukup baik, namun cara ini mempunyai banyak kekurangan, diantaranya morbiditas dari sisi donor.* ***Tujuan:*** *Tujuan dari penulisan ini adalah untuk menjelaskan tentang prinsip-prinsip dasar tissue engineering, hal-hal yang berperan dalam proses regenerasi serta keuntungan dan kerugian tissue engineering dibandingkan dengan autogenous bone graft.* ***Tinjauan pustaka:*** *Saat ini penggunaan tissue engineered bone merupakan suatu strategi inovatif yang telah dikembangkan dan memberikan suatu alternatif dalam proses regenerasi tulang. Tissue engineering atau rekayasa jaringan merupakan suatu istilah yang digunakan untuk menjelaskan bagaimana suatu jaringan dihasilkan dengan cara isolasi dan kultur sel dalam berbagai matriks porous absorble. Tissue engineering akan melibatkan tiga elemen kunci (tissue engineering triad) yaitu Scaffold (matriks), molekul-molekul signal (growth factors) dan sel-sel (osteoblast, fibroblast, dll).* ***Kesimpulan:*** *Teknik tissue engineering akan memfasilitasi proses awal penyembuhan tulang sehingga proses regenerasi jaringan akan tercapai.*

Kata kunci: *Tissue engineering, autogenous bone graft, defek tulang*

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INTRODUCTION

The need of bone regenerating is gradually increasing as the quality of life is improving and the consequence the life expectancy is also increasing. Now, regeneration of bone tissue is still a challenging in cranio-maxillofacial surgery. The surgical treatment is commonly conducted for repairing bone defect caused by trauma, tumor, infection or any abnormal bone growth.[1, 2]

Transplantation is a procedure to anticipate the above problems. In order to repair the bone defect, transplantation can be conducted by using many grafts, such as autogenous bone graft (graft derived from the patient's body), allogeneic bone graft (graft obtained from donor), and bone matrix that has been demineralized (demineralized bone matrices) or synthetic biomaterial, like metal, ceramics, polymer, and composites.[1–3]

Until now, the use of autogenous bone graft still becomes the first option for repairing the bone defect and regenerating, and is also commonly used for reconstruction in oromaxillofacial surgery. The advantages of autogenous bone graft are that there is no immunogenic reaction and that it has good osteogenicity and osteoinductivity. Besides, autogenous bone graft can recruit mesenchim cells and then induce them to differentiate into osteogenic cells through osteoinductive growth factors.[1–3]

Though the use of autogenous bone graft has many advantages, there are still many main weaknesses, such as morbidity of donor, continual pain after the surgery, hypersensitivity, infection, and paresthesia. The complication can occur in 10–30% patients. Besides that, bone obtained is also limited.[2,3]

Another alternative is by using allograft. The use of allograft can eliminate the weaknesses of autogenous bone graft, but the quality of bone obtained from allograft is worse than that is from autogenous bone graft. Allograft has worse cell cellularity degree, worse revascularization, bigger resorbsion level, and slower bone formation than those in autogenous bone graft. The most serious disadvantages is that there is immunogenic reaction potency and viral transmission risk for the patients.[3]

Though processing technique like demineralization, freeze-drying method, and irradiation can eliminate the immune response of patients, the processing can also disturb the graft structure and reduce the potency of inducing the bone recovery process (osteoinductivity) while there is still possibility of disease transmission.[3]

In order to anticipate those weaknesses, the new alternative technology for reconstructing the bone defect through tissue engineering technique by using bone marrow stem cells is developing now.[3–6] Many studies on animals have shown that tissue engineering can produce bone, either in non-bone environment (ectopic bone formation) or in bone environment (orthotopic bone formation).[3–6]

Tissue engineering is actually a new multidisciplinary in medical, surgery, molecular and cellular biology, polymer and physiology chemistry. Therefore, the objective of this study is to analyze the principles of tissue engineering as well as the advantages and disadvantages of tissue engineering compared with the transplantation of autograph or allograft.

Tissue engineering

Tissue engineering is tissue regenerating in body involving cells, biologic mediators, such as growth factors of synthetic or biologic matrix that can be implanted into the patient's body in order to regenerate certain tissue.[1] Tissue engineering is multidisciplinary field using biologic principles and engineering technique for improving a substitute material that can repair and maintain the function of bone tissue.[1] It involves the use of synthetic polymers in order to facilitate the regenerating process of tissue. These polymers then will be absorbed and substituted by natural and physiologic tissues.[1]

Many studies of tissue engineering have actually been conducted either in vitro or in vivo, for instance: Caplan[7] who said that mitotic isolation and expansion from autologous stem cells can cause faster and more specific reparation of bone tissue. Friedenstein *et al*.[5] moreover, shows that a specific cell group, which is a colony forming fibroblast unit or mesenchim cells located in bone marrow can differentiate into many different cell types, including osteoblast. Quarto *et al*.[8] published the first clinic paper that reports the repairment of bone defect by using autologous bone marrow stromal cells. Next, Schimming & Schmelzeisen[9] conducted the first study on human beings showing that periosteum-derived osteoblast can form lamellar bone in 3 months after transplantation. Urist[10,11] then showed that bone tissue contains specific growth factors that can induce the bone formation in ectopic sites (non bone environment).

Tissue engineering actually involves 3 key elements (Tissue Engineering Triad) which are: scaffolds (matrix), signalling molecules (growth factors), and cells (osteoblast, fibroblast). By combining those three elements, the process of tissue engineering can be conducted (Figure 1).[2]

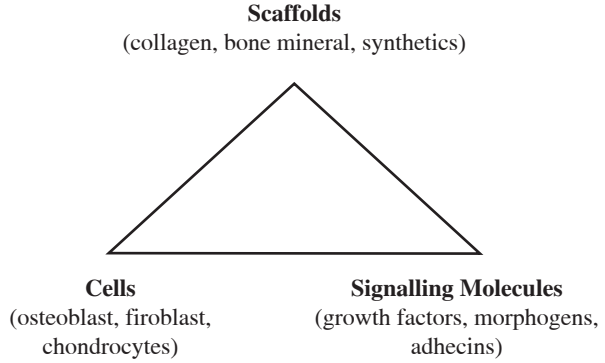

Figure 1. Tissue engineering triad.[2]

The brief procedure of tissue engineering involves the following stages: first, cells (osteoblast, fibroblast) and signaling molecules (protein growth factors) are

induced into scaffolds or highly biodegradable matrix, and then those are cultured in vitro. After being cultured, those scaffolds are induced or implanted into a defected bone in order to induce the growing of new bone in vivo. Those cells then will adhere into scaffolds, multiply or regenerate themselves, differentiate from non-specific or primitive cells into specific cells that have bone function, and continually organize into normal and health bone cells. Finally, after engineering health new bone, those scaffolds then will degrade (Figure 2).[3]

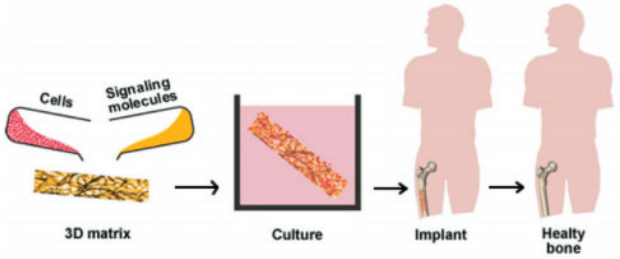

Figure 2. The role of *scaffold* as *guidance* in the process of tissue engineering.[3]

However, it must be remembered and understood that we cannot harvest some cells like osteoblast, and then culture them for forming a complete or whole bone. In tissue engineering, there are three important components: matrix, cell and soluble regulator.[1-3]

Matrix (porous structure)

Matrix in bone tissue engineering is involving many biomaterial groups, such as synthetic polymers, natural polymers, ceramic, and composites. Synthetic polymer is an organic or inorganic structure . This material is widely used in biomedical field. Its characteristics are degradable/ absorbable and non degradable/non absorbable. For instance, degradable synthetic polymer is polylactic acid and polyglycolic acid that have got hydrolysis into lactate acid and glicolate acid. Nowadays, degradable synthetic polymers that are being improved are polycaprolactone, polyanhydrides, and polyphosphazenes. Meanwhile, non degradable synthetic polymers that are being improved are polytetrafluoroethylene (PTFE), polymethylmethacrylate (PMMA), and polyhydroxyethylmethacrylate (PHEMA). These materials are commonly used for making dentures, arthroplasty, and cranioplasty, as well as used as cements in orthopedic prosthesis. PTFE, moreover, is commonly used for subcutan augment material and guide bone regeneration in order to regenerate bone by making line for osteoblast cells.[1, 2,12-14]

Ceramics are materials that have osteoinductive porous structur. These materials are widely used in dentistry and in tissue engineering. Ceramics commonly used in dentistry are alumina (Al_2O_3) and hydroxyapatite (HA). Alumina is very resistant to corrosion, and its biocompatibility is very good and strong. Meanwhile, hydroxyapatite is ceramics with calcium phosphate as the basic materials and has been used more than 20 years in medical field and dentistry. Hydroxyapatite is a main inorganic component of bone that is osteoinductive, biocompatible, and biodegradable, but has low mechanical power. Degradation of hydroxyapatite is controlled by many chemical structures. Besides hidroxyapatite, materials of ceramics commonly used are Tricalcium phosphate (TCP). Tricalcium phosphate can be degraded faster than hydroxyapatite.[1,2, 12-15]

Natural polymer is extracellular protein which is often used as bone graft. Natural polymer includes collagen (type I, II, III, IV), glycosaminoglycans copolymer, polysaccharide hyaluronic acid (Hy) and chondroitin sulfat. Polysaccharide hyaluronic acid is glycosaminoglycans found in synovial liquid and kartilago which can induce chondrogenesis and angiogenesis. If it is combined with collagen, it acts as matrix in bone regeneration. Chondroitin sulfate is glycosaminoglycans found in kartilago functioning as scaffolds in tissue engineering. The mechanical strength of collagen matrix is little and its size is not enough to cover defect. Collagen can be osteoinductive especially if it is combined with bone marrow.[1, 2,5,8,13-15]

Composites is a combination between ceramics and polymer. For example, Collagraft is a combination between collagen type I (95%) and collagen type III (5%) taken from bovine and mixed with HA. Collagraft is mostly used in orthopedic surgery. In craniomaxillofacial, Bio-OSS is often used and it is combination between collagen bovine and de-organified bovine bone. Combination between collagen and ceramics made from calcium functions as osteoinductive i.e. a function of matrix found in bone that supports adhesion, migration, growth, and cell differentiation.[1, 2, 5,8, 12-16]

A matrix has some roles during the tissue regeneration in vivo. Structurally, matrix can support the defect so that it can sustain its shape from defect and keep distortion away from the tissue. It can function as barrier for the tissue growth. It also functions as regulator of insoluble cell function through its interaction with other receptor cells. It can function as scaffolds to migrate and proliferate the cells in vivo or implant the cells in vitro.[1, 3,6]

Cells

Dynamics of bone metabolism is a remodeling process that continually occurs through 3 main cells: osteoblast, osteocyte and osteoclast. Osteoblast is a cell that has a role to synthesize and organize deposition and mineralize extracellular matrix of bone. The activity and differentiation of osteoblastic are organized by either systemic or local hormones, growth factors, ions, lipid and steroid**.** Osteoblast, pre-osteoblast and osteoblastic work to investigate transduction signal. Proliferation and differentiation of osteoblast cells are modulated by transforming growth factor beta (TGF-b) and bone morphogenetic proteins (BMPs) that are very important in bone homeostasis.[1,3,5, 17–19]

Osteocytes is a cell that has high differentiation with alkaline phosphatase activity, PTH receptor and functions as mechanosensory cell**.** The mechanical stimulus can interfere the bone structure and the bone mass. Osteocytes has lacuno-canalicular in bone porosity that mediates

mechanosensory system**.** Mechanosensory system of osteocytes in bone responds any changes. Consequently, there is a flow of interstitial liquid through osteostitic canalicular tissue. This flow will initiate the electrokinetic and mechanic signal. Then, the secretion of molecule signals will take place, for examples, insulin-like growth factor, IGF-1, prostaglandin G/H synthase, PGE2 and nitrit oxide which contributes to coordinate metabolic response from adjacent cells: osteoblast, osteoclast. Osteocytes has a role in cellular organization of bone that responds the changes of mechanics by augmenting and reducing from bone apposition. Osteocytes do not resorb dentine surface in vitro. This indicates that osteocytes do not have a role in calcium homeostasis.[1,3,6, 20-22]

Osteoclast is multinuclear cell from hemopoietic cell. It's function is to to resorb bone. The bone resorbsion by osteoclast is the result of blend from acid intravesical cytoplasm and plasma membrane.[1,3,6]

Soluble regulators

Soluble regulator is soluble molecule either used with or without another biomaterial as delivery system. There are some examples of soluble regulators such as growth factors–polypeptide mitogens, and differentiation factors (e.g.bone morphogenetic protein).[2, 11] Some functions of soluble regulators are stimulate cell diffusion and infiltrate in the defect, stimulate particular differentiation cell, stimulate angiogenesis process and act as chemoattractan for certain cells.[2, 6, 11]

In dentistry, platelet-derived growth factor has shown significant roles in tissue healing in which the role of growth factor in periodontal tissue engineering has shown mitosis effect, migration, matrix synthesis, and differentiation of periodontal ligament cells and osteoblast. In addition, BMP is frequently used with biomaterials like collagen, tricalcium phosphate or HA to surpass the bone defect.[1,11, 18, 21-24]

DISCUSSION

Bone tissue engineering has important role to overcome clinical problems especially dealing with bone defect retrieval by requiring 3 important elements: matrix, cell and soluble regulator/signaling molecules. In tissue engineering there are various approaches depending on the cell source e.g. autologous (taken from the patients), allogeneic (taken from donors) or xenograph (taken from animal); whether the scaffolds are used or not, such as the use of growth factor in the defective tissue found in small defect area. In larger defect area, matrix as structural factor is more needed; whether the scaffolds are implanted with cultured cells before the surgery or those cells are embedded in matrix and implanted when the surgery takes place.[1-3]

In bone tissue engineering there are two approaches: growth factor like bone morphogenic protein (BMP) and transforming growth factor (TGF), and osteogenic cells like stem mesenchim cells (mesenchymal stem cell). Bone marrow is the source of osteogenic cells that has high proliferation and large capacity to differentiate.On the first approach (growth factor based), bone morphogenic proteins from TGF- α are used. The weakness of this approach is that it needs high concentration to obtain osteoinductive effect. Besides, its side effect is greater and the cost is expensive. On the second approach (cell-based approach) which is considered to be more interesting, combination between osteogenic cells and biomaterial scaffolds through ex vivo may trigger the growth of tissue structures in three dimensions.[3, 9, 22–27]

In bone tissue engineering, osteogenic potential and mesenchymal stem cells (MSC) have widely been studied. These cells can easily be isolated from various tissues like fat tissue (adipose), muscle from the edge blood and bone marrow. MSC does not only have ability to proliferate in a culture but also to change immature progenitor cells through several ways, for examples, osteogenic, chondrogenic or adipogenic.[7, 25–27]

Previous studies are conducted on some animals as specimen/invitro concerning tissue engineering on jaw bone/alveolus. One of them is conducted by Li *et al*.[27] that studied repairing process on mandibula defect by applying bone tissue engineering on rabbit. Osteoblast cells taken from the rabbit's bone morrow are cultured and implanted in scaffolds in the form of allogeneic demineralized bone in order to form tissue engineering bone graft through in vitro which is used to repair bone defect in mandibula.

Vesala *et al*.,[28] evaluated a variety of absorbable materials in order to direct bone regeneration on cranium bone defect by applying self reinforce poly-L, D-lactide 96/4 (SR-PLA96) implanted on the rabbit's cranium bone defect. From the study, it is obtained that on the 48th week the defect on cranium bone is perfectly covered.

A study by Weng *et al*.,[29] involved human's condyle TMJ as model by applying a mixture between synthetic non woven mesh poly-glycolic acid fibers and polylactic acid in methylene chloride as scaffolds implanted with osteoblast cells from periosteum bovine for 12 weeks. After that, it is evaluated in two ways: macroscopic and microscopic. The result of the study shows that bone forming and cartilage take place and the bone tissue or cartilago found in condyle is normal.[29]

Similar study reconstructing mandibula in human by applying titanium mesh filled with hydroxyapatite, rhBMP7 and bone marrow stromal cell in order to stimulate osteogenesis process on mandibula bone. In the follow-up process, repairing the defect on mandibula shows good result so that, as consequence, the quality of patient's life will be increased.[30]

Another study by Weng[29] involved dog's alveolar mongrel bone which has resorbtion due to periodontal disorder. The study applies bone marrow stromal cell (BMSC) mixed with calcium alginate that is used to form gel which functions as scaffolds in bone tissue engineering. After it is evaluated for 4 weeks of post-surgery, mature

bone has been formed and on the 12th weeks the forming of similar bone has normally taken place.

Since Friedenstein *et al*.[5] published the similar study, it has been known that mesenchymal stem cells (MSCs) can be used to engineer mesenchim tissue like bone and cartilago Therefore, researchers around the world work hard to obtain proper carrier for those cells. Bone transplantation is conducted so that the bone regeneration will occur.[5, 18]

Bone marrow is the source of MSC. In addition, it is the source of osteogenic cells taken by simple aspiration procedure. This method is more minimal invasive than method which assembles osteogenic cells by biopsy from calvarium. Besides bone marrow, periosteum, bone trabeculae taken from fat tissue and stem cell taken from dental pulp show osteogenic potentials.[31–32] Caplan,[7] have combined MSCs with scaffolds to produce bone matrix after being implanted.

To gain success in tissue engineering, four conditions are required: number of cells with adequate osteogenic capacity, proper scaffolds to implant cells, factors to stimulate osteogenic differentiation in vivo, and sufficient supply of blood vessels. The first three conditions can be applied by tissue engineering while the fourth condition depends on patients like defect size. The lack of supply in blood vessels leads to the cell death after being implanted. This may cause the bone tissue engineering on the patients failed.[3,8, 27, 31]

The use of MSCs in tissue engineering can be the best solution for regeneration in medical future in the near future. Dental and maxillofacial surgeon often deal with large bone defect which is difficult to reconstruct so that they optimally need either bone tissue and biomaterial to restore structure and tissue function. Hence, reconstructive maxillofacial needs an innovation in the form of studies or researches to seek biocompatible material which can be used in tissue engineering. The use of autogenous bone has become the main option to repair the bone defect, but difficulty in gaining enough amount of bone often appears. Procedure to gain autogenous bone will bring some pain, anatomical restraint, and morbidity on donor domain. Therefore, bone tissue engineering has important role to solve problems in clinics especially problems in bone defect repairing.[18, 26, 32]

It is concluded that tissue engineering will facilitate the healing process of bone so that the tissue regeneration will be obtained. The biggest challenge in tissue engineering is how to ensure that angiogenesis has important role in tissue regeneration where cells without sufficient supply will die and the regeneration will not be obtained.

New biomaterials are needed to give response for unknown object and degrade perfectly in expected time. Knowledge about bone tissue engineering should be developed more. Thus, it needs further research, better materials of analysis, more realistic in vitro studies, better tissue developing through in vivo and non invasive approach.

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