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

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ABSTRAK


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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.  

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.  

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.  

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.

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 resorption 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.

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.

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. 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).

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.

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. 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.

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

Scaffolds

(collagen, bone mineral, synthetics)

Cells

(osteoblast, fibroblast, chondrocytes)

Signalling Molecules

(growth factors, morphogens, adhecsins)

Figure 1. Tissue engineering triad.

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 healthy new bone, those scaffolds then will degrade (Figure 2).\(^3\)

![Figure 2. The role of scaffold as guidance in the process of tissue engineering.\(^3\)](image)

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 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.\(^1,2,12-15\)

Ceramics are materials that have osteoinductive porous structure. These materials are widely used in dentistry and in tissue engineering. Ceramics commonly used in dentistry are alumina (\(\text{Al}_2\text{O}_3\)) 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 hydroxyapatite, 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 sulfate. Polysaccharide hyaluronic acid is glycosaminoglycans found in synovial liquid and cartilage 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 cartilage 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.\(^1,2,5,8,12-16\)

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 osteostotic 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.\(^1\)\(^{13}\)\(^{6}\)\(^{20}\)\(^{22}\)

Osteoclast is multinuclear cell from hemopoietic cell. It’s function is to to resorb bone. The bone gosborision 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 from donors) or xenograph (taken from animal); whether e.g. autologous (taken from the patients), allogeneic (taken from donors) or xenograph (taken from animal); whether

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 morphogenetic proteins from TGF-\(\alpha\) 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 adipogetic.\(^7\)\(^{25}\)\(^{27}\)

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 resorption 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. published the similar study, it has been known that mesenchymal stem cells (MSCs) can be used to engineer menenchim tissue like bone and cartilage. 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. 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. Caplan, 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.

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.

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.

REFERENCES


