Chapter 2 - Clinic of membrane and bone graft substitute

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I. Introduction

In order to achieve an implant prosthesis that is both aesthetically and functionally agreeable, it is desirable for the quantity and quality of the bone to be sufficient with soft tissues. An implant installation to bone structures with such favorable conditions would result in a firm integration with the bone, and one harmonious co-existence with the surrounding gingivae structures and the neighboring natural teeth would be possible. Unfortunately, these ideal cases are rare in the clinical settings, as the alveolar bone resorption is induced upon tooth loss. In implant treatments conducted to replace the extracted tooth that has resulted from periodontal disease damage, the alveolar bone is often found to have been resorbed both vertically and horizontally and sufficient amount of bone is difficult to be obtained. The progress of bone resorption that results from loss of teeth can rapidly occur within a short period of time in the regions where the alveolar bone is thin (particularly the anterior region of both mandible and the maxilla). The buccal alveolar bone is thinner in the alveolar region of the anterior teeth than the labial side. In case of tooth loss the buccal bone resorption is often found to occur along with the vertical bone resorption leading to a general collapse of the buccal side of the bone. In cases where such resorption of the bone has affected the alveolar bone structure, the implant prosthesis with an aesthetic outcome can become difficult to be achieved. These issues can be overcome by conducting alveolar bone augmentation procedures in conjuction with the implant installation.

The reason for bone augmentation procedures to have widely spread as a dental clinical procedure can be said to be due to the development of bone fillers and membrane materials. The rise in application of this method is also due to the synergistic effect with the increased need for the alveolar bone regeneration to be established with high predictability. The scientific research for viable bone fillers that aid the bone regeneration are a subject of high interest where a significant number of materials have been developed and are applied in the clinical settings. The main subject of current research lies in the development of cell therapy with bone fillers combined with growth factor agents and application of stem cells. With regards to the bone fillers with growth factor agents, there are some that have already been licensed for use in field of dental surgery. Unfortunately, the majority of these bone fillers and membranes have not yet been approved under the Pharmaceutical Affaires Law in Japan, therefore cannot be used in the dental clinical settings in this country. For this reason, there is a general lack of knowledge and difficulty in gaining updated information on bone regeneration therapy at present, and are progressively becoming left behind from the global clinical advancements, reminiscent of national isolation period. I still believe that sufficient understanding of the forefront of clinical sciences is essential in order to provide the patients with the best possible treatment.

The objective of this chapter is not to advertise or to explain the use of the bone filler agents or the membranes in current use, globally. It is intended to introduce the theory of alveolar bone regeneration therapy for the success of the implant treatment, to provide the readers with accurate clinical scientific knowledge.

II. Alveolar bone regeneration therapy

There are three factors that cannot be forgotten when performing bone regeneration therapy. The osteogenicity, osteoinductivity, and osteoconductivity (Fig 1-2-1). In theory, the presence of these three factors should result in bone regeneration. Osteogenicity indicates the presence of the cells that are able to form the bone. For example, bone fragment is extracted with the bone forceps from the maxillary tuberosity to be used as bone filling agent. The maxillary tuberosity is mainly cancellous bones that consist of osteocytes and bone marrow stromal cells. Here, the bone filling agent becomes an own bone with a high osteogenic ability. Osteoinductivity refers to the property that can induce the bone marrow stromal cells that reside in the host bone or allografted bones for a specific differentiation of the osetocytes. The bone marrow stromal cells are undifferentiated mesenchymal cells that have the ability to differentiate into adipocytes, fibroblasts, chondrocytes, and myocytes in addition to osteocytes. However, with the activation of bone morphogenic proteins (BMPs), these set of cells can selectively differentiate into osteocytes and achieve osteoconductivity. Such property is known as the osteoinductive abiliy, and the typical components are BMP-2 and BMP-7. The osteoconductivity refers to the property of the material to provide an environment where the cells can proliferate and differentiate to secrete the substrates. In short, it is the ability of the material to accommodate the cells acting as a scaffold for the cells to proliferate and divide, or construct blood vessels, accordingly.

Take a blood clot in an tooth extraction socket as an example. The blood clot formation is the initial step, and the most important of the wound healing /bone regeneration process. The blood clot is constructed of the complex mesh of fibrins. In the interior of the blood clot, platelets can be found to be activated to secrete cytokines of significant variation. Amongst these cytokines, there are those with a role to draw the stromal cells from the surrounding bone marrows towards the extraction cavity; those that facilitate the proliferation process; or those that attract the vascular cells for capillary constructions. The undifferentiated stromal cells that have been accumulated by the cytokines proliferate further and differentiate within the blood clot made up of the fibrins to extend the bones. Therefore to summarize, osteoconduction is the responsibility of the fibrin mesh, and the osteoinduction is orchestrated by the cytokines secreted by the platelets.

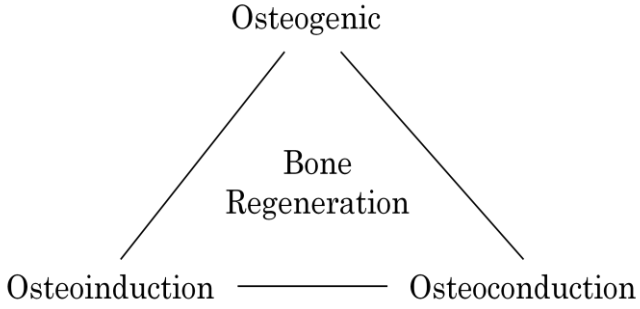

Fig.1-2-1　Three factors of osteoanagenesis

III. GBR and membranes

The extracted socket becomes filled with blood clots, which eventually becomes covered with the gingivae. The internal structure of the jaw is undergoing continuous osteogenesis for the extraction socket to be healed, but it is often found in the clinical settings that the healing of the socket is associated with the

collapse of the bone structure. Two reasons can be thought as the cause for this phenomenon. First, the rate of healing of the gingivae is faster than that of the osteogenesis, therefore the gingivae structure invading the internal structure of the extraction socket results in the prevention of the osteogenic process. Second, the blood clot constricts in the wound healing process and forming the indentation itself. Whichever the order, the cause is the gingivae invasion into the space that is required for the extension of the bone. Therefore by implementing a method to maintain this space by preventing the gingivae invasion, as much bone augmentation can be possible. The solution to this problem for both "exclusion of the soft tissues", and "preservation of space" is the use of membranes, and the theory for this was established by Nyman in 1982[1]. In their method, they inserted the membrane with the aim to regenerate the periodontal tissues, and termed it periodontal guided tissue regeneration (GTR), but the membrane applied for bone augmentation/ bone regeneration, this should be referred to as guided bone regeneration (GBR). This technique was discussed with the extraction socket healing as an example, but the fundamentals of this technique "the exclusion of the soft tissues and the preservation of the space can facilitate the osteogenesis" is applicable and one that is essential for all of the bone augmentation/ regeneration methods, and one that should be the foundation for success of the GBR method to facilitate the implant treatment.

An ideal membrane is one that has the following properties:

1) Processibility, such that it can be trimmed with ease and be applied to even the complicated surgical surfaces.
2) Promote the formation of a stable blood clot
3) Support the attachment of the flap (gingival flap)
4) Has the resistance to modification during its function in the oral cavity for the preservation of space
5) Retain these properties till the healing process of the tissues is complete
6) One that can be removed easily

The membrane materials can be largely divided into those that are nonabsorbable and absorbable. The Millipore filter and the expanded polytetrafluoroethylene (ePTFE) membrane are classed under the nonabsorbable types. ePTFE membrane consists of a titanium frame, designed specifically with the aim to "preserve space". The non-absorbable membrane is characterized with a high biocompatibility and with the ability to maintain its function within the physiological settings for a long time. In addition to these properties, the titanium frame of the ePTFE membrane is specialized with its ability to preserve the amount of space. The drawback to the non-absorbable type is the necessity of the secondary surgery to extract the membrane, and the incidence of exposure of this type has been reported to be higher than its counterpart [2].

Absorbable membrane can be further divided into those composed of synthetic polymers or collagens, where Type I is the most prominent or there are others that consist of both Type I and Type III. The raw materials used are usually of the animal origin such as the skins and tendons of pigs and cows. The collagen membranes are able to be digested by the endogenous collagenases within the body eventually, but remain functioning for at least a month, and up to half a year in some types. The synthetic polymer membranes include polylactic acid (PLA) polymers, Polyglycolic acid (PLA/PGA), and Polyglactin-910 (polyglactin-910). These synthetic polymer types undergo hydrolysis within the body to be absorbed

eventually, but remain functioning for few months to half a year. The absorbable membranes, especially the collagen type, are highly compatible with the biological tissues and attach the flap well therefore can prevent the exposure of the membrane. In addition, since it is absorbable, in the body, the secondary surgery for the extraction of the membrane is not required. On the other hand, the absorption rate of the membranes varies amongst the individuals therefore it is not possible to accurately determine the time that it remains in function. Another disadvantage is that with this type lacks in the strength therefore bone filling agents may be required to preserve the space required.

There are a variety of membranes, as have been mentioned here, but the main types applied under the clinical settings involve collagen membranes and ePTFE membranes.

The comparable studies that have investigated the differences in outcomes in the application of the collagen membrane and the ePTFE membranes for bone augmentation in the cases of bone fission, the results indicated that there were no differences in their abilities to facilitate bone augmentation ion those where there were no exposures of the membranes. Where exposures did occur, it was found that collagen membrane to be the more suitable of the two types [3)].

The application of membranes in implant treatment is usually for bone augmentation/ regeneration purposes, but can also be used as the recovery measure in situations where the schneiderian membrane comes unstuck during sinus-lift procedures (Fig. 1-2-2). The membrane must be the absorbable type in this case. Ensure a complete cover of the perforation with the absorbable membrane, and stabilize it with the application of the bone filling agents before closing the flap.

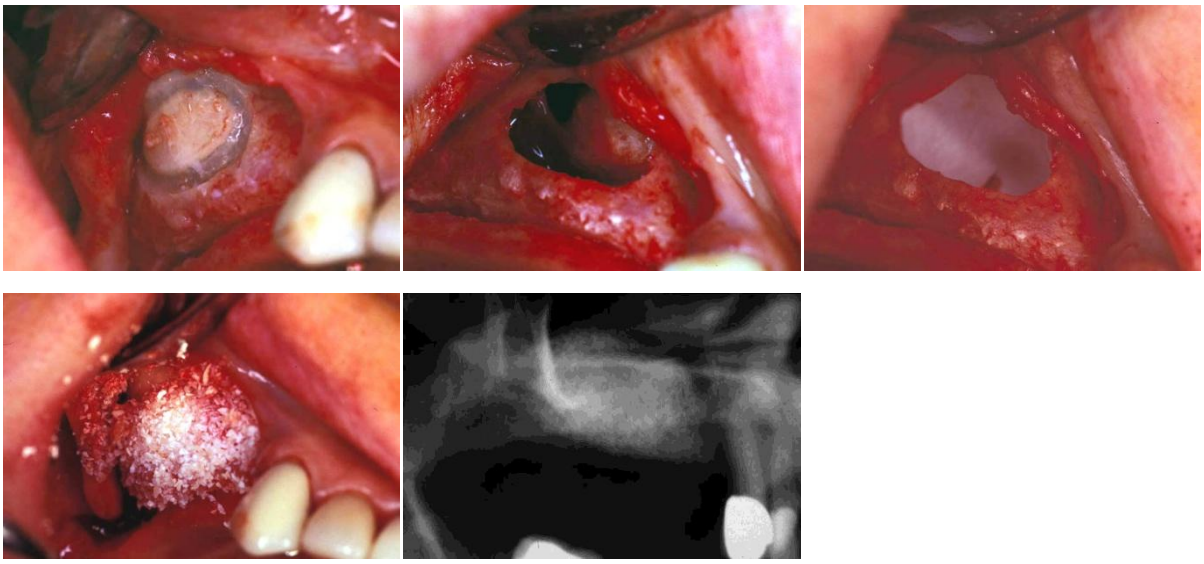

Fig. 1-2-2-a to e. A case where the perforation of the schneiderian membrane was closed with the application of the absorbable membrane:

a. Sinus-lift procedure was initiated with lateral fenestration surgery.

b. A large perforation resulted at the elevation stage of the schneiderian membrane (the black region at the 12 o'clock position is the perforated region).

c. Covering the perforation with absorbable collagen membrane.

d. Bone filling agent was added on the top, with the placement of the membrane.

e. The localization of the bone filling agents to underneath the sinus mucosa can be observed in the radiograph.

IV. Importance of "space preservation"

The preservation of secure space is the key to a successful outcome of bone augmentation with GBR technique. In comparing the clinical outcome of the ePTFE that have been strengthened with the titanium frame and that without, those with the adjunct has a better outcome [5]. This is owing to the ability of the titanium frame to protect the space required for the bone augmentation. Other recommended means to secure the preserved space are to use bone screws and bone tack to stabilize the ePTFE membrane. Provided that the application of membrane itself is sufficient to achieve adequate bone augmentation/ regeneration, there is no need for the adjunct tools, however, the reality is that the blood clot constriction, and the exertion of pressure from the membranes make this difficult to accomplish this. In particular, as in the case illustrated in Fig. 1-2-3, the even the force applied for suture can be enough to exert pressure on the membrane and restrict the amount of space possible. For this reason, it is often recommended to support the membrane with the addition of the bone filling agent in order to gain sufficient bone augmentation/ regeneration. Given that that bone filling agent itself can promote the bone augmentation process, or more specifically, the higher the osteoconductivity and osteoinductivity, a satisfactory bone augmentation and regeneration can be accomplished.

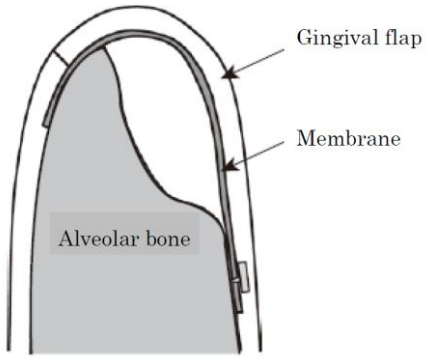

Fig.1-2-3
It is difficult to secure the amount of space required with only a membrane and blood clot, in cases such as this, thus it is desirable to pack bone graft substitutes.

V. Bone filling agents

The requirements for materials to be used as bone fillers:

1) Highly biocompatible
2) Stabilize blood clot and preserve the space
3) Has osteoconductive and osteoinductive abilities that support angiogenesis, proliferation of the bone marrow stromal cells and division of osteoblasts
4) Become a part of the host bone structure by its degradation

The bone filling agents can be largely separated into alloplast, xenograft, allograft and autograft types. The bone filling agents, with the exception of the own bone, have been processed into granules to have the ability to adopt to fill even the complex bone deficient structures. They have

also been referred to as the granular bone graft materials. The sizes of these particles vary from those that are within 100 to 300 μm diameters up to those of 300 to 1000 μm diameters. The smaller the diameter of the granules, the larger the surface area can be used, and the larger the diameter of the granular graft particle, the slower the degradation.

A. Alloplast

There are absorbable and non-absorbable types amongst the alloplast materials. Bioceramics, β - tricalcium phosphate (TCP), hydroxyapatite (HA), calcium sulfate, calcium carbonate calcium carbonate (derived from corals), and calcined gypsum are examples of the absorbable type, and porous HA, high-density HA, bioactive glass, and hard tissue replacement polymer (HTR polymer) (based on polymethyl methacrylate (PMMA)) are the non-absorbable types. The biocompatibility of the alloplasts reduces its risks of infection derived from the material, and with the added advantage of the manufacture in large quantities, makes it a viable option; however there have not been many reports of successful outcome with its application for the GTR/GBR procedures. Furthermore, its osteoconductive ability is not one that is superior to the other bone grafting substituents. Application of alloplasts alone for GTR/GBR is not recommended, at present.

B. Xenograft

Xenografts are bone graft substituents of other animal origin, where in the field of dentistry uses bones from cows as the raw material. The proteins are decomposed by undergoing heat and chemical treatments, then are processed into the granular shape without any modifications to the structures of inorganic materials. As the structure of the inorganic matter remains as the natural HA, it provides with an osteoiconductive environment that is more suitable for the osteogenic process. The bone graft material acts as the initiator of the neonatal bone formation, and a significant amount of time is required for the absorption of the material [6].

A type of xenograft material that has coated the natural HA with a synthetic peptide (P-15) is also present. The Type I collagens act as a natural cell scaffold with a specialized peptide structure, and the P-15 has been synthesized to mimic this structure. The theory here is that the P-15 coat should improve the compatibility of endogenous HA with the other cells. These bone graft material use the bone of the cows as the raw material, but has been reported to be devoid of the prion proteins that induce Bovine spongiform encephalopathy (BSE), and therefore with no risks of its infection [7, 8].

C. Allograft

The allograft uses the human bone that has been donated to the tissue bank. Since the raw material is of the same species, its similarities in their basic organic and inorganic structures can be applied directly to those of the host site, making this type of graft effective for osteogenesis. In the United States, allograft transplant had been applied to over a million cases by 2004. There are decalcified freeze-dried bone allograft (DFDBA), freeze-dried bone allograft (FDBA), and solvent-treated human HA to this type. These bone graft materials have excellent osteoconductive and osteoinductive abilities. In the 1990s, a study to quantify the amount of

BMP contained in DFDBA was conducted which reported that the amount of BMP contents differed significantly according to the age of the donor and the treatment method that subsequently led to the disparity in the osteoinductive abilities. The present belief however is that the amount of BMP contained in the DFDBA is not sufficient to precipitate osteoinduction, questioning the ability of the allograft material with its osteoinductive ability. The FDBA have not undergone decalcification therefore requires a longer period of time for its absorption into the body than with the DFDBA. Meanwhile the solvent-treated human HA has been treated via the tutoplast method without either of the decalcification nor freeze-dry processes. This tutoplast treatment is thought to retain the original organic and inorganic structures, therefore have strong oseteoconductive ability to promote the osteogenesis. The DFDBA, FDBA, and human HA are widely used in clinical settings with their efficacy proven (Fig.1-2-4). In addition to the granule-forms, block-forms are also present that are able to be used in place of chin or ramus grafts.

The one factor that should be considered is the risk of infection from the bone graft material. Theoretically, the transmission of infection is possible even after the material have undergone thorough screening and disinfection treatments. The risk of transmitting HIV infection has been reported to be 1/8,000,000 and risk lower than this.

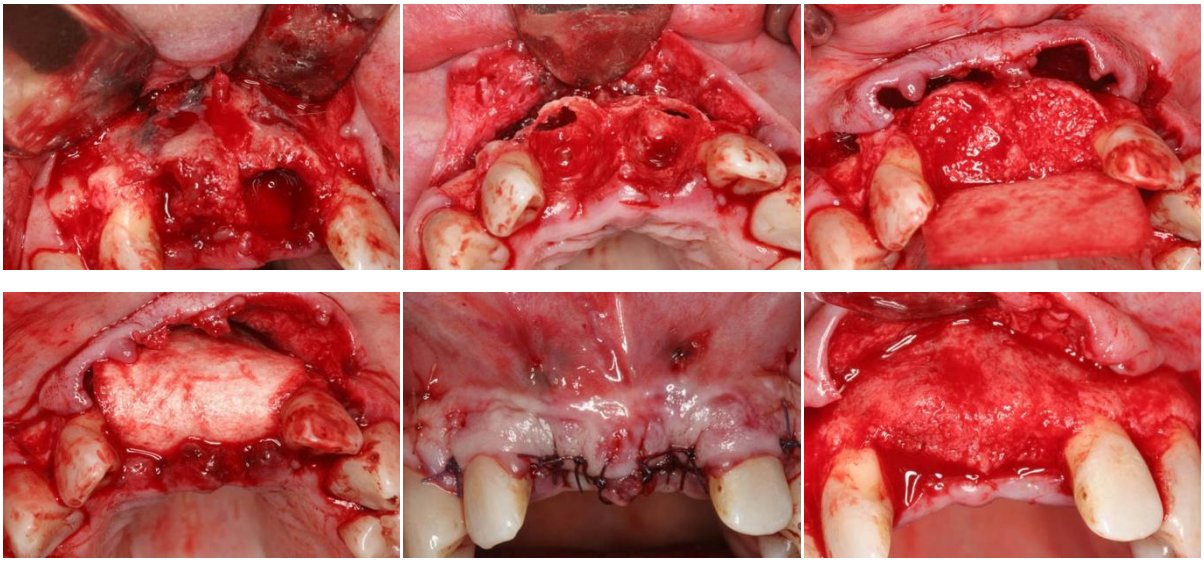

Fig.1-2-4-a to f. A case where the extraction socket preservation method on the anterior maxilla was conducted with absorbable collagen membrane.

a. A large amount of abnormal granulation tissues in the extraction socket and the apical area
b. The abnormal granulation was removed completely, which revealed thin labial alveolar crest. By closing the gingival flap without the any treatment, the aesthetic outcome with an implant treatment would become difficult due to the indentation on the labial side of the anterior maxilla.
c. Trim the membrane to be inserted under the palatal gingival flap, and upon determining the location, fill the extraction socket and its surroundings with the human HA.
d. Cover the bone graft material with the membrane, followed by the gingival flap.
e. Relax the gingival flap before closing with absorbable suture.

f. A significant amount of regenerated bone could be found half a year later.

D. Autograft

Autograft refers to the graft of own bone, usually extracted from other parts within the oral cavity, iliac bones or fibulas. The safety and its attachment ability are both effective on top of their osteogenic, osteoconductive, and osteoinductive abilities.

The bones from the intraoral cavity can be obtained from maxillary tuberosity, mandibular torus, buccal sides, and mental region, but those extracted from the maxillary tuberosity are high in osteoblasts and bone marrow stromal cells, therefore is the most suitable for GBR. Autograft is the gold standard of bone graft material with its numerous advantages but its drawback is the limited amount of bone quantity that is available. For this reason the combination of allograft and the xenografts are often used in the clinical settings since it is not recommended to combine the autograft with the other graft materials. When applying autograft, it should be spread to an area of bone or the exposed section directly, then spread a layer on top of this. The bone fragments that were extracted from the implant surgery must be preserved in the physiological saline solution till the end of the surgery (Fig. 1-2-5). If the GBR is required to mend the bone dehiscence resulting from implant surgery, this should be treated with the autograft.

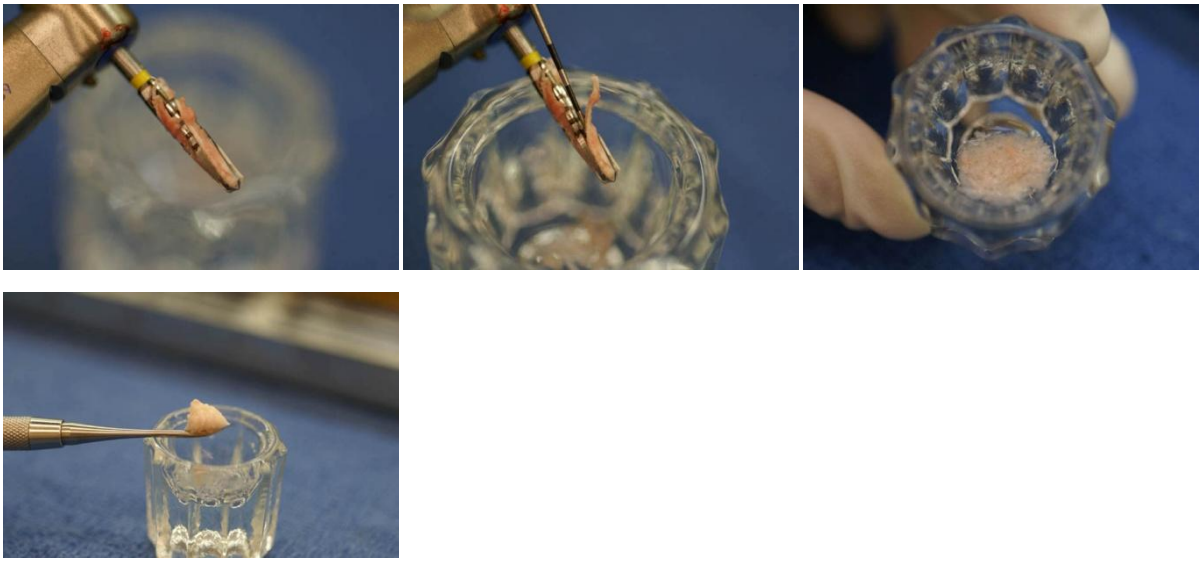

Fig. 1-2-5 a to d.

a. The drilling for the implant cavity results in the bone fragments to come attached to the drill blade. Limit the water infusion and avoid suction of the bone fragements.
b. Remove the bone fragments with the perio-probe, and preserve the fragments in the physiological saline solution to prevent it from drying
c. Own bone that has been obtained with the 2 mm, 2.5 mm, 3.2 mm and 3.7 mm drill blades.
d. A sufficient amount of graft material for a small area of bone deficiency after the implant installation.

VI. New generation of bone graft agents

The new generations of bone graft materials are biomaterials that have encompassed the idea of tissue engineering, and this is thought to evolve as the main stream therapy. The growth factors play a major role in this process (Fig. 1-2-6). The growth factors act as the source of osteoinductivity, and attract the undifferentiated stromal cells, stimulate its proliferation and its differentiation. The growth factors are not included in the bone graft materials except in those of the autograft agents. Platelet rich plasma (PRP) and plasma rich in growth factors (PRGF) have drawn attention in the recent years owing to their high level of growth factors included in these materials. In Japan, the research into the application of fibroblast growth factor-2 (FGF-2) in the regeneration of the periodontal tissues has been conducted extensively.

A. BMP-2 and collagen sponge

Bone morphogenic proteins (BMPs) are a potent inducing agent of osteogenesis. BMP-2 and BMP-7 have been particularly focused due to their success in the treatment of intractable fractures in United States and Australia. It is a recent development that BMP-2 has been approved for its use in bone regeneration therapy (GBR and sinus lift) in the field of dentistry. It is a medicinal product that has not yet been approved in Japan. The method for application of this agent is to add to the collagen sponge to the area of bone augmentation/regeneration. BMP-2 and collagen sponge are both synthetic materials therefore have no risks of infection.

B. PDGF and β-TCP

Platelet derived growth factors (PDGF) plays important roles in wound healing, angiogenesis, cell proliferation and regulation of osteocytes, and a large number is contained in the platelets. The PRP which is the blood plasma enriched with platelets contain 5 to 10 times the concentration of PDGF, whereas in the Gem 21 (growth factor enhanced matrix-21) contains 1000 times that of the PRP. This is highly concentrated PDGF is applied in a mix with the alloplast, β-TCP. Its application to the regeneration of the periodontal tissues has been approved, but this has not been applied in Japan.

VII. Quality of regenerated bone

There have always been conflicting views in the long-term preservation of the implants with the bone that had undergone regeneration processes in the same way as the original, innate bone. The recent findings have reported that the survival rate of the implants over five years were found to be on average 96% with the bone that had been regenerated with GBR technique, therefore confirming there to be no significant difference between those that have been implanted to the innate bone.

VIII. Final word

Many different kinds of membranes and bone graft materials have been developed, but the key to a successful outcome with the GBR technique is thought not to be in the selection of the materials, but in the complete closure and the healing of the area operated. In case of the

membrane exposure, it can also contaminate the bone grafting material, leading to the worsened state of the bone. The meta-analysis conducted by Machtei showed that the exposure of membrane in GTR method does not lead to a huge failure but the membrane exposure in GBR method have led to a significant setback. The average growth of the bone without the membrane exposure was indicated to be 3 mm, whereas the growth of 0.5 mm was noted where the membrane exposure did occur 11).

The key factors for a complete closure of the gingival flap and its healing have been listed as follows:

1) Conduct a tension-free closing of the gingival flap, having fully released the structure.
2) The incision should be formed that is widened at the bottom to ensure sufficient supply of blood.
3) Apply horizontal mattress suture, and draw in the gingival flap as a whole structure.
4) Avoid overloading the bone graft agents

The alveolar bone regeneration is an area of particular interest from the clinical setting as well as research. A number of scientific findings have been derived from the clinical studies applying membranes and bone graft materials. The main emphasis has been placed on the application of the growth factors and in the development of scaffolds that are highly osteoconductive (Fig. 1-2-6).

Using animal subjects, trials for the application of the growth factors, which have been induced with the genetic engineering, into scaffolds for its transplantation to the host site are being conducted. It is an area that can expect a great development hereafter, and the future of implants is optimistic.

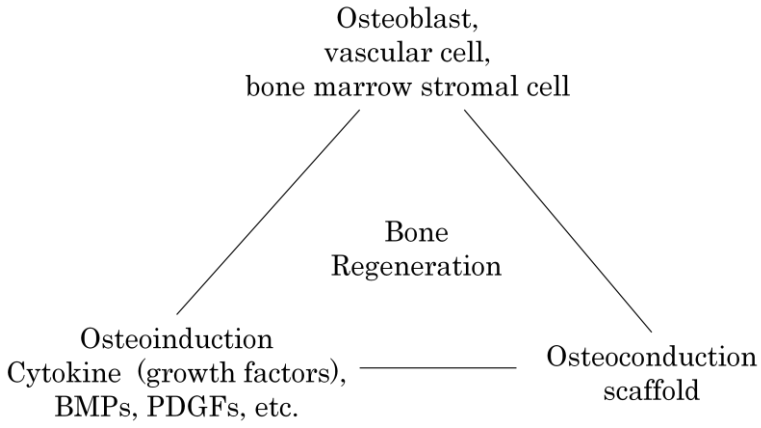

Fig.1-2-6 Three factors of the new generation of bone regeneration.

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