Review Article

Recent advances in tissue engineering for regeneration of oral tissues

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Recent development of biomedical engineering as well as basic biology and medicine has enabled us to induce cell-based regeneration of body tissue assisted with the self-repairing potential tissue or substitute biological functions of damaged organs with cells. For successful tissue regeneration, it is indispensable to give cells an environment suitable for induction of cell-based tissue regeneration. Tissue engineering is a newly emerging biomedical technology to create the environment for tissue regeneration with various biomaterials. This paper overviews recent researches and clinical data about oral tissues regeneration based on tissue engineering briefly explaining the key technologies of tissue engineering.

Rec.3/9/2006, pp82-91

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Key words tissue engineering, regeneration, oral tissues, dentistry

Introduction

When body tissue or organ is severely injured or largely lost, or becomes functionally wrong, it is clinically treated with either reconstruction surgery or organ transplantation. Reconstruction surgery always depends on biomaterials or biomedical devices that have been artificially prepared. However, they cannot substitute all of the biological functions, even for a single organ, and consequently cannot prevent progressive deterioration. Although there is no doubt that the surgical therapy has saved and improved countless lives, there are many problems to be clinically resolved. One of the largest problems for organ transplantation is the shortage of donor tissues or organs. Additionally, permanent immunosuppessive medication often causes various side-effects, while virus transfection is not completely ruled out. One promising approach to tackle these problems is to promote the self-healing potential of patients themselves for induction of body tissues and organs regeneration. The technology and methodology of biomedical sciences to induce tissue regeneration are called " Tissue engineering ". The basic concept of tissue engineering was originally introduced by R. Langer and J. Vacanti[1]. Several technologies and methodologies of tissue engineering to induce the regeneration of various tissues have been reported so far to demonstrate their scientific and clinical feasibility[2, 3].

Fundamental Technologies for Tissue Engineering

There are three key factors constituting body tissue: (i) cells,

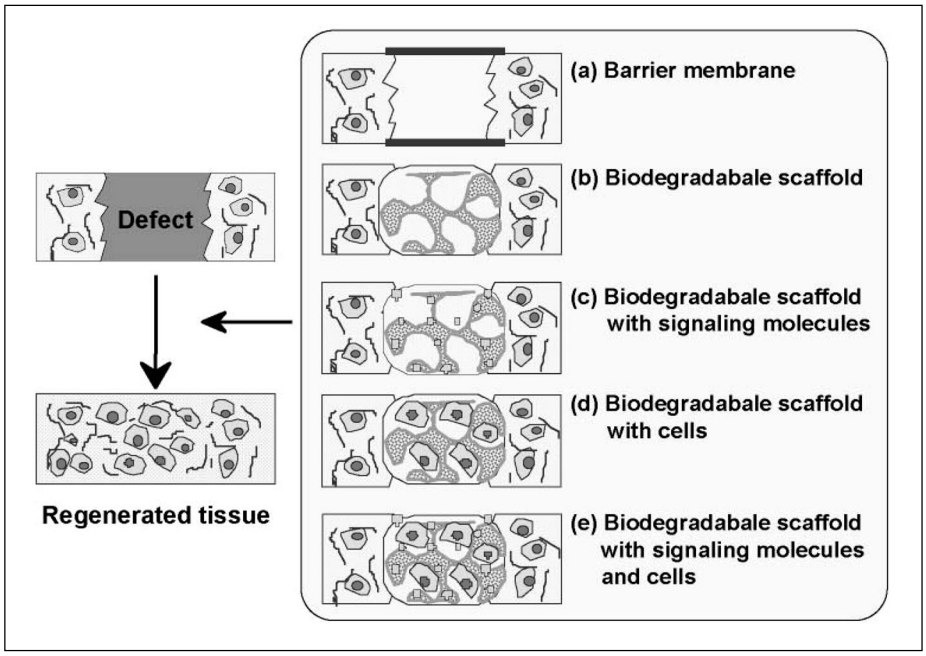

Fig.1 Several strategies to induce tissue regeneration at defect space with barrier membrane, cell scaffold, signaling molecules, and cells.

(ii) the extracellular matrix (ECM) for cell proliferation and differentiation (natural scaffold) and (iii) signaling molecules. Tissue regeneration is induced by making use of the three factors and the combination. To achieve the tissue regeneration, there are five fundamental technologies or methodologies of tissue engineering.

In the case that the tissue surround a defect space is healthy and biologically potential for regeneration, only supplying a physical barrier membrane to protect the space will result in tissue regeneration (**Fig.1a**). Physiologically, a body defect is occupied rapidly with the fibrous tissue produced by fibroblasts which are ubiquitously present in the body and can proliferate rapidly. This is one of the typical wound healing processes to temporarily fill and emergently repair the defect space. To prevent this tissue in-growth, a barrier membrane to make a space for tissue regeneration is required. The typical example is to use the membrane of guided tissue regeneration (GTR)[4] and guided bone regeneration (GBR)[5] in the field of periodontology and oral implantology.

Generally, it is difficult to naturally regenerate and repair a large-size tissue defect only by supplying the physical barrier membrane around the defect (**Fig.1b**). Therefore, to induce tissue regeneration at the defect site, one possible way is to artificially build an environment for cells to induce tissue regeneration by providing a scaffold of artificial ECM, which initially assists cell attachment and the subsequent proliferation and differentiation. The scaffold ECM is not only functions as a sterical support for cells, but also provides a temporal environment to promote their proliferation and differentiation, which may contribute to tissue regeneration and organogenesis. For example, only by using a collagen sponge of scaffold, it has been practically possible to induce regeneration of the skin dermis, trachea, esophagus, and dura mater[3].

When the tissue around a defect does not have the inherent potential to regenerate, tissue regeneration cannot be always expected if only the scaffold is supplied to the defect. Cells and/or signaling molecules (e.g. growth factors, cytokines, chemokines, and genes etc.) should be used in combination with scaffold to accelerate tissue regeneration (**Fig.1c-e**). Combination of cells isolated from the blood vessel, cartilage, and small intestine with biodegradable scaffolds results in the *in vivo* regeneration of respective tissues. Moreover, cells with a high potential for proliferation and differentiation, so-called " stem cells ", are prepared and applied to a tissue defect to induce tissue regeneration therein. For example, human mesenchymal stem cells (MSC) isolated from the bone marrow are commercially available[6], while some clinical researches with MSC have begun. There are some cases in which growth factors are needed to promote tissue regeneration. Growth factors are commercially available and have been experimentally applied for regeneration purpose. Although there are some cases where growth factor is required to promote tissue regeneration, the direct injection of growth factor in the so-

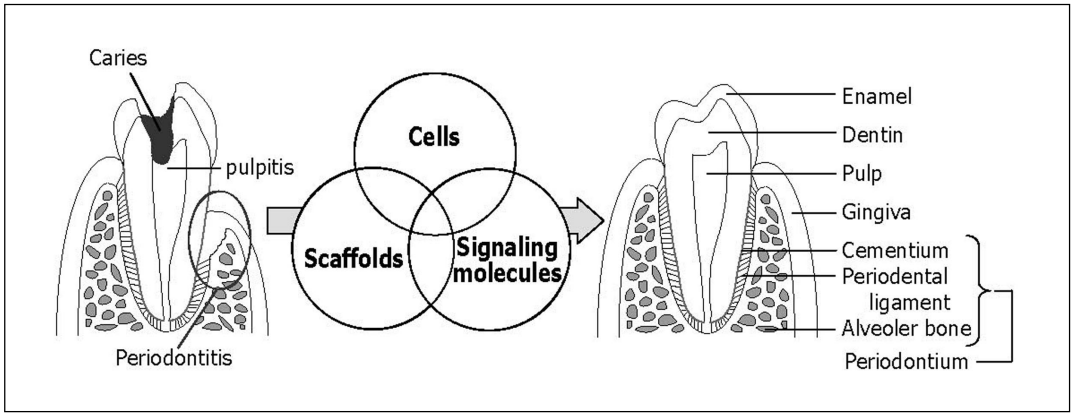

Fig.2 Critical factors necessary for regeneration of tooth and the surrounding tissues.

lution form into the site to be regenerated is generally not effective, because the growth factor rapidly diffuses from the injected site and is enzymatically digested or deactivated. To enable the growth factor to efficiently exert its biological functions, controlled release technology of drug delivery systems (DDS) technology of is strongly required. For example, basic fibroblast growth factor (bFGF) is reported to have a variety of biological activities[7] and be effective in enhancing wound healing through induction of angiogenesis and regeneration of bone, cartilage and nerve. When bFGF was incorporated into a gelatin hydrogel of the controlled release carrier and subcutaneously implanted into the back of mice, significant angiogenic effect was observed around the site implanted, in contrast to the control mice injected with bFGF solution or an empty gelatin hydrogel[8]. This angiogenic therapy for leg ischemia has been permitted by the ethics committee of Japanese university hospitals and the clinical experiment has been begun[3].

The question relevant to dental clinicians is " What type of clinical application will tissue engineering have in dentistry? " There are no clear answers for that, but it is highly expected that tissue engineering will bring about a therapeutical revolution in dentistry in near future. The idea and procedure of tissue engineering have been widely applied to many different types of oral tissues including tooth, periodontium, gingiva, and bone, and will be more expanded in dental clinics (**Fig.2**). The present review summarizes advance of tissue engineering in regeneration of oral tissues which has been reported experimentally and clinically.

Oral Tissues Regeneration with Tissue Engineering Technologies

The loss of natural teeth poses the partial destruction of facial skeleton with accompanying distortion of the appearance, morphology, and function of the soft tissues. The teeth lost is often causes by caries, trauma, and periodontal disease, or teeth may be congenitally missed. Over 40% of people receive prosthodontic treatments including dentures and bridges for tooth missing at ages of 45-54, and about 80% at ages of 65-74[9].

Dental caries is a common dental problem in which the enamel and dentin matrixes are damaged. When the caries is deep and the dental pulp is exposed or pulpitis, the pulp is clinically removed in many cases. While synthetic materials have been successfully utilized as restorative materials for many years in dentistry[10], they cannot always compensate the normal structure and function of dental tissues lost nor lead to remodeling in the face of ongoing insult or stimulation. Moreover, the teeth endodontically treated were lost more frequently than other teeth[11] and pulpal involvement may hasten tooth loss[12]. Therefore, the regeneration of enamel, dentin, dental pulp, and their complex is a big final goal of surgical dentistry and endodontics.

1. Enamel

Enamel, the exterior coating of vertebrate teeth, is a biomineral with remarkable hardness and resistance to physical and biochemical attack. The special features of enamel result from its composite nature, as it is composed of substituted hydroxyapatite, and organic macromolecules[13]. The enamel organ epithelium, including the ameloblasts, remains as a protective layer on the tooth crown until to eruption of tooth loss. Therefore, the enamel does not naturally regenerate in contrast to the dentin. So far, various attempts have been undertaken to harden enamel surfaces[14] and remineralize tooth minerals, which include tooth treatment with fluoride[15], calcium phosphate[16] or apatite particles[17]. Basic investigation about the mineralization of fluorapatite in gelatin gels enabled an artificial formation of spherical composite particles that show remarkable similarity to the

enamel in terms of morphology and chemical composition[18]. Yamagishi et al. prepared a white crystalline paste of modified hydroxyapatite (HA), which chemically and structurally resembles natural enamel, and the paste was used to repair an early caries lesion of lower premolar tooth. They have shown that the synthetic material could reconstruct enamel without prior excavation, in process where early caries lesions are not only repaired according to the healing, but also their reoccurrence was prevented by strengthening the natural enamel[19].

2. Dentin and Dental Pulp

The production of dentin and dental pulp have been also attempted experimentally by using tissue engineering technologies. There are many ways in which dentin and dental pulp lost are tried to regenerate based on tissue engineering concept.

Once damaged, dentin does not undergo remodeling, different from bone which remodels throughout the postnatal life. However, after tooth eruption, dentinal damage caused by mechanical trauma, exposure to chemicals or disease processes induces the formation of reparative dentin, a poorly organized mineralized matrix that serves as a protective barrier to the dental pulp. Conventional protection of exposed pulp by a capping procedure with calcium hydroxide have been widely used in clinical practice. Initially, calcium hydroxide leads to low-grade irritation to the pulp tissue, then reparative dentinogenesis is initiated; a layer of odontoblast-like cells is formed in association with the superficial calcification and a tubular mineralized matrix is secreted in a polar predentin-like pattern[20]. Several trials have been performed as the potential options for the pulp capping with demineralized dentin matrix[21], collagen[22], HA[23], and α-tricalcium phosphate (TCP)[24]. Synthetic extracellular matrixes may also be a potential scaffold for reparative dentinogenesis[25]. Recently, mineral trioxide aggregate (MTA) (ProRoot™ MTA), including fine hydrophilic particles of tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide, was developed as a root end filling material and a filling material to repair root furcation perforations. The MTA of biocompatibility hardens in the presence of moisture and prevents microleakage of microbe while it promotes reparative dentin formation[26].

Tissue engineering technologies with growth factors and cell scaffolds to assist the regeneration of viable endogenous pulp tissue in order to facilitate reparative dentin formation. Bone morphogenetic protein (BMP) formed a large amount of osteodentin accompanied with tubular reparative dentin[22, 27]. Recombinant human insulin-like growth factor (IGF)-I with a collagen membrane induces complete dentin bridging and tubular dentin formation[28]. Osteodentin accompanied with homogeneous and well-mineralized atubular reparative dentin was seen after capping treatment with bone sialoprotein[29]. Hard tissue formation at a distance region from the capping was found after the placement of enamel matrix derivatives (EMD) in the pulp exposed[30]. Intrapulpal implantation of Millipore filters containing transforming growth factor-β (TGF-β) induced specific dentinogenic events in the proximity close to the implants. Implantation of Millipore filters containing other growth factors, such as bFGF or IGF-II, showed increased dentinogenic effect at a distance region from the implant[31].

It is thought that progenitor cells are recruited from the dental pulp to develop the supportive connective tissue and terminally differentiated into odontoblasts. Several studies have demonstrated that highly proliferative pulp cells isolated from the adult pulp showed the potential of differentiation into odontoblasts and mineralization *in vitro*[32]. Gronthos et al. have isolated the dental pulp stem cells (DPSC) of highly proliferative activity from the adult human dental pulp[33]. Miura et al. have also isolated the stem cell from human exfoliated deciduous teeth (SHED)[34]. It is demonstrated that the DPSC and SHED showed the ability to generate a dentin/pulp-like complex *in vivo* when co-transplanted with HA/TCP particles subcutaneously into immunocompromized mice. Transplantation of DPSC and SHED transplants could form the vascularized pulp tissue surrounded by a well-defined layer of odontoblast-like cells, aligned around mineralized dentin with their processes extending into tubular structures[33, 35].

3. Periodontium

The regeneration of tooth supporting tissues which are structurally lost with periodontal disease progression is one of the final dreams for periodontists. Periodontal regeneration, i.e., the formation of new bone and new cementum with supportive periodontal ligaments, is a concrete goal of several periodontal therapies. Bone grafting and GTR are two techniques with the most histologic documentation of periodontal regeneration. A cellulose membrane (Millipore filter®) was the first biomaterial barrier applied for the periodontal GTR and the membranes of expanded polytetrafluoroethylene (ePTFE) (Gore-Tex®)[36] and biodegradable materials including synthetic polymers (Resolut®, Atrisorb®, GC membrane®etc.) and collagens (Biomed®, Ossix®, KOKEN Tissue Guide®etc.) have been developed and applied clinically[37]. Kikuchi et al. have prepared a composite membrane of β-TCP and poly (L-lactide-co-glycolide-co-ε-caprolactone) to maintain their mechanical strength and reported successful bone regeneration with the composite membrane[38]. Moreover, there are some research reports about membranes combined with

the DDS technology of growth factors. Milella et al. prepared an asymmetric membrane of poly (L-lactic acid) (PLLA) combined with an alginate film. This membrane not only prevents soft tissue ingrowth, but also functions as a release matrix of TGF-β to promote osteogenesis. TGF-β incorporated into the alginate membrane retained the biological activity when it was tested *in vitro*[39]. Lee et al. have reported the preparation of moldable porous PLLA-TCP membrane containing platelet-derived growth factor (PDGF)-BB and induced remarkable bone regeneration at an early healing stage[40].

Bone replacement grafts, such as autografts, allografts (freeze-dried bone allografts: FDBA and demineralized freeze-dried bone allografts: DFDBA), xenografts (bovine, porcine, horse), and alloplasts (HA, TCP, and bioactive glass etc.), are therapeutic strategies having widely used for the correction of periodontal osseous defects during the last 30 years. Bone grafts substitutes provide clinically considerable improvements in periodontal osseous defects compared with the surgical debridement alone. However, no periodontal regeneration has been achieved by using the bone substitutes, although the healing was enhanced and the probing depth decreased through the formation of a long junctional epithelium[41]. Presently, the substitutes are used combining with growth factors to promote periodontal regeneration.

Promising activities of growth factors in promoting periodontal wound repair have been demonstrated by preclinical and clinical studies. PDGF[42], IGF-I[42], bFGF[43], BMP-2[44], BMP -7[45], BMP -12[46], and EMD[47] show positive effects to stimulate periodontal regeneration. PDGF is one of the growth factors studied from long ago in terms of the wound healing because it is a potent mitogenic and chemotactic factor for mesenchymal cells *in vitro*. Animal experiments with non-human primates parallel to the clinical experiment indicate that PDFG alone was as effective as the PDGF/IGF- I combination in promoting periodontal regeneration, while no significant effect was found for IGF alone[42]. Presently, recombinant human PDGF combined with the TCP carrier (GEM 21S®) is FDA approved and commercial available. Recombinant human bFGF of potent angiogenesis[48] in the solution (Fibrast® spray) is on the Japanese market for the treatment of decubitus, a chronic skin ulcer. It has been demonstrated that the growth of immature periodontal ligament (PDL) cells and the mRNA level of laminin in PDL cells are promoted by bFGF stimulation[49]. Murakami et al. have experimentally confirmed periodontal tissue regeneration including new bone and cementum formation after topical application of recombinant bFGF to the furcation defects of beagle dogs molar. EMD (Emdogein®) is approved by FDA as a factor for periodontal regeneration[47]. EMD is one of the enamel matrix proteins harvested from developing porcine teeth. A recent report addresses the EMD effect on the stimulation of angiogenesis at a periodontal wound[50]. This factor may also accelerate periodontal regeneration. The potential of platelet-rich plasma (PRP) containing autologous growth factors of PDGF, TGF-β , and bFGF has been introduced for bone and periodontal regeneration[51]. PRP can be readily separated by the centrifugation of autologous blood, while platelets are enriched by 338 percent in PRP preparation and the concentrations of PDGF and TGF-β in PRP are 41.1 and 45.9 ng/ml, respectively[52]. Okuda et al. report that treatment with a combined PRP and HA for a human intrabony periodontal defect led to significant bone regeneration compared with HA alone[53].

Another approach of tissue engineering is for promoted periodeontal regeneration is with cells. Zhao et al. have demonstrated that transplantation of cloned cementoblasts with a polylactide-co-glycolide acid (PLGA) carrier repaired a large size of periodontal alveolar bone defect in rodents[54]. Kawaguchi et al. use autologous bone marrow MSC in combination with atelocollagen to regenerate cementum, periodontal ligament, and alveolar bone in an alveolar bone defect of dogs[55]. Akizuki et al. report that a PDL cells sheet fabricated using a temperature-responsive cell culture dish was effective in periodontal tissue regeneration at the dehiscence defect of root[56]. Multipotential stem cells isolated from the human periodontal ligament (PDL stem cells: PDLSCs) showed a differentiation potential into cementoblast- like cells or adipocytes[57].

4. Whole tooth tissues

If the whole tooth tissues can be regeneration, it is an ideal goal expected in dentistry. The tooth regeneration is one of the therapeutic demands stronger than the regeneration of each tissue like dentin and periodontium, but it is practically difficult based on only the present knowledge of basic biology and medicine. It is recognized that tooth development is performed as the result of mutual interaction between the dental epithelium and mechenchyme. Epithelial tissues produce enamel, while dental mesenchyme differentiates into pulp and dentin. Classical investigations of tooth growth were performed for tooth bud explants *in vivo* and *in vitro*[58]. However, there are many unknown factors contributing to this development process. Duailibi et al. have prepared a suspension of mixed cells from third molar tooth buds of porcine and seeded the suspension into a sponge of biodegradable copolymer. When the cell-sponge construct was implanted into the omentum, random generation of small teeth was observed[59]. The cell suspension prepared from the third molar tooth of canine was combined with a non-woven fabric of PGA

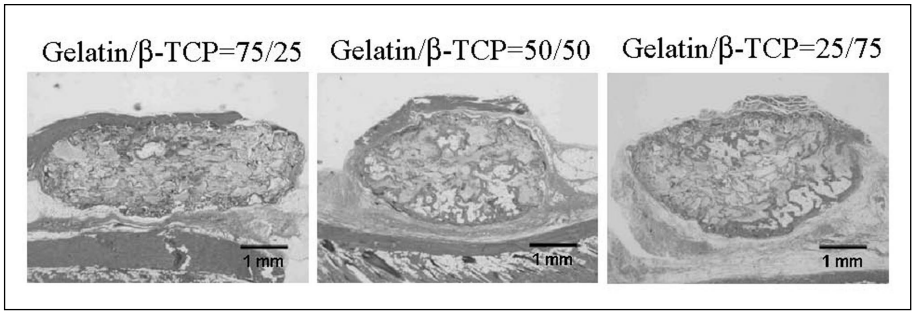

Fig.3 Histological cross-sections of subcutaneous tissue around the implanted site of gelatin-βTCP sponges incorporating BMP-24 weeks after implantation into the back subcutis of rats.

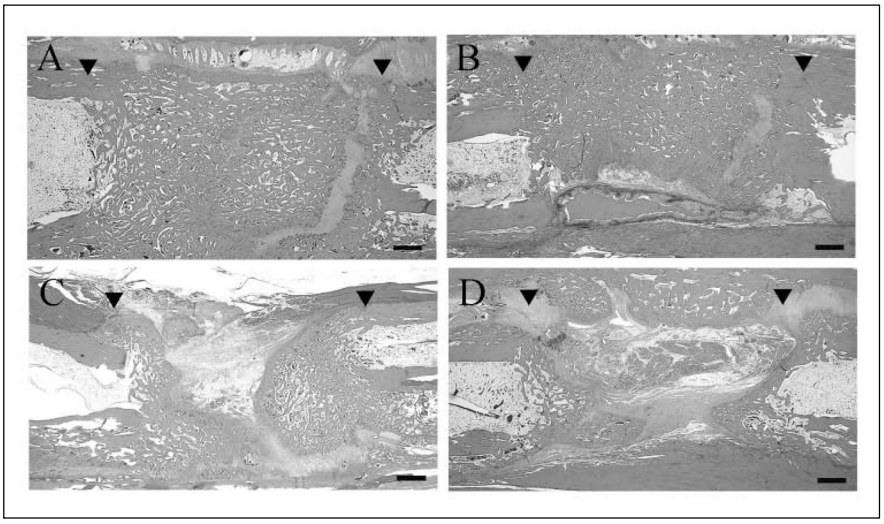

Fig.4 Histological sections of rabbit ulna defects 4 weeks after application with a gelatin hydrogel incorporating PRP (A), a fibrin glue incorporating PRP (B), free PRP (C) or without any application (D). An arrow head indicates the edge of ulna bone resected. The bar length is 1.0 mm.

fibers. When the construct was transplanted into the same defect from which the tooth buds were taken out, the hard tissue of tubular dentin and bone was regenerated[60]. However, the teeth regenerated were small and showed the shape and structure different from those of normal tooth[59].

5. Gingiva

Root dentin exposure by gingival recession often results from tooth brush abrasion and causes potential sensitivity to physical stimulation, i.e. hot, cold, sweets, and touch. In this case, subepithelial connective tissue graft (CTG) harvested from the palatal mucosa has been clinically used for root coverage[61]. Ideally, the root coverage corrective procedure requires not only esthetical replacement of lost gingival, but also the functional attachment to the root surface previously denuded. However, the present procedure does not always satisfy the therapeutic requirements. In addition, there are some clinical points to be improved, such as the donor-site morbidity and severe pain. The palatal mucosa of patients is too thin to obtain a sufficient volume of CTG from the donor site. To tackle the problems, the scaffold of biomaterials with or without growth factors and cells has been used for the root coverage therapy. Acellular dermal matrix (ADM) allograft is a CTG substitute for root coverage[62]. Griffin et al. used a collagen sponge containing PRP, combined with a coronally positioned flap procedure for two gingiva recession cases to demonstrate complete root coverage[63]. Okuda

et al. have reported about the cells sheet of human cultured gingival epithelial as an autologous grafting material for a patient with chronic desquamative gingivitis[64].

6. Maxillomandibular bone

For maxillomandibular bone defects caused by bone fracture, tumor resection, and congenital skeletal defect, reconstruction procedure is clinically indespensable. In such cases, dental implants cannot be clinically applied because of the proximity to the maxillary sinus at the implanted site and insufficient height or width of residual alveolar bone. Generally, bone graft is performed to augment the alveolar ridge and maxillary sinus floor (sinus lifting) for the placement of dental implant. So far, autologous bone has been considered as the gold standard graft material because of the inherent osteoinductive and osteoconductive properties. However, the donor-site morbidity and supply limitations are disadvantages for the autologous bone graft. Recently, allografts, xenografts, and alloplastic grafts, alone or their combination with autologous bone are applied for bone regeneration or augmentation[65]. In addition, new scaffold fabrication technologies have been developed to facilitate the repair of critical-sized and three-dimensionally complex cranial defects[66]. Yamamoto et al. have desired biodegradable gelatin-TCP sponges incorporating BMP-2 for the controlled release of BMP-2 to demonstrate the superior osteoinduction activity *in vivo*[67] (**Fig.3**).

Tissue engineering technologies with growth factors and cells are promising to promote bone regeneration. It has been demonstrated that the controlled release of growth factors is one of the key DDS technologies contributing to enhance their *in vivo* biological activity. Recently, gelatin hydrogels have enabled various growth factors to achieve their controlled release and consequently enhance their biological functions, resulting in promoted regeneration of tissues and organs[68]. A gelatin hydrogel enabled BMP-2 to induce the ectopical or orthotopical formation of bone tissues by the *in vivo* control release even at low doses which the BMP-2 solution was not effective. It should be noted that the BMP-2 dose to induce bone regeneration at a monkey skull defect was as low as that of rabbit and rat skull defects[67]. Successful bone regeneration by this hydrogel system of bFGF[69] and PRP[70] (**Fig.4**) has been reported. This hydrogel system of controlled release not only can release a single growth factor, but also two types of growth factors at the same time or in a different time profiles or concentrations. The synergistic effect on the bone regeneration and angiogenesis was achieved by the dual controlled release of bFGF and TGF-β1 or bFGF and HGF[71].

For bone regeneration with osteogenic cells, Kinoshita et al. have reported successful reconstruction of continuity defects in the canine mandible by combination of particulate cancellous bone and marrow (PCBM) and a PLLA mesh tray[72]. Ueda et al. have succeeded in the regeneration of alveolar crestal bone with a mixture of autologous MSC, PRP, and TCP improved[73]. A novel scaffold of PLGA combined with autologous MSC was reported on osteogenesis at a segmental mandibular defect and enhanced penetration of the bone with blood vessels[74]. A segmental mandibular defect was repaird by combination of autologous bone marrow, bone substitute, and BMP-7[75]. We have recently succeeded in the regeneration of bone tissue with complete vascular network by a prefabrication procedure of a vascularized bone graft composed of PCBM, a vessel bundle, and biodegradable membrane[76, 77].

Concluding remarks and future directions

Tissue engineering is a biomedical technology or methodology to realize the third medical therapy, so-called regenerative medicine therapy, following reconstruction surgery and organ transplantation. It is highly expected that tissue engineering can be clinically applied to recreate dental tissues and bone lost or injured as well as physiologically correct skeletal bone defects. Recent rapid advent of dental sciences will allow to make clear the biological mechanism of dental regeneration phenomena and enable to make use of biological signaling molecules related the phenomena in future. Undoubtedly, clinical applications of the novel scientific knowledge and the signaling molecules will be accomplished technological and methodological contrivance with biomaterials. To realize regeneration medical therapy based on tissue engineering, substantial research collaborations among materials, pharmaceutical, biological, and medical dental sciences, and clinical medicine and dentistry need to mature scientific knowledge and technologies of tissue engineering and the related fields. The real objective of tissue engineering improves the quality of life for patients suffering from diseases as soon as possible.

Acknowledgments

This paper is supported in part by Establishment of International COE for Integration of Transplantation Therapy and Regenerative Medicine (COE program of the Ministry of Education, Culture, Sports, Science and Technology, Japan).

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