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Cell Sheet Engineering for Periodontal Regeneration

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Additional information is available at the end of the chapter

1. Introduction

Periodontitis is a world-wide infectious disease that destroys the tooth-supporting attachment apparatus, which consists of alveolar bone, cementum, and periodontal ligament. Recent studies have reported numerous associations between periodontitis and systemic diseases, such as cardiovascular disease (de Oliveira et al., 2010) and diabetes mellitus (Lalla and Papapanou, 2011), as well as a higher risk of preterm low birth-weight babies (Offenbacher et al., 1996). Furthermore, researches have recently shown that Bisphosphonate-Related Osteonecrosis of the Jaws (BRONJ) is also associated with severe periodontitis (Vescovi et al., 2011). Therefore, periodontal treatment may not only contribute to oral hygiene but also improvement of systemic conditions (Seymour et al., 2007). Conventional treatments, such as scaling, root-planing, and surgical cleaning, have been performed to remove the bacteria and contaminated tissue. However, these procedures frequently result in the formation of a weak attachment, a condition termed “long junctional epithelium (LJE)” (Caton et al., 1980), wherein the patients tend to present with a recurrence of disease without maintenance therapies (Axelsson and Lindhe, 1981). To overcome this problem, various regenerative therapies, such as guided tissue regeneration (GTR) and enamel matrix derivative, have been introduced in clinical practice. The use of cell-occlusive membranes for GTR is regarded as the first generation of periodontal regeneration, whereas the development and use of growth factors and endogenous regenerative technology for periodontal regeneration is regarded as the second generation of periodontal regeneration (Ishikawa et al., 2009). However, the outcomes of these studies were limited and associated with poor clinical predictability (Esposito et al., 2009). Therefore, stem cell-based approaches for periodontal regeneration have been studied and translated into clinical settings as the third

generation. In this chapter, we would like to describe the principles of “Cell Sheet Engineering” and its application of clinical settings, featuring our recent translational research for periodontal regeneration.

2. “Cell Sheet Engineering (CSE)”

The cell delivery for periodontal regeneration is usually performed with the combination use of cells and scaffolds, although the location and the differentiation of transplanted is difficult to control. In contrast to approaches that utilize scaffolds, we have developed an alternative technology for cell transplantation using temperature responsive culture dishes, which we call “Cell Sheet Engineering”.

2.1. Intelligent surface of *N*-isopropylacrylamide (PIPAAm) and fabrication of cell sheets

Poly(*N*-isopropylacrylamide) (PIPAAm) is a temperature responsive polymer that has been widely utilized for novel biomedical applications. We have developed a PIPAAm-grafted surface as a smart biointerface wherein cell attachment/detachment can be easily controlled by simply changing the temperature (Okano et al., 1995; Yamada et al., 1990). This surface is slightly hydrophobic under cell culture conditions of 37 °C, but readily becomes hydrated and hydrophilic below its lower critical solution temperature (LCST) of 32 °C. Cells can adhere, spread, and proliferate similarly to that on ungrafted tissue culture grade polystyrene surfaces at 37 °C (Figure 1A), and cells detach from the surface by reducing temperature below LCST, making it possible to harvest the cells from the culture surfaces without the use of proteolytic enzymes (Figure 1B).

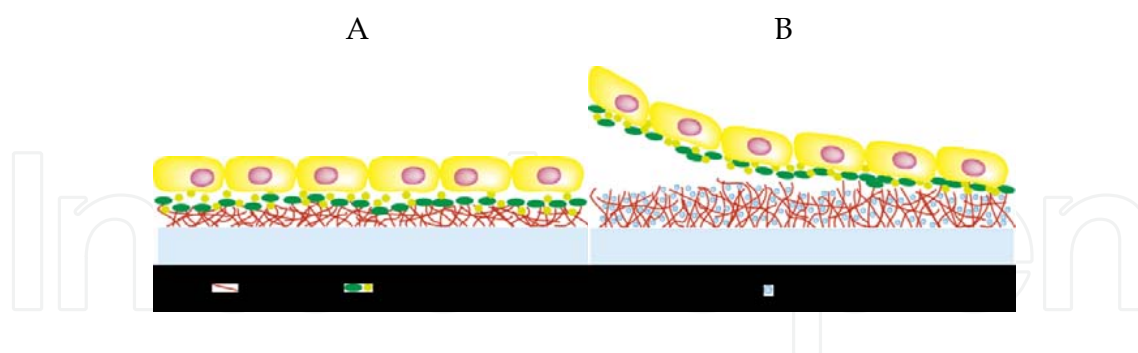


Figure 1. The principle of “Cell Sheet Engineering”.

A: Cells can attach and proliferate on grafted surface of the temperature responsive polymer (poly (*N*-isopropylacrylamide: PIPAAm) at 37 °C, wherein PIPAAm is extensively dehydrated and compact. B: At temperatures below 32 °C, cells with extracellular matrix proteins spontaneously detach from the temperature responsive culture dishes, wherein PIPAAm is fully hydrated with an extended-chain conformation. A simple temperature change can control cell attachment/detachment without any damages. Modified and reprint from Iwata et al., 2013.

The application of this technology has enabled the retrieval of confluent cultured cells, such as keratinocytes (Yamato et al., 2001), corneal epithelial cell sheets (Nishida et al., 2004a), and oral mucosal epithelial cells (Ohki et al., 2006) in the form of a “cell sheet”. The epithelial cell sheets are multi-layered and preserve the integrity of proteins such as E-cadherin and laminin 5 that are typically destroyed in the process of enzymatic treatments (Yamato et al., 2001). In addition, recent studies revealed that epithelial cell sheets can be fabricated using temperature responsive culture inserts without feeder layers (Murakami et al., 2006a; b), thereby eliminating xenogeneic factors for animal-free cell transplantation (Takagi et al., 2011).

To fabricate thick tissues, cell sheets can be stacked in layers because they can connect to one another very quickly. A study demonstrated that bilayer cardiomyocyte sheets were completely coupled 46 ± 3 min (mean \pm SEM) after the initial layering (Haraguchi et al., 2006), suggesting that multi-layered cell sheets can communicate and become synchronized as functional tissues. Based on this study, multi-layered transplantation was performed (Shimizu et al., 2006b). When more than three cardiomyocyte sheets were layered and transplanted into the subcutaneous space in rats, the appearance of fibrosis and disordered vasculature indicated the presence of fibrotic areas within the transplanted laminar structures. Although the rapid establishment of microvascular networks occurred within the engineered tissues, this formation of new vessels did not rescue the tissues when the thickness was above 80 μ m. Using a multiple-step transplantation protocol at 1 or 2 day intervals resulted in rapid neovascularization of the engineered myocardial tissues with a thickness of more than 1 mm (Shimizu et al., 2006b), and these results led us to fabricate prevascularized cell sheets (Sekine et al., 2011). Recent studies demonstrate that the combination of different types of cells, for example an endothelial cell sheet sandwiched with other types of cell sheets, can lead to pre-vascularization *in vitro*, which may allow the graft to survive and function (Haraguchi et al., 2012; Pirraco et al., 2011). Furthermore, the three-dimensional manipulation of fibroblast cell sheets and micro-patterned endothelial cells with a gelatin-coated stacking manipulator produced microvascular-like networks within a 5-day *in vitro* culture (Tsuda et al., 2007). Non-patterned endothelial cell sheets and other types of cell sheets with a fibrin gel manipulator can also produce pre-vascular networks both *in vitro* (Asakawa et al., 2010) and *in vivo* (Sasagawa et al., 2009).

2.2. Cell sheet transplantation in animal models

From the beginning of the 21st century, various types of cells have been extracted, cultured in temperature responsive dishes, and fabricated as cell sheets. Transplantation has been performed, and the efficacy of these cell sheets was evaluated in most of the studies.

2.2.1. Corneal regeneration

Limbal stem-cell deficiency by ocular trauma or diseases causes corneal opacification and loss of vision. To recruit limbal stem cells, a novel cell-sheet manipulation technology that takes advantage of temperature responsive culture surfaces was developed (Nishida et al., 2004a). The results reveal that multi-layered corneal epithelial cell sheets were successfully

fabricated and that their characteristics were similar to those of native tissues. Transplantation of these cell sheets induced corneal surface reconstruction in rabbits. For patients who suffer from unilateral limbal stem deficiency, corneal epithelial cell sheets can be cultured from autologous limbal stem cells. When the objective is to repair the bilateral corneal stem cell deficiency, autologous oral mucosal epithelial cells are utilized to create oral mucosal epithelial cell sheets. The cell sheets contain both cell-to-cell junctions and extracellular matrix proteins, and can be transplanted without the use of any carrier substrates or sutures. Therefore, oral mucosal epithelial sheets were examined as an alternative cell source to expand the possibilities of autologous transplantation. Autologous transplantation to rabbit corneal surfaces successfully reconstructed the corneal surface and restored transparency. Four weeks after the transplantation, epithelial stratification was similar to that of normal corneal epithelia, although the keratin expression profile retained characteristics of the oral mucosal epithelium.

2.2.2. Cardiac regeneration

To enhance the function of cardiac tissue, neonatal rat cardiomyocyte sheets were fabricated and examined (Shimizu et al., 2002). When 4 sheets were layered, spontaneous beating of the engineered constructs was observed. When they were transplanted subcutaneously, heart tissue-like structures and neovascularization within the contractile tissues were observed. The long-term survival of pulsatile cardiac grafts was confirmed for more than one year in rats (Shimizu et al., 2006a). Another study was performed to create thick tissue in rats (Shimizu et al., 2006b). However, the thickness limit for the layered cell sheets of subcutaneous tissue was ~80 μm (3 layers). To overcome this limitation, several transplantations of triple-layer grafts were performed, resulting in an approximately 1 mm-thick myocardium with a well-organized microvascular network. Other types of cell sheets were also examined to improve cardiac function. Adipose-derived mesenchymal stem cells in mice (Miyahara et al., 2006) and skeletal myoblasts in dogs, rats, and hamsters (Hata et al., 2006; Hoashi et al., 2009; Kondoh et al., 2006) were transplanted as cell sheets, demonstrating the efficacy of the method for cardiac repair.

2.2.3. Cartilage regeneration

Chondrocyte sheets applicable to cartilage regeneration were prepared using cell sheet manufacturing technique that takes advantage of temperature responsive culture dishes. The layered chondrocyte sheets were able to maintain the phenotype of cartilage and could be attached to sites that exhibited cartilage damage. The cell sheets act as a barrier for preventing the loss of proteoglycan from these sites and for protection against catabolic factors in the joints of rabbits (Kaneshiro et al., 2006).

2.2.4. Esophageal regeneration

With the recent development of endoscopic submucosal dissection (ESD), large esophageal cancers can be removed using a single procedure. However, complications, such as postoperative inflammation and stenosis, frequently occur after an aggressive ESD procedure,

which can considerably affect the quality of life of the patient. Therefore, a novel treatment combining ESD and the endoscopic transplantation of tissue-engineered cell sheets created using autologous oral mucosal epithelial cells, was examined in a canine model (Ohki et al., 2006). The results confirm the efficacy of the novel combination of the endoscopic approach with the potential treatment of esophageal cancers that can effectively enhance wound healing and possibly prevent postoperative esophageal stenosis.

2.2.5. Hepatocyte regeneration

To address the demand for therapeutic benefits for patients suffering from liver disease, the development of new therapeutic applications is crucial. Therefore, hepatic tissue sheets transplanted into the subcutaneous space of mice have been investigated, resulting in the efficient engraftment of the surrounding cells, as well as the formation of a two-dimensional hepatic tissues network, which was stable for more than 200 days (Ohashi et al., 2007). The engineered hepatic cell sheets also showed several characteristics of liver-specific functionality, and the use of bilayered sheets enhanced these characteristics.

2.2.6. Fibroblast sheet transplantation for sealing air leaks

In thoracic surgery, the development of postoperative air leaks is the most common cause of prolonged hospitalization. To seal the lung leakage, use of autologous fibroblast sheets on the defects was demonstrated to be an effective treatment for permanently sealing air leaks in a dynamic fashion in rats (Kanzaki et al., 2007). Using roughly the same procedures, pleural defects were also closed by fibroblast sheets in pigs (Kanzaki et al., 2008).

2.2.7. Mesothelial cells for the prevention of post-operative adhesions

Post-operative adhesions often cause severe complications such as bowel obstruction and abdominopelvic pain. The use of mesothelial cell sheets was investigated to prevent post-operative adhesions in a canine model (Asano et al., 2006). Mesothelial cells were harvested from tunica vaginalis (Asano et al., 2005) and cell sheets were fabricated on a fibrin gel. The results demonstrated that mesothelial cell sheets are effective for preventing post-operative adhesion formation.

2.2.8. Retinal Pigment Epithelial (RPE) cell regeneration

The retinal pigment epithelium (RPE) plays an important role in maintaining the health of the neural retina. RPE cell sheets were fabricated as a monolayer structure with intact cell-to-cell junctions, similar to that of native RPE (Kubota et al., 2006). In the transplantation study, RPE cell sheets attached to the host tissues in the subretinal space were more effective than the use of injected isolated cell suspensions in rabbits (Yaji et al., 2009).

2.2.9. Urothelial regeneration

Augmentation cystoplasty using gastrointestinal flaps may induce severe complications such as lithiasis, urinary tract infection, and electrolyte imbalance. The use of viable, contig-

uous urothelial cell sheets cultured *in vitro* should eliminate these complications. Canine urothelial cell sheets were grown and their structures were shown to be appropriate (Shiroyanagi et al., 2003). Urothelial cell sheets were autografted onto dog demucosalized gastric flaps successfully, with no suturing or fixation, and generated a multi-layered urothelium *in vivo* (Shiroyanagi et al., 2004). The novel intact cell-sheet grafting method rapidly produced native-like epithelium *in vivo*.

2.2.10. Islet regeneration

To establish a novel approach for diabetes mellitus, pancreatic islet cell sheets were fabricated and transplanted in rats (Shimizu et al., 2009). Laminin-5 was coated on temperature responsive dishes to enhance the initial cell attachment, and the presence of specific molecules, such as insulin and glucagon, was also observed in the recipient site.

2.2.11. Thyroid regeneration

For hormonal deficiencies caused by endocrine organ diseases, continuous oral hormone administration is indispensable to supplement the shortage of hormones. To verify the cytotherapeutic approach, cells from rat thyroid were spread on temperature responsive culture dishes, and cell sheets were created (Arauchi et al., 2009). Rats were exposed to total thyroidectomy as hypothyroidism models and received the thyroid cell sheet transplantation 1 week after the total thyroidectomy. The transplantation of the thyroid cell sheets was able to restore the thyroid function 1 week after the cell sheet transplantation and the improvement was observed long after the surgery.

2.3. Cell sheet transplantation in human clinical trials

In Japan, 6 clinical trials using cell sheet engineering technology have been started or have already been completed.

2.3.1. Corneal reconstruction

The first clinical trial of the cell sheet engineering technology involved a corneal reconstruction using autologous mucosal epithelial cells, and the results were published in 2004 (Nishida et al., 2004b). Oral mucosal tissue was harvested from 4 patients with bilateral total corneal stem-cell deficiencies. Subsequently, cells were cultured for two weeks using a mitomycin C-treated 3T3 feeder layer and transplanted directly into the denuded corneal surfaces without sutures. The results demonstrated that complete re-epithelialization of the corneal surfaces occurred, and the vision of all patients was restored. Recently, autologous oral mucosal epithelial cell sheets cultured with UpCell-Insert technology (CellSeed, Tokyo, Japan) without the feeder layer were transplanted into 25 patients for the treatment of corneal limbal epithelial deficiency in France. The safety of the products was established during the 360-day follow-up, and the results confirmed its efficacy for reconstructing the ocular surface. (Burillon et al., 2012).

2.3.2. Endoscopic treatment of esophageal ulceration

Using a canine model (Ohki et al., 2006), autologous oral mucosal epithelial cell sheets were fabricated using the UpCell-Insert technology. After performing the esophageal endoscopic submucosal dissection to remove superficial esophageal neoplasms, cell sheets were transplanted, resulting in the complete prevention of stricture formation in patients with partial circumferential resection (Ohki et al., 2009; Ohki et al., 2012).

2.3.3. Improvements in ischemic cardiomyopathy

Autologous myoblast cells from a patient's thigh were fabricated as cell sheets, and these cell sheets were transplanted into end-stage dilated cardiomyopathy patients in need of left ventricular assist systems (Sawa et al., 2012). The myoblastic cell sheets were transplanted into the affected part of the heart in the patients. The first patient was successfully treated and discharged from the hospital without requiring a ventricular assisting device.

2.3.4. Cartilage regeneration

A clinical trial for cartilage regeneration began in 2011 at Tokai University, Japan. In this study, autologous chondrocytes and synoviocytes were co-cultured with the UpCell-Insert technology. After a period of cultivation, co-cultured cell sheets were combined into three layers and transplanted into the cartilage defects of patients.

2.3.5. Nasal mucosa epithelial cell sheet transplantation to the middle ear bone for preventing hearing loss

A clinical trial for preventing hearing loss began in 2014 at The Jikei University, Japan. Autologous nasal mucosa epithelial cell sheets were transplanted to the surface of bone of the middle ear, and inhibit such as the hyperplasy of granulation tissue and bone, and the progression of fibroblast within middle ear cavity, which induce hearing loss after the surgery of otitis media.

3. Periodontal regeneration

Our laboratory started to introduce cell sheet engineering for periodontal regeneration since sometime after 2000. A key event in periodontal regeneration involves the formation of periodontal ligament and cementum complex (MacNeil and Somerman, 1999), which is a thin surface structure that anchors the tooth to the alveolar socket. Several studies have demonstrated that the cell sheet engineering approach can deliver functional cells in the form of a thin layered sheet, wherein the extracellular matrices, cell-cell junctions, and cell-matrix interactions are well-preserved (Kumashiro et al., 2010). Thus, we have attempted to regenerate this periodontal attachment apparatus based on the technology of "cell sheet engineering" (Yang et al., 2007).

3.1. Small animal studies

Human PDL (hPDL) cell sheets were successfully created using temperature responsive dishes, and the characteristics of hPDL cell sheets were investigated (Hasegawa et al., 2005). In this study, explant culture methods were utilized for the primary culture of hPDL cells. The hPDL cell sheets cultured with ascorbic acid were recovered from the culture dishes as a contiguous sheet accompanied by abundant extracellular matrix components, including type I collagen, integrin $\beta 1$ and fibronectin. Then, hPDL cell sheets were transplanted as cell pellets into a mesial dehiscence model in athymic rats. Four weeks after surgery, newly formed immature fibers with obliquely anchored dentin surfaces were observed in all the experimental sites, whereas no such findings were observed in any control sites (Figure 2). These results suggest that this procedure based upon the principles of cell sheet engineering can be applied to periodontal regeneration.

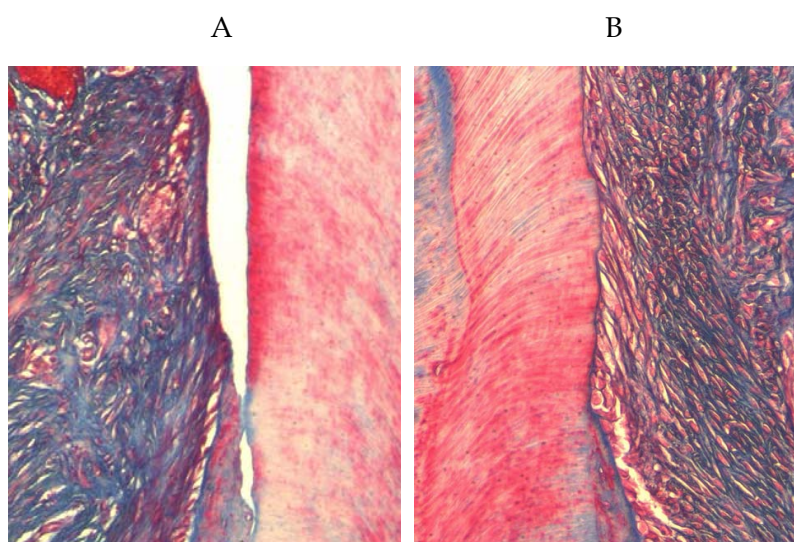


Figure 2. PDL regeneration at 4 weeks postsurgery.

A: Nontransplanted control site. B: hPDL transplanted experimental site. Regeneration of periodontal ligament-like structure was observed only in the experimental site. Azan staining. Modified and reprint from Hasegawa et al., 2005.

Next, the optimal culture condition was examined. Because the osteoinductive medium, which contains 50 $\mu\text{g/ml}$ of ascorbic acid, 10 mM β -glycerophosphate, and 10 nM dexamethasone, enhanced both osteoblastic/cementoblastic and the periodontal differentiation of PDL cells *in vitro*, we compared hPDL cell sheets cultured in the absence and presence of these osteoinductive supplements in a xenogeneic transplantation model (Flores et al., 2008a). Three layered hPDL cell sheets were constructed with fibrin gel and transplanted with a human dentin block into the back of a subcutaneous athymic rat. The constructs were excised for histological investigation 6 weeks after the transplantation. The three-layered hPDL cell sheets-dentin block constructs induced a new cementum-like hard tissue on the surface of the dentin in more than 60% of the samples. Collagen fibers were inserted perpen-

dicularly into the newly formed cementum-like tissue, and this orientation resembled the native Sharpey's fibers. In addition, the regenerative potential of hPDL cell sheets cultured with the osteoinductive medium was confirmed, when hPDL cell sheets were transplanted onto the root surface of periodontal defects in athymic rat mandibles (Flores et al., 2008b). The results indicate that most of the specimens in the experimental group exhibited a newly-formed cementum and a new attachment of collagen fibers to the cementum layer. No clear cementum layer was observed in the control group (in the absence of osteoinductive supplements). As shown in these experiments, hPDL cells cultured with osteoinductive medium could contribute to the simultaneous regeneration of cementum and PDL.

3.2. Large animal studies

Based on the successful results from small animal studies, we next utilized canine periodontal defect models. Dog PDL (dPDL) cells were extracted using collagenase/dispace digestion. Four individual dPDL cells were successfully isolated and expanded *ex vivo*. Cells were cultured in a standard medium with osteoinductive supplements for 5 days, because longer cultivation induced spontaneous detachment of cell sheets from the UpCell Surfaces. Three-layered dPDL cell sheets were fabricated with woven polyglycolic acid (PGA) for cell sheet transfer. This PGA product has a number of advantages, including: 1) cell sheets can be easily peeled from temperature responsive dishes, because cell sheets can be attached to the fibers of the woven PGA, 2) the shrinkage of cell sheets can be prevented, 3) easy stacking of multi-layered cell sheets can be achieved in a short period of time (see the video attached to the manuscript (Iwata et al., 2009)), 4) easy adjustment of different sizes of cell sheets can be used to cover any defect shape by simply trimming the cell sheets, 5) the ability to make contact on hard tissues and curved surfaces, and 6) the transplant is visible to the operators. dPDL cell sheets were transplanted into the surface of dental roots containing three-wall periodontal defects in an autologous manner, and bone defects were filled with porous beta-tricalcium phosphate (β -TCP). Cell sheet transplantation regenerated both new bone and cementum connecting with the well-oriented collagen fibers, while only limited bone regeneration was observed in the control group where cell sheet transplantation was not performed. These results suggest that PDL cells have multiple differentiation properties that allow for the regeneration of periodontal tissues composed of hard and soft tissues.

Next, we evaluated the safety and efficacy of PDL cell sheets in a one-wall infrabony defect model (Tsumanuma et al., 2011), which is considered to be a severe defect model (Kim et al., 2004). In this study, we also compared the differences in the periodontal healing of various cell sources. PDL cells, bone marrow derived mesenchymal stem cells, and alveolar periodontal cells were obtained from each animal, three-layered canine cell sheets were transplanted in an autologous manner, and bone defects were filled with porous β -TCP with 3% type I collagen gel to stabilize the graft shape. Eight weeks after transplantation, significantly more periodontal regeneration was observed in the newly formed cementum and well-oriented PDL fibers more in the PDL cell sheets group than in the other groups. These results indicate that PDL cell sheets combined with β -TCP/collagen scaffold serve as a promising tool for periodontal regeneration.

3.3. Optimization of human PDL cells

To protect human rights as subjects in clinical trials, the protocol of cytotherapy should be designed based on Good Clinical Practice (GCP) and Good Manufacturing Practice (GMP). Culturing hPDL cells from a single tooth is essential in performing our clinical trial. However, appropriate method for the extraction and expansion of hPDL cells are still not well understood. Thus, we determined the optimal method of isolation and expansion of hPDL cells and then examined their gene expression levels and differentiation potentials, and eventually validated the common characteristics of hPDL cells from 41 samples (Iwata et al., 2010). The hPDL cells were successfully extracted with collagenase/dispase, and then clonal proliferation was performed. Typically, 10 to 100 colonies were observed for a few days after the initial spreading. hPDL cells exhibit the ability to be highly proliferative when cultured at a low cell density. The cells were subcultured for 3 to 4 days, reaching one million cells in 2 weeks. Then, cells were spread on temperature responsive dishes to create a cell sheet in the presence of the osteoinductive medium. Cell sheets were harvested 2 weeks after spreading because the mRNA expression of osteogenic marker genes was strong after that period of time. Quality assurance tests were performed on at least 7 samples, and then the standard phenotypes of hPDL cell sheets were determined.

According to the GCP and GMP guidelines, hPDL cell sheets were created from three healthy volunteer donors at the GMP-grade Cell Processing Center (CPC) in our university (Washio et al., 2010). GMP-grade reagents and certified materials were used for culturing the hPDL cells. The safety and efficacy of “the product (hPDL cell sheets in this case)” was validated for a clinical trials. Prior to performing the cell culture, autologous serum was prepared from the donors. The hPDL cells were cultured under xeno-free conditions, and cell sheets were fabricated using the temperature responsive dishes. Culture sterility was confirmed using conventional tests. Safety was evaluated using the following tests: 1) the soft-agar colony-formation assay, 2) transplantation into nude mice, and 3) the karyotype test (Yoshida et al., 2012). The efficacy of the cell sheets was verified by transplantation with a dentin block into SCID mice. All of these tests revealed that hPDL cell sheets created at the CPC were safe and exhibited the ability to regenerate periodontal tissues. Another set of three hPDL cell sheets from healthy volunteer donors were created at the CPC to optimize the procedures.

3.4. The clinical trial

After approval on the 5th of January 2011, our clinical trial called “Periodontal regeneration with autologous periodontal ligament cell sheets” was initiated to treat patients presenting with the following ailments: 1) infrabony defects with a probing depth of more than 4 mm after the initial therapy, 2) radiographic evidence of infrabony defects, and 3) a redundant tooth that contains healthy periodontal tissue as a cell source. All patients provided written informed consent according to the GCP. Exclusion criteria included the following: 1) relevant medical conditions contraindicating surgical interventions (e.g., diabetes mellitus, cardiovascular, kidney, liver, or lung disease, or compromised immune system), 2) pregnancy or lactation, and 3) heavy tobacco smoking (more than 11 cigarettes a day). The primary out-

come of this trial is to evaluate the safety and efficacy of autologous transplantation of periodontal ligament cell sheets. As of the end of May in 2014, 10 cases of autologous PDL cell sheets were transplanted, and the healing process took place uneventfully.

4. Conclusion

The applications of cell sheet engineering for regenerative medicine are mentioned. Various types of cells have been examined and most of them improved the functions of recipients, suggesting that cell sheet engineering can be an alternative strategy for the therapy of tissue engineering. The implementation of robotic systems that allow the safe mass production of sterile cell sheets automatically, as well as further collaboration between researchers and medical professionals will make “cell sheet engineering” the leading edge solution for regenerative medicine (Elloumi-Hannachi et al., 2010).

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