Chapter 6

Bone Marrow Mesenchymal Cell Contribution in Maintenance of Periodontal Ligament Homeostasis

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Additional information is available at the end of the chapter

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Abstract

In general, remodeling phenomenon of the periodontal ligament (PDL) is occurring in all times. Thus, in the chapter, the word "maintenance" was used, and the chapter title is "Maintenance of Periodontal Ligament Homeostasis." Our experimental data on the remodeling of the PDL with cell acceleration at the furcation area in this experimental model are recovered using the cells in situ and the bone marrow-derived cells (BMCs). BMC migration into the PDL tissues using green fluorescent protein (GFP) bone marrow-transplanted model mouse was examined. BMCs have abilities of cell migration and differentiation into tissues/organs in the body. The immunohistochemistry revealed that GFP-positive cells were detected in the PDL. GFP-positive cells were also positive to CD31, CD68, and Runx2 suggesting that fibroblasts differentiated into osteoclasts and tissue macrophages. In this way, Notch signaling involvement considered in our tentative examinations revealed that the experimentally induced periodontal polyp was examined; the cytological dynamics of the cells in granulation tissue are mainly from migration of undifferentiated mesenchymal cells of the bone marrow and differentiate into the tissue-specified cells. Furthermore, the data suggest that cell differentiation is due to Notch signaling.

Keywords: periodontal ligament (PDL), homeostasis, cell migration, bone marrow-derived cells, notch signaling

1. Introduction

In general, "homeostasis" of the periodontal ligament tissues (PDL) is occurring in any times, due to the mechanical stress to the tissue including the physiological and pathological occlusal stress and/or orthodontic mechanical stress, and so on. Thus, in the above sentences, the word "homeostasis" was used in the chapter. From a view point of developmental biology, there are well-known facts that the main component of PDL fibroblasts is from neural crest-derived neuroectodermal cells. From the point of the fact, at the period of the maintenance of the PDL, there are numerous cell proliferations that occurred in the regional tissues. It is a very important problem. Thus, at first, we introduce the chosed GFP bone marrowtransplanted model mouse (Tsujigiwa's model [1, 2]) shown in **Figure 1** (GFP mouse model: upper).

I used the ddY mouse as an experimental animal and chosed a histopathology in a periodontal ligament of the mouse that received mechanical stress by Waldo methods. The immunohistochemistry manifestation situation of heat shock protein 27 (HSP27) and phosphorylated HSP27 (p-HSP27) was investigated until that and at most 24 hours later. A periodontal ligament fibroblast was both low in HSP27 and p-HSP27 in the control group. But HSP27 was manifested 10 minutes later after a PDL fibroblast caused mechanical loading on the tension side of experimental group. The strongest appearance was detected 9 hours later after mechanical load was led. p-HSP27 was also weaker than HSP27, but it was manifested in a time-depending way. These results suggest that HSP27 and p-HSP27 are manifested by activation of a PDL fibroblast on the tension side for maintenance of the

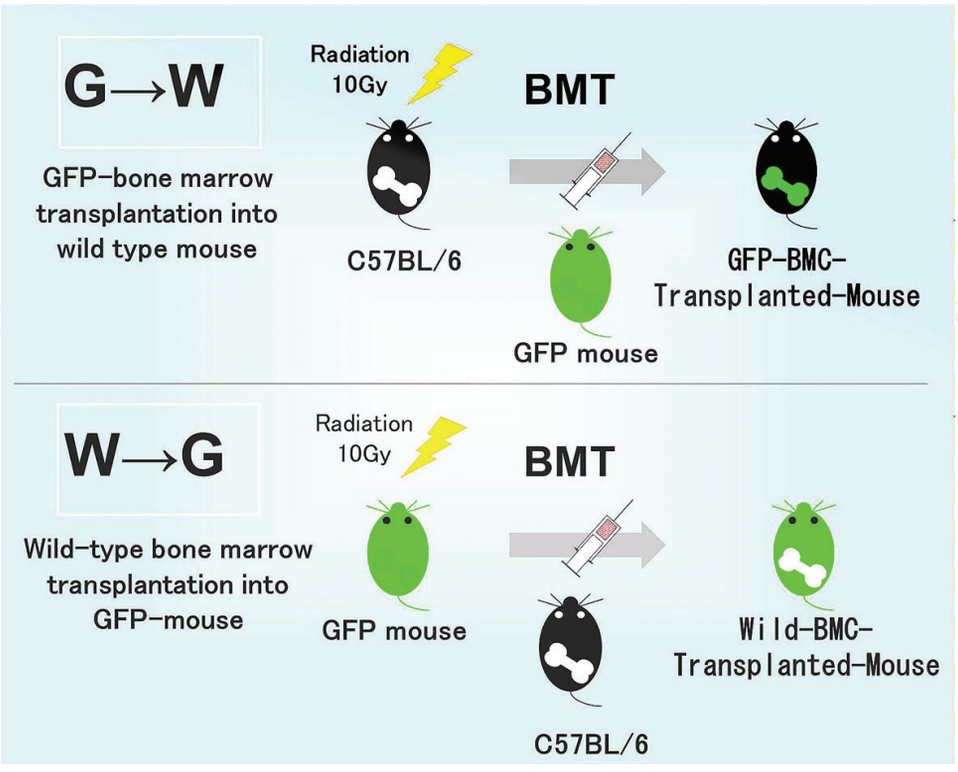

Figure 1. GFP-transplanted model mouse schematic diagram.

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homeostasis of the periodontal ligament. Therefore, the facts suggest that these proteins will act on this as molecular chaperone for activation of an osteoblast and maintenance later.

We have investigated using the GFP mouse, the cell dynamics of the periodontal ligament in the past decade. The following experiments have been carried out: (1) Orthodontic mechanical stress causing injury of the periodontal ligament tissues. (2) Occlusal trauma of the periodontal ligament. In the examinations, histopathological changes were observed at the course of the experiments. Furthermore, we used the GFP bone marrow-transplanted mouse for examination of the cell supplying source to the regional periodontal ligament tissues from bone marrow-derived cells (BMDCs). (3) Experimentally induced periodontal polyp-contained cells are mainly from migration of undifferentiated mesenchymal cells of the bone marrow and differentiate into the tissue-specified cells. (4) Furthermore, the cell differentiation is due to the expression of Notch signaling. The result also suggests that the PDL fibroblasts in granulation tissue are differentiated in the periodontal ligament-specified cells from bone marrow-derived mesenchymal cells.

2. GFP-BMDCs into the PDL-received orthodontic mechanical stress

The periodontal ligament (PDL) is usually remodeling at physiological in condition. Furthermore, orthodontic treatment results to mechanical stress inducing reorganization of PDL collagen bundles. The examination results in "movement of an orthodontics tooth." The mechanical stress communicated to PDL causes a reaction of organization and causes "movement of a tooth." This reaction of PDL is the one for maintenance of a homeostasis. A histology-like reply and production of the copying factor which controls a cell differentiation and various morphogenesis phenomena are studied more widely in recent years [3, 4]. It becomes clear that it's able to bring manifestation of a remodeling of a periodontal tissue and the activated molecule which replies to various mechanical stress and inflammation to maintain a homeostasis [5–10]. Our experimental method was based on our previous reports [4, 5]. The Waldo method of inducing mechanical stress load in mouse periodontal tissues was followed. Under general anesthesia, the mouse was inserted between the maxillary molars to induce persistent mechanical stress. A separator was inserted between M1 and M2 of the right maxillary molars to ensure the mechanical stress due to pressure over a period of time. After each experimental time, the periodontal tissues of the left maxillary molar region (untreated side) were used as controls. In this experiment, the distal buccal root of the maxillary first molar was the observation part. The schematic diagram, macro-view, and histology are shown in **Figure 2**.

Therefore, we focused on the expression of various HSPs that maintain homeostasis during injury. HSPs are one of the factors recognized that is transiently enhanced by heat shock [11]. It is also called stress protein because it is not only enhanced by heat shock but also by ischemia; other pathological changes such as infections and inflammation and radiation; physical stress such as light; stress from enzymes, heavy metal ion, arsenic, arsenic acid, methanol, and active oxygen; and stress from chemical and various amino acid derivatives [12, 13]. When the chromosomes of the salivary gland of *Drosophila* were at high temperatures, HSP

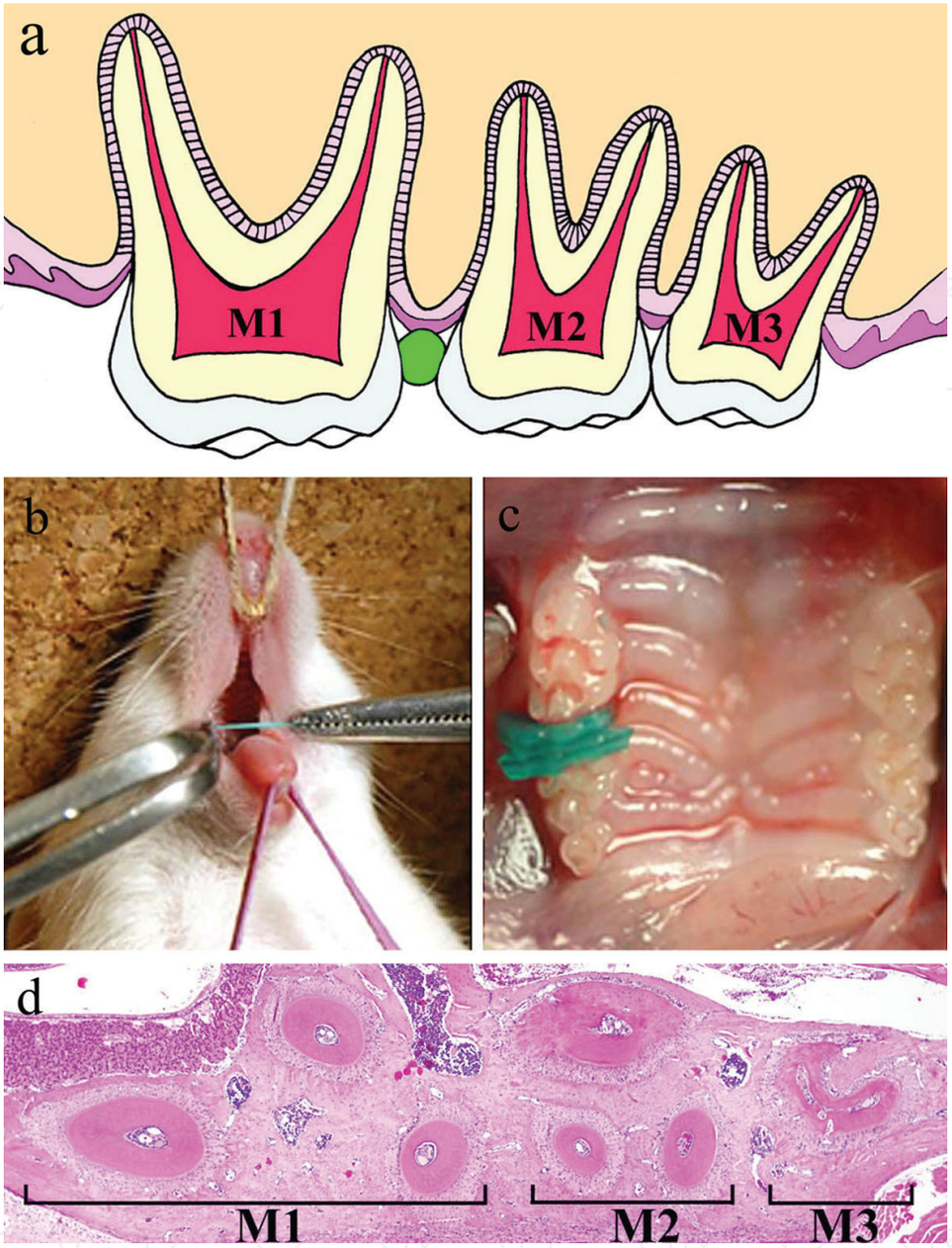

Figure 2. Experimental schema (a), macro-view (b, c), and histology (d). Quotation alteration of literature #5.

strongly expressed [14]. After that, the isolation of synthesized HSP by a SDS-polyacrylamide gel electrophoresis first by *Drosophila*. Some of the main protein which was also led by heat shock method of treatment in non-salivary glandular system was regarded as HSP together. A puff of the mRNA history of HSP was copied. After that a high study is reporting that the colon bacillus kept during the environment, leaven, and a gene of other mammalian cells are able to receive a heat stress and preserve a gene more than they are concerned with HSP [14]. The representation of these HSPs is then a common phenomenon [15]. HSPs have molecular ancillary and functional antiapoptotic capacity [16, 17] from ancient times. That is, it is a protein that develops to escape cell death in harsh conditions for cell survival. Many HSPs also

express many cells in response to stress, suppression, and repair of proteins whose properties have been altered. In addition, HSPs are essential proteins to maintain various cellular life functions between cell differentiation and growth and presence. It is a widely distributed intracellular equilibrium protein, which is regularly expressed under various conditions in in vitro and in vivo experiments. Depending on the molecular weight, HSP is classified into high molecular HSP (HSP 110, HSP 90, HSP 70, and HSP 40–60 families) and low molecular HSP (HSP 20 family). These are polypeptides of tens to hundreds of kDa. Many researches have been done on expression and function of these HSPs and various sites. That is, high molecular HSPs such as HSP 70 and HSP 90 have a role of assisting the maturation of proteins. That is, it temporarily binds to immature state protein and acts as a molecular chaperone. However, small molecular HSPs that function as molecular chaperones have not yet been reported [21]. HSP 27 belongs to the low molecular HSP family. Firstly, HSP 27 was found in the actin polymerization. HSP 27 is known to exist at high levels in non-stimulated vascular smooth muscle and skeletal muscle cells. From this, it is believed that HSP 27 plays a role in maintaining blood pressure and other physiological effects in the vascular system [18–20].

We reported that BMCs migrated into the PDL regions; PDL fibroblasts, hemendothelial cells,osteoclasts, and Langerhans cells migrated into the PDF regions. These cells differentiate into their tissue-specific cells [21–23]. The facts are that BMCs are not mesodermal cells and they are derived from neural crest cells. This differentiation into specialized cells may be done on the spot by expression of odontogenic genes [24]. According to a classical tissue engineering technique, the tooth-like structure is created based on biodegradable polymer scaffolds by transplantation of dental pulp cell pellets or BMC extracted directly from dental embryonic cells and dental pulp stem cells separated by enzyme treatment [25].

We examined the transplanted BMC migration into the PDL. The IHC revealed that GFP-positive cells were detected in the PDL tissues. A number of GFP-positive cells appeared on mechanically loaded periodontal tissue, especially on the tension side of the experimental group. On the other hand, little GFP-positive cells appeared in the control group. From the above results, we analyzed how they differ from the experimental group and control group [25]. Thus, these data indicated that orthodontic mechanical stress acts as a possible promoting factor of transplanted bone marrow-derived cell migration into periodontal tissues and of differentiation to fibroblasts [26].

On the other hand, mice transplanted with bone marrow cells of GFP transgenic mice were used to observe GFP-positive cells in dental pulp of mouse incisors, PLD, oral epithelial Langerhans cells, pulp fibroblasts, dental vascular endothelial cells, and osteoclasts. GFP-positive cells in the dental pulp are dendritic cell-like cells, and some odontoblast-like cells also showed a positive response to GFP. It is clear that BMCs have the ability to differentiate into teeth and related connective tissues. GFP-positive cells histopathologically differentiated into several cell types. Fluorescent immunohistochemistry (FIHC) and tartrate-resistant acid phosphatase (TRAP) staining techniques showed that these cells were detected as osteoclasts and macrophages. In addition, GFP-positive cells gathered in adjacent blood vessels. This data suggests that GFP-positive BMCs migrate to periodontal tissues and differentiate PDL tissue-specified cells.

GFP-positive cells were detected in PDL in both experimental and control groups in this study. In the experimental group, a number of GFP-positive cells were found in the PDL tissue and intermittently stimulated intermittent mechanical stress. However, there were few GFP-positive cells in the control group. This result was significantly larger between the experimental group and the control group. This suggests that orthodontic mechanical stress induces GFP-positive transplanted BMCs into the PDL tissues.

BMC migrate from bone marrow tissue and different types of tooth-related cell types including odontoblasts [27], osteoclasts [28], and PDL [29, 30]. Osteoblasts and osteoclasts maintain and reconstruct cancellous bone surrounding the marrow tissue. BMCs from the bone marrow are closely involved in the repair of tissues to maintain periodontal tissue homeostasis of PDL fibroblasts. Furthermore, mechanical stress strongly induces cellular activation of these PDLs. Teeth can be produced from non-odontogenic stem cells. This establishes the basic principle that bone marrow stem cells are also involved in tooth embryogenesis. As a future therapeutic possibility, these cells include transplantation to a tooth defect site or transplantation into a patient's bone marrow with developmental abnormality, which may lead to a new approach to tooth and jaw bone regeneration.

3. GFP-BMDCs into the PDL received occlusal trauma

Regarding the examination results of periodontal ligament in experimental occlusal trauma mouse model, we have reported the cytological behavior of the related regions. The experimental model diagram is shown in **Figure 3**. Periodontal connective tissue remodeling occurred due to traumatic occlusal overload [31]. In the remodeling course, the fibroblasts act as an important role. In the process of PDL remodeling phenomenon, fibroblasts make an important role such as collagen synthesis. Heat shock protein 47 (HSP 47) is a protein that acts as a molecular chaperone in procollagen biosynthesis and maturation. Type I collagen is a major component of PDL. Therefore, in our study, the expression of IHC of HSP 47 on the experimental-induced periodontal tissue of traumatic occlusion was investigated. That is, an experimental occlusal trauma model was developed and experimented. Expression of HSP in occlusal trauma periodontal tissue was performed using immunohistochemistry (IHC). The results indicated that fibroblasts had high HSP expression in response to excessive traumatic occlusion. HSP 47 is thought to play an important role in the maintenance of fibroblast homeostasis exposed to traumatic occlusion. HSP 27 and HSP 70 have detailed observation results on damaged periodontal tissue by Muraoka et al. [5–7]. Based on the experiments of Fujii et al. [31] and Takaya et al. [32], we carried out the IHC study on the histopathological changes of mouse dental tissue and on HSP.

The experimental outline is shown in **Figure 3**. After that, changes in the periodontal ligament were observed over time. Then after, the micro plus screw was removed at day 4 after implantation, and the subsequent tissue changes were observed. Histopathological examination: It was observed that a fibroblast and a spindle-shaped cell have high density in a periodontal ligament of the control group. An erythrocyte was filled by a capillary. Periodontal ligament

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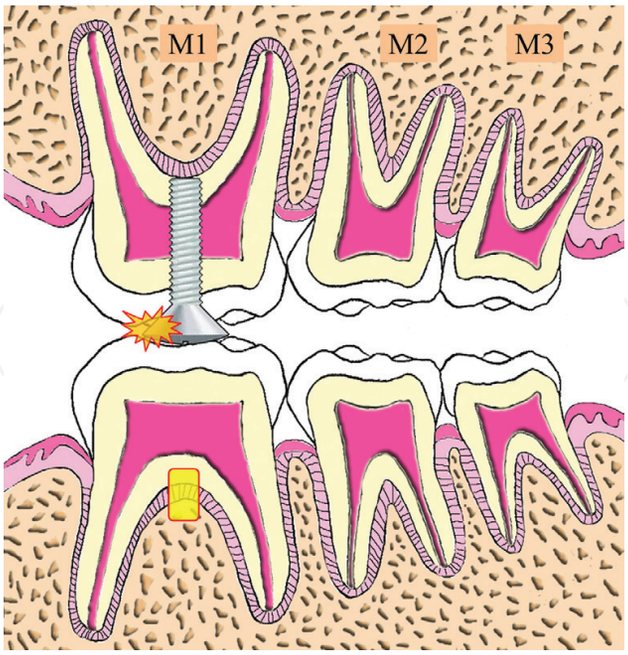

Figure 3. Diagram of the mouse traumatic model.

fiber was arranged irregularly. An osteoclast could see conspicuously in an alveolar bone. The furcation was lined by acellular cementum. At experimental group on day 1, the capillary which swelled was filled with an erythrocyte. The amount of deeply stained cells with round nuclei increased. More osteoclasts were observed on the glassy surface of the alveolar bone.

There are several kinds in a stem cell; a stem cell differentiates into various cells of a human body and has a special nature. The stem cell and a marrow-derived cells (BMCs) also possess the differentiation special quality of the plural. Many researchers reported that BMCs might relate into retinal vessels, myoblasts, hepatocytes in the liver, Purkinje neurons, cardiac muscle in the heart, and airway epithelial cells in recent years [33, 34]. A stem cell can be used in the field of the regenerative medicine; so to regenerate an organ, the stem cell is very important for treatment of various diseases [35]. For treatment of an end limb ischemia and an ischemia disease including myocardial infarction, a try at a local delivery of BMCs is studied [36, 37].

It is stated that occlusal trauma is defined as damage resulting from tissue changes within the PDL as a result of abnormal occlusion forces. It has been proven for many years by many researchers that occlusal trauma may cause various destructive biological reactions to the tissue of PDL [37–40]. A lot of researchers reported cytological kinetic examinations of PDL tissue regarding occlusal trauma PDL, but they have not been fully performed regarding establishing an experimental system with animals that can be used in a very versatile manner; we have constructed an experimental system with a mouse with respect to the occlusal trauma model. We reviewed the organization of PDL from the perspective of cytological kinetics [40]. We then performed a histopathological and also immunohistochemical study. **Figure 4** shows histopathology and IHC results.

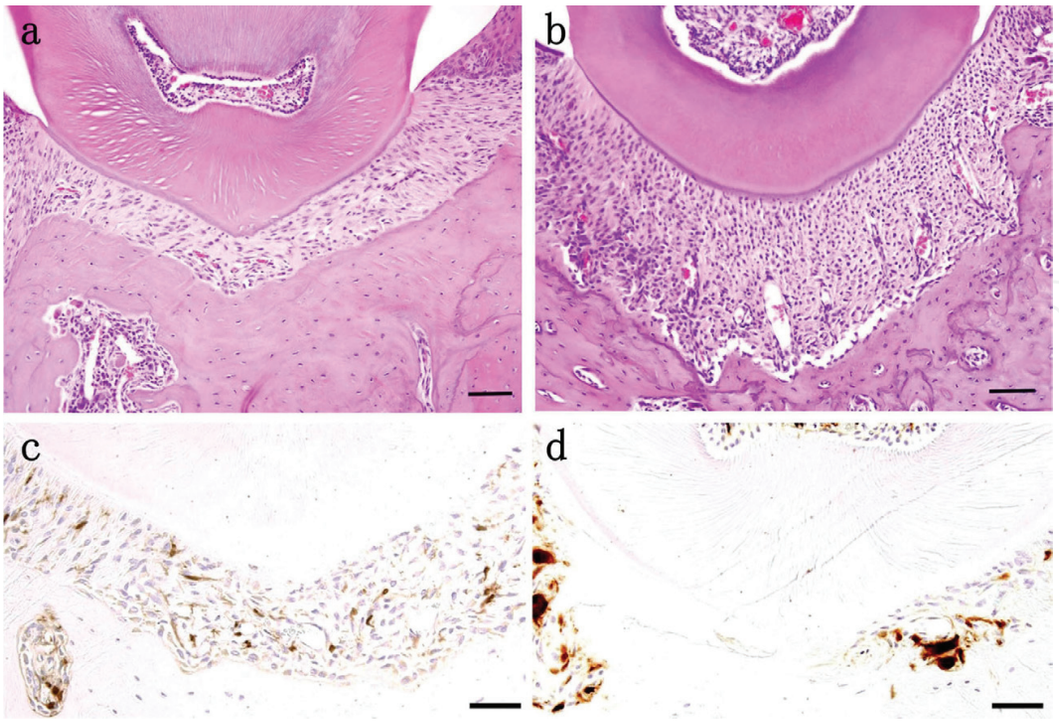

Figure 4. Histopathology of control region (a) and experimental region (b) of the day 4 specimen. Scale bar = 50 μm. IHC of GFP. Control specimen (c) and experimental day 7 specimen (d). Scale bar = 50 μm. Quotation alteration of literature #32.

Eleven 7-week-old ddY male mice and eight 7-week-old bone marrow-transplanted female C57BL/6 genealogy mice from GFP transgenic mice (GFP mice), for a total of 19 mice, were used in this study. Histopathological examination showed as followed. Control group specimens showed the PDL maintained a constant width; the major fibers arranged in orderly cementum and alveolar bone. Spindle-shaped fibroblasts that appeared in PDL were collagen fiber bundles. In the relatively dense cell nucleus, PDL, there was a congested capillary. Furthermore, the cellular cementum could be clearly confirmed. On day 4 of the experimental group, PDL was somewhat compressed, and evident capillary vessel filling was confirmed. In spindle-shaped fibroblasts, those with deeply stained circular nuclei of hematoxylin increased in number. Multinucleate giant cells appeared mainly on the alveolar bone surface. It gradually absorbed bone tissue and made some blanks. In the experimental group, the circular nucleus cells decreased considerably on day 7 from day 4. Vascular hypertrophy developed. Multinucleate giant cells were expressed on the alveolar bone surface of Howship's lacunae. On day 4 of the experimental group specimens, the regression of hyaline degeneration area had expanded. Furthermore, the cellular cementum destruction was evident in the expansion of PDL areas. On day 14 of the experimental group, the cementum absorption region by multinucleated giant cells and the alveolar bone surface rapidly expanded remarkably. There was a decrease in cells with circular nuclei. Cells in which both the nucleus and the cytoplasm are spindle-shaped are increasing again. The width of PDL became wider.

Using the cytological kinetics method, we analyzed the nuclear occupancy to compare all cell numbers. The area examining the occupancy rate analyzed related PDL experiments and

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control groups in the histopathological photographs. The result was markedly increased on the day 4 of the experimental group. Compared with the experimental group, experimental group on day 7 and 14 decreased but mostly of the same degree share, and they were not significant to compare with the control group.

GFP-positive cells were sparse in the control group and the experimental group on days 4 and 14. These cellular contours are PDL cells with circular nuclei. According to the digital image analysis method, the number of GFP-positive cells increased in the experimental group day 7. The results of image analysis of GFP-positive cells of PDL on the day 7 of the experimental group showed a considerably larger increase in comparison with the control group.

In the progress of periodontal disease [41], things such as dental plaque and tartar caused by tooth deposits are common, but it is well-known that occlusion abnormalities such as traumatic occlusion are also important. Histopathological examination of PDL has been conducted [42–46] so far. These were done using rats, mice, macaque monkeys, and Beagle dogs. However, the report did not find a focus point at cytological kinetics of periodontal ligament due to excessive occlusal loading. Thus, we focused the cytological kinetics in the periodontal tissues by excessive occlusal loading.

GFP-IHC specimens shows, although the positive rate of GFP was considerably high on the day 7 of the experimental group, that there was almost no significant difference in the day 14 of the experimental group as compared with the control group. All cells constituting individual tissue cells of GFP transgenic mice have green fluorescent protein. Even if any types of cell differentiate transplanted bone marrow-derived cells, they can be traced by GFP. It is a technique of immunofluorescent staining. Such cells were identified from bone marrow-transplanted cells. At first it was osteoclasts and macrophages. It is reported later in the results of the previous experiment that many GFP-positive cells migrate to the PDL tissues in mice and differentiate into in situ specific cells. Furthermore, it is easy to imagine that dendritic cells and PDL fibroblasts, which migrate into the PDL, differentiate into PDL-specific cells.

In the experiment, bone marrow-derived cells showing GFP-positive cells in PDL of the root bifurcation region subjected to occlusal trauma on the day 7 of experimental group increased. It is clear that the majority of GFP-positive cells are osteoclasts and macrophages by previous studies. It is not only PDL damage due to continuous excessive occlusal trauma. It causes remodeling of alveolar bone and PDL and mobilization of bone marrow-derived cells is necessary for it.

According to many studies, PDL remodeling phenomenon due to acceleration of cell activation is caused by excessive traumatic occlusion stress on the day 4 of the experimental group on the tooth PDL damaged part in the root furcation region. In the experimental group day 7, PDL remodeling mechanism is done by osteoclasts and macrophages. The cells are present in the furcation area as GFP-positive BMDCs. Therefore, when gingivitis has not been caused at all or only very slightly, the PDL stressed with traumatic occlusion; it will be constructed by BMDCs.

4. Differentiation of BMDCs into the PDL peculiar cells

The IHC view is as follows. BMDC is GFP-positive except for an endothelial cell of micro-capillarity. PDL cells and/or cells spindle in shape on the alveolar bone surface are mostly GFP-positive (**Figure 5a**). Furthermore, the micro-capillaries are positive for GFP in some occasions of the periphery (**Figure 5b**).

Cells of various cell types could be identified by immunofluorescence double staining check for GFP. Green fluorescent GFP-positive cells as orange fluorescence by fluorescence immunohistochemical double staining of GFP-S100A4 (**Figure 5c**) and GFP-Runx2 were consistent with red fluorescent S-100 A 4-positive and Runx2-positive cells (**Figure 5d**). Fusiform cells toward the root direction were arranged in a bundle. When superimposing, the nucleus stained blue, and the cell of that cell was stained orange. The enlarged view of **Figure 6** also clearly shows both positive for GFP and CD 31 and proposes PDL capillaries (**Figure 5e**).

Recently, many studies using undifferentiated mesenchymal cells (UDMC) of the bone marrow are done in the repair and regeneration of tissues, bones, and other organs around the

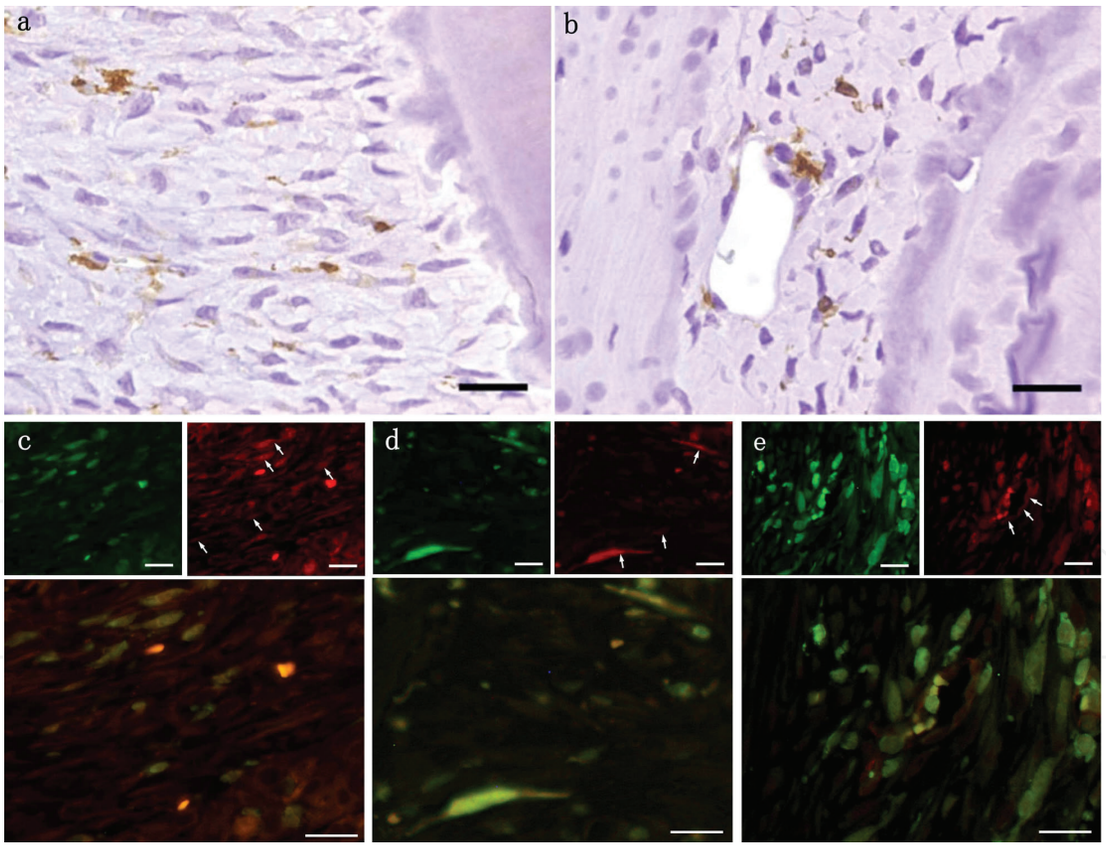

Figure 5. IHC results showing GFP-positive round- or spindle-shaped cells within the PDL tissues (a, scale bar = 50 μm) and GFP-positive products existing within the vascular endothelial cells (b, scale bar = 50 μm). Regarding the lower layer photographs (c–e), enlarged FIHC images of PDL, 6 months specimen. GFP (green) and merged image (c) of S-100 A4 (red) result fibroblast. GFP (green) and Runx2 (red) view (d) shows clearly both positive suggesting PDL fibroblasts, and GFP (green) and CD31 (red) image (e) suggests endothelial cells. Scale bar = 100 μm. Quotation alteration of literatures #26 and 43.

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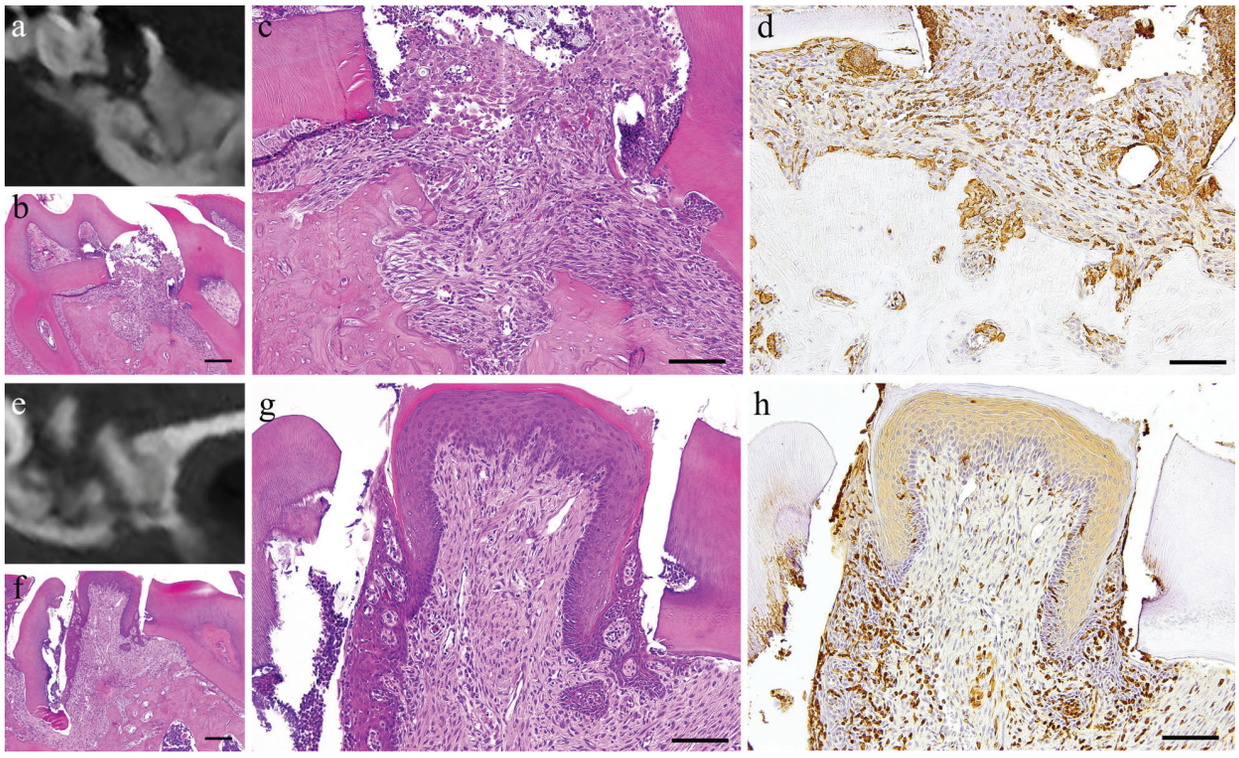

Figure 6. Micro-CT of day 4 specimen (a), histopathology view (b, c), and IHC of GFP (d). Micro-CT of day 7 specimen (e), histopathology view (f, g), and IHC of GFP (h). Scale bar = 50 μm. Quotation alteration of #44.

teeth. Regarding the alveolar bone and the PDL regenerations, it was described for the differentiation of UDMC from bone marrow into cartilage [11, 12]. Experiments on BMDCs using GFP mice showed that after cell transplantation, it differentiated into salivary gland epithelial cells and myoepithelial cells capable of playing a role not only of olfactory cells but also salivary gland tissue [13]. Histopathological examination of experimentally loaded mice in orthodontic treatment showed that bone marrow stem cells increase bone remodeling capacity [3].

Tomida et al. reported that PDL cells increased after 1–5 weeks of mechanical stress. In the current study, the cell number was calculated by counting GFP-positive cells in the control group and the experimental group [47]. The results showed that the number of GFP-positive cells in the experimental group was much higher than that in the control group. The increase in the number of cells in PDL after mechanical stress loading strongly suggests that it occurred because BMDCs were transferred to PDL. Using GFP mouse experiments, Tsujigiwa and others indicated that the transplanted BMDCs migrated to pulp and differentiated into cells constituting pulp tissue [1]. By using the same experiment, Muraoka et al. [48] showed that the transplanted BMDCs migrated to PDLs and later differentiated into PDL cells. However, the biological response of mesenchymal cells in response to mechanical stimuli also caused changes in cell morphology [48]. Movement of transplanted BMDCs using GFP mice was reported to be due to mechanical stress [49].

The results of the study showed that the mechanical stress promoted the increase in the number of cells in both pressure and tension sides of the PDL. Morphological changes of the

extracellular form were not detected, but the number of cells increased in a short period of 1 week. To determine if an increase in cell number is made by migration of BMDCs, using Tomida's method [47], GFP-positive cells were counted.

The number of GFP-positive cells immediately after stress load showed a gradual increase. It had increased over time until 6 months. It is certain that BMDCs were supplied to the PDF for a long period of time. Furthermore, when each cell was characterized by double immunofluorescence staining, Tsujigiwa et al. [1, 2] showed that the transplanted BMDCs, GFP-positive cells such as osteoclasts, were stained and identified. This confirmed that it moved and differentiated into the bone remodeling site.

Applying the method of Muraoka [48], cell identification by double immunofluorescence staining with GFP-CD31, GFP- CD68, and GFP- Runx2 were performed. As a result, it was possible to distinguish between osteoclasts and macrophages. Furthermore, since some GFP-positive cells expressing CD31 were found, they were derived from BMDCs and differentiated into hemangioendothelial cells. Similar results were obtained for macrophages by CD68. Furthermore, since Runx2 represented fibroblasts [3, 4], respectively, the expression of Runx2 was executed. As a result, it was clear that it was GFP-positive and expression of Runx2, and the cell morphology was a certain swimming; so it was a PLD fibroblast which strongly suggested that it had migrated from the bone marrow.

5. Notch signaling in cell differentiation of the BMDCs in the PDL

The GFP mouse model examination revealed that the cells were derived from mesenchymal cells of the bone marrow. Furthermore, these cells differentiated into the tissue-specific PDL fibroblasts, blood capillary endothelial cells, etc. Notch is a membrane-bounded protein, which regulates the differentiation gen for changing the cell type [49]. However, there have been no reports on the component cells of periodontal polyp-granulation tissues [9].

In usual dental clinical practice, perforation of the floor of the dental pulp suddenly occurred during a dental treatment. In case of a large perforation, it cause chronic granulomatous growth in the regional portions [9]. Granulation tissue grows in the periodontal ligament (PDL) region from the perforated dentin causing periodontal polyp. Regarding the PDL polyp, our previous histopathological and immunohistochemical examinations were done [4–6]. The data using an experimental system on GFP mouse bone marrow transplantation model revealed that the cells were derived from mesenchymal cells of the bone marrow. Furthermore, these cells differentiated into the tissue-specific PDL fibroblasts, blood capillary endothelial cells, etc. (**Figure 6**).

In general, Notch signaling is necessary for cell fate determination, cell proliferation, and differentiation [56, 57]. Therefore in this study, we examined using the method of Matsuda, et al. [9]. We examine the expression of Notch in the experimentally induced pulp polyp component cells, using observation of histopathology and immunohistochemistry methods. Histopathological observation of 2-week specimens, the spindle-shaped cell proliferation was evident with some neutrophils in the specimens. Within these cells, the relatively round nucleus-having cell and some capillaries were observed.

The IHC examination of Notch expression of the 2-week specimens, elongated, and spindle-shaped cells with spindle nucleus were positive to Notch1 (**Figure 7a, b**). From 1 to 6-month specimens, the spindle-shaped cells were also positive to Notch1. Notch1-positive reaction was continuously detected (**Figure 7c, d**). In contrast, as observed in the control, the dental pulpal tissues of the non-treated teeth were completely negative, although some nonspecific positive reactions existed (**Figure 7e, f**). Furthermore, the physiological PDL was slightly positive to Notch (**Figure 7e**).

Generally Notch is an important regulation signaling of morphogenesis. It was reported that Notch1 is a transmembrane protein necessary for cell fate determination, etc. [49]. Thus, we examined the relationship between the cell differentiation in the periodontal polyp component cells and Notch signaling in the present study. According to the present results: (1) spindle-shaped fibroblastic cell of the pulp polyp tissues was almost Notch1-positive reactive and (2)

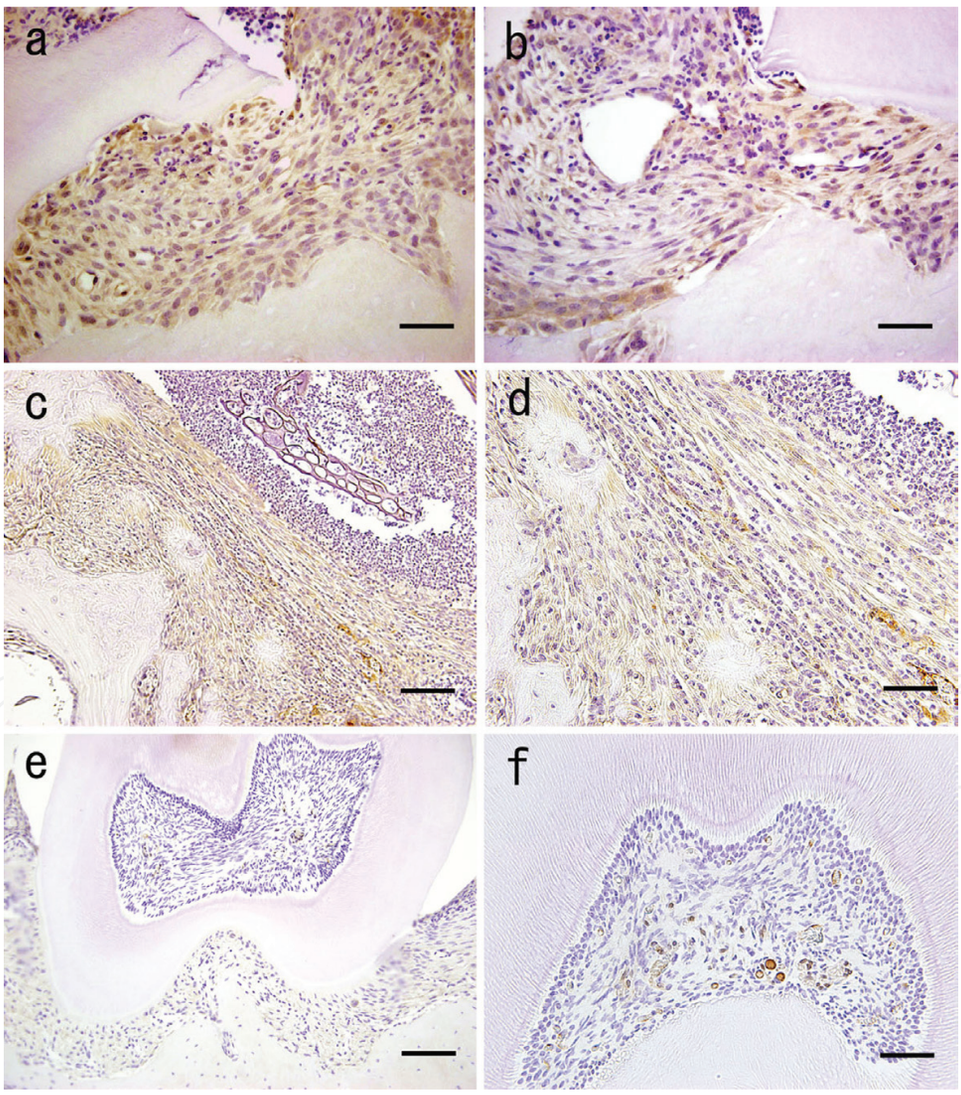

Figure 7. IHC images of notch expression. (a) Granulation tissue area, scale bar = 50 μm; (b) granulation tissue area, scale bar = 50 μm; (c) fibrous rich area, scale bar = 100 μm; (d) enlarged view of c, scale bar = 50 μm; (e) control areas (dental pulp and periodontal ligament tissues), scale bar = 100 μm; and (f) control areas (dental pulp and periodontal ligament tissues), scale bar = 50 μm. Quotation alteration of #44.

these reactions strongly suggested that the cell differentiation was caused by the Notch1 signaling. The reactions mean that the PDL is always received and controlled by Notch signaling.

6. Conclusions

In general, remodeling of the periodontal ligament (PDL) tissue is occurring in all times. Thus, in the above sentences, the word "remodeling" was used in the section "Maintenance of Periodontal Ligament Homeostasis." Our experimental data suggest that the remodeling of periodontal ligament with cell acceleration at the furcation area in this experimental model has recovered using the cells in situ and the bone marrow-derived cells (BMCs). BMC migration into the PDL tissues using BMC transplantation model was examined. BMCs have abilities of cell migration and differentiation into tissues/organs in the body. The immunohistochemistry revealed that GFP-positive cells were detected in the periodontal tissues, both in the experimental and control specimens. These results suggest that orthodontic mechanical stress accelerates transplanted BMC migration into the PDL tissues. GFP-positive cells were also positive to CD31, CD68, and Runx2 suggesting that fibroblasts differentiated into osteoclasts and tissue macrophages. In this way, Notch signaling involvement was considered in our tentative examinations.

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Conflict of interest

The authors have declared that there is no conflict of interest.

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