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Chapter

Regeneration of Dentin Using Stem Cells Present in the Pulp

Toshiyuki Kawakami, Kiyofumi Takabatake, Hotaka Kawai, Keisuke Nakano, Hidetsugu Tsujigiwa and Hitoshi Nagatsuka

Abstract

Dentin is one of the major hard tissues of the teeth. Dentin is similar to bone in texture, but it is different from bone tissue histologically. It is formed by odontoblasts; however, these cells are present in a limited area in the human body and are not found anywhere other than the dental pulp. It is difficult to collect and proliferate mature odontoblasts for regenerative medicine. However, odontoblast are necessary for regenerating dentin. It is known that odontoblasts differentiate from mesenchymal stem cells in the dental pulp during tooth development. Dentin can be generated using the stem cells present in the pulp. Many stem cells are recruited from the bone marrow to the teeth, and it is possible that the stem cells present in the pulp are also supplied from the bone marrow. Herein, we explain the mechanism of stem cell supply to the teeth and the possibility of dentin regeneration by specific cell differentiation induction methods.

Keywords: dental pulp cell, odontoblast, bone marrow-derived cell, mesenchymal stem cell, differentiation induction

1. Introduction

Stem cells have the special property of differentiating into different types of cells. In the human body, stem cells are present in organs with a high regenerative and proliferative ability. Bone marrow is an organ that supplies blood cells throughout the body. The bone marrow is also rich in stem cells. The special nature of stem cells in the bone marrow is that they can migrate to all tissues throughout the body. Bone marrow-derived cells (BMDCs) that appear in peripheral tissues have the characteristics of stem cells and show multiple differentiation. Recently, many researchers have shown that BMDCs may differentiate into a variety of organs, including skeletal muscle, hepatocytes, neurons, myocardium, mucosal epithelial cells, and blood vessels [1, 2]. This is an important fact because such cells can be used to regenerate organs for treating various diseases [3]. The local supply and delivery of BMDCs has been extensively studied for treating ischemic diseases, including peripheral tissue ischemia and myocardial infarction, and has been attempted as a therapeutic tool [4, 5].

In previous studies, we used bone marrow transplant animal models in which bone marrow cells were transplanted from green fluorescent protein (GFP) transgenic mice to investigate the ability of BMDCs to be distributed and differentiated in the bones and teeth. GFP-positive cells were observed diffusely in the pulp and

periodontal ligament of mouse incisors, Langerhans cells in the oral epithelium, stromal fibroblasts, blood vessels, and osteoclasts in the tooth area [6]. Tissue stem cell differentiation is triggered by a variety of stimuli. We have shown previously that orthodontic mechanical stress and artificial inflammation stimulate the cells of the periodontium and dental pulp to express various cell differentiation factors. The osteoblast marker alkaline phosphatase (ALP), runt-related transcription factor 2 (Runx2), which induces osteoblast differentiation, and heat shock proteins (HSPs), which are involved in homeostasis and cell differentiation, may be strongly expressed in cells exposed to injurious stimuli [7–12]. Moreover, periodontal tissue and dental pulp respond to mechanical stresses and inflammation, causing periodontal tissue remodeling and expression of cell differentiation-related factors [13]. Furthermore, orthodontic mechanical stress on the periodontium causes activation of hard tissue-forming cells in the pulp tissue [10, 11]. This indicates the potential of dental pulp stem cells to differentiate into hard tissue-forming cells upon stimulation. Moreover, mechanical stress and inflammation on the periodontal tissue affect the local recruitment of BMDCs [13, 14].

Recently, dental pulp has been proposed as a promising source of pluripotent mesenchymal stem cells for use in a variety of clinical applications. We isolated stem cells from the pulp, which is rich in BMDCs. The isolation and culture of dental pulp cells is an important factor. We attempted to establish cells that induce dentin-like hard tissue from the cells present in rat dental pulp and succeeded in establishing cells that resemble odontoblasts under *in vitro* conditions. These cells were named as the tooth matrix-forming GFP rat-derived cells (TGCs). The TGCs were maintained in cultures over 80 passages without showing any changes in morphology or properties. Physiological dentin has a characteristic structure, which is derived from the cell polarity of odontoblasts. However, the TGCs form dentin-like hard tissue under *in vivo* conditions but do not lead to the induction of polarized odontoblasts. Conversely, the geometric structure of biomaterials is considered important for inducing cell differentiation and tissue formation. Focusing on the importance of the geometry of artificial biomaterials in inducing cell differentiation and hard tissue formation, we have already succeeded in developing new honeycomb tricalcium phosphate (TCP) structures with holes of various diameters. We used the honeycomb TCP as a scaffold to induce TGCs into odontoblasts for the purpose of inducing odontoblasts with cell polarity [15].

2. Recruitment of BMDCs to periodontal tissue and dental pulp

Caries are a major cause of pulpitis. When the tooth crown is destroyed by caries, the pulp cavity is perforated. High proliferative activity of the pulpal tissue results in chronic inflammatory hyperplasia [16]. Granulation tissue grows from the pulp and forms periodontal polyps that can grow from inside the pulp cavity to outside the pulp. Experimental histopathological studies have long been performed on periodontal polyps, including histological analysis and treatment [17–20]. However, there is a lack of knowledge about the origin of the cells present in the pulp. As a result, using an experimental system of GFP mouse bone marrow transplantation, this study revealed that the cells were derived from bone marrow mesenchymal cells. Our research group used an experimental system of GFP bone marrow transplanted mice to study the migration and differentiation of cells in different parts of the oral cavity and teeth. Muraoka et al. showed that the BMDCs migrate to periodontal tissue and differentiate into periodontal ligament cells, such as macrophages and osteoclasts [21]. Tomida et al. showed the pluripotency of BMDCs, which migrated to periodontium, after the application of orthodontic mechanical

stress loading [13]. Kaneko et al. also reported the differentiation of BMDCs into cell components of periodontal tissue [14]. In our study, the method suggested by Osuga et al. [22] was used for the formation of granulation tissue through chronic inflammation in the dental pulp of GFP bone marrow transplanted mice [16]. Observations by micro-computed tomography (m-CT) [23, 24], histopathology, and immunohistochemistry were followed over time. Immunohistochemistry revealed notable results. Oka et al. used rats to observe periodontal tissue reactions in the tooth roots associated with dental pulp perforation [17]. In a similar study, Imaizumi et al. examined the spread of inflammatory lesions in the periodontal ligament [18]. Other experiments have shown the formation of inflammatory lesions in the pulp because of perforation of the pulp cavity, examining the types of cells that appear with inflammation and the evolution of the inflammatory state [19, 20]. A detailed histopathological examination by Nakamura et al. showed continuous granulation tissue growth in the pulp [25]. Thus, periodontal polyps are considered suitable for observing cell dynamics in the regeneration and repair of dental pulp tissue. The focus of previous studies was on histopathological examination, and the origin of the cellular components of pulp granulation tissue was not mentioned. Recently, it has been widely reported that mesenchymal stem cells derived from bone marrow migrate to various organs and play a role in tissue formation. To histologically examine the *in vivo* recruitment of bone marrow-derived undifferentiated mesenchymal cells, these cells need to be marked. GFP transgenic mice express GFP in all cells of the body. Therefore, in wild-type mice transplanted with GFP mouse bone marrow, it is possible to trace BMDCs using GFP as a marker [26, 27]. We used an experimental system using GFP bone marrow transplanted mice to perforate the pulp cavity of the maxillary left first molar and induce pulpitis. With the development of pulpitis, the origin of the cellular components involved in the growth of periodontal polyps was investigated.

The periodontal polyp model used in our experiment was based on Osuga's method [22]. Anesthetized GFP bone marrow transplanted mice were secured to the plate, and a hole was made in the crown of the maxillary left first molar using a dental cutting device. Thereafter, the pulp was histologically observed over time for 2 weeks, 1 month, 3 months, and 6 months.

At 2 weeks, granulation tissue proliferation with neutrophil infiltration occurred just below the pulpal perforation. Round to short oval cells appeared around the granulation tissue. The granulation tissue was composed of fibroblast-like cells, capillaries, and chronic inflammatory cells (**Figure 1**). Immunohistochemical examination of GFP revealed that GFP-positive cells made up the majority of the small oval cells that emerged around the granulation tissue. A small number of fibroblasts also showed a GFP-positive reaction (**Figure 1-d**).

At 1 month, the granulation tissue that proliferated in the pulp cavity increased. The number of short oval cells increased around the granulation tissue, and the number of fibroblasts and capillaries, which are the cellular components that make up the granulation tissue, also increased (**Figure 2**). Compared to the tissue at 2 weeks, the total number of GFP-positive cells increased. Most of the GFP-positive reactions occurred in small oval cells. However, the number of positive reactions in fibroblasts also increased (**Figure 2-d**).

At 3 months, the number of fibroblasts in the granulation tissue increased the most, and collagen fiber proliferation was also apparent. The number of capillaries also increased the most. Conversely, the number of small oval cells decreased (**Figure 3**). The number of GFP-positive cells in the tissue was the highest, with positive reactions occurring in small oval cells and fibroblasts (**Figure 3-d**).

At 6 months, the continuous growth of granulation tissue resulted in an increase in mature fibroblasts and collagen fibers. However, inflammatory cells, capillaries,

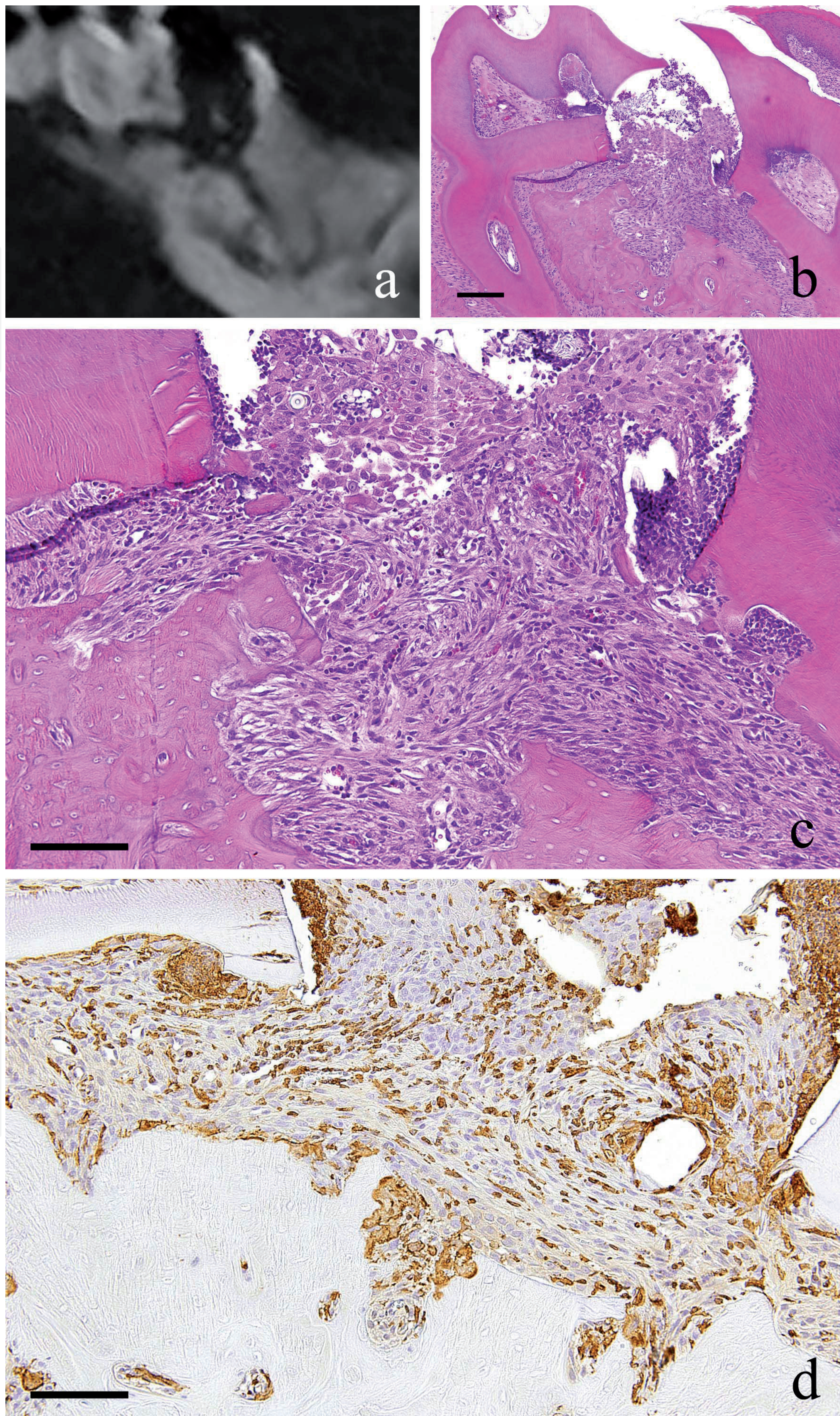

Figure 1.
2-week-specimen. a: m_CT image; b: Histopathological view of the same part of a, Scale bar = 200 μm; c: Enlarged view of b, Scale bar = 100 μm; d: IHC for GFP, Scale bar = 100 μm.

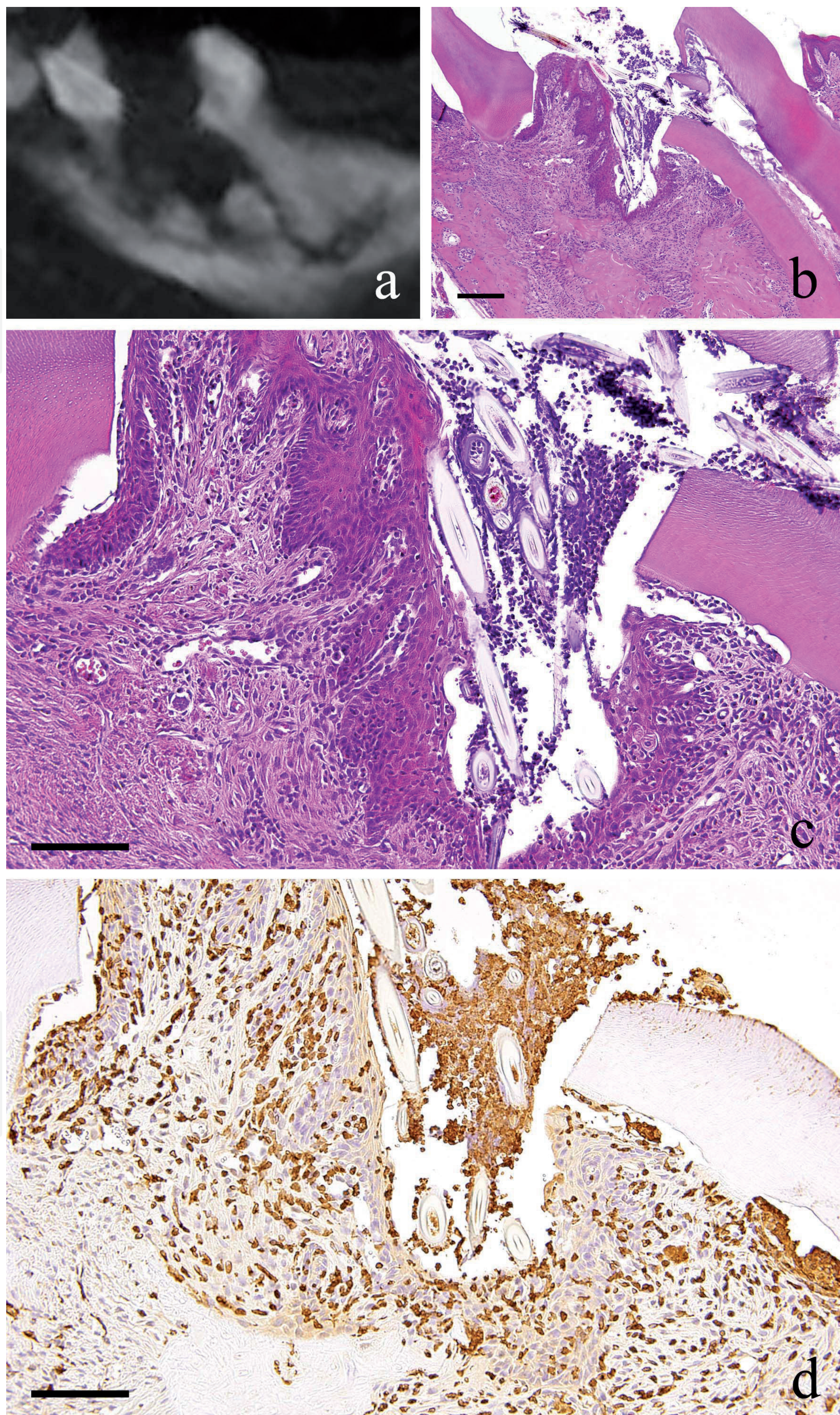

Figure 2.
1-month-specimen. A: m_CT image; b: Histopathological view of the same part of a, scale bar = 200 μm; c: Enlarged view of b, scale bar = 100 μm; d: IHC for GFP, scale bar = 100 μm.

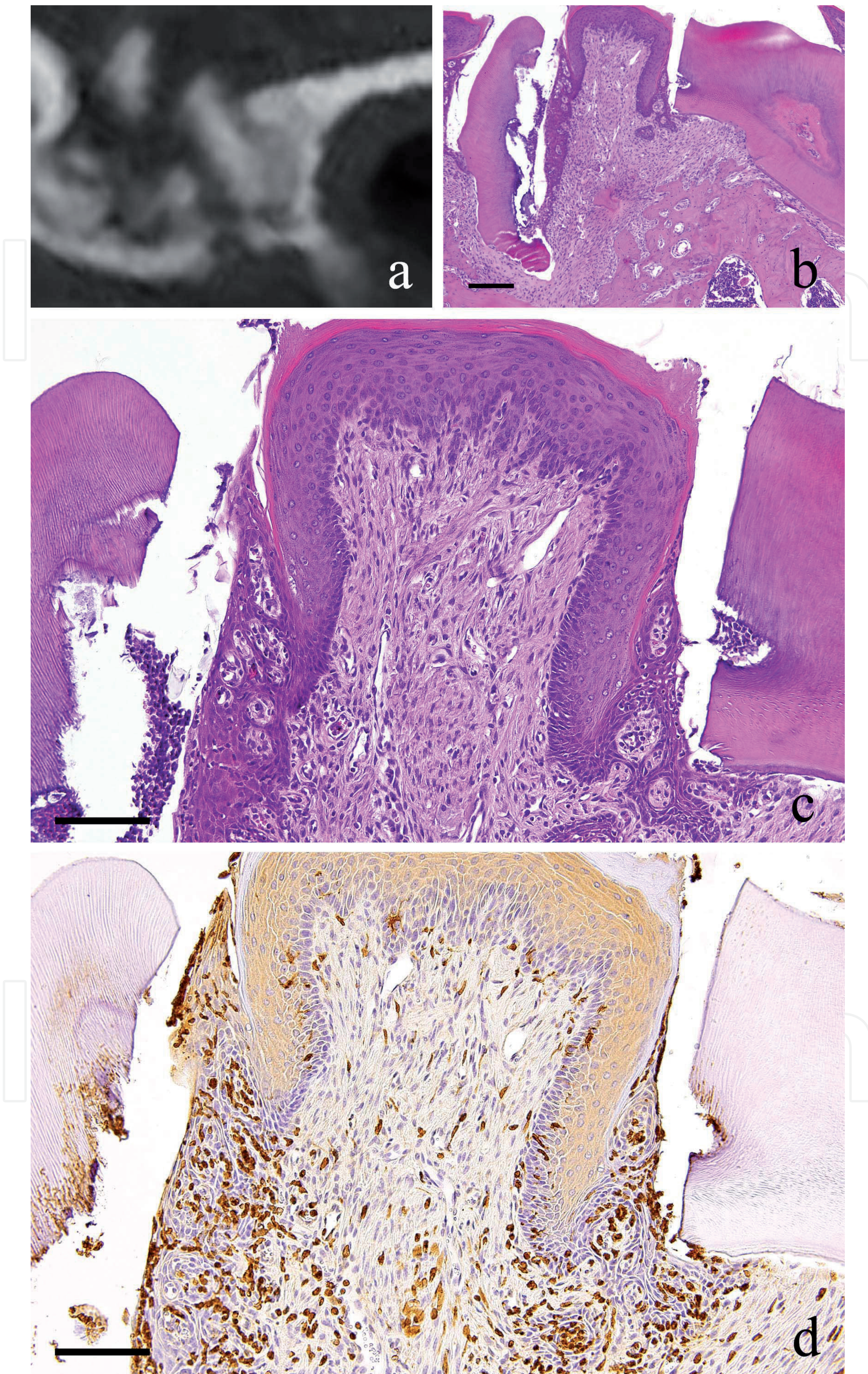

Figure 3.
3-month-specimen. A: m_CT image; b: Histopathological view of the same part of a, scale bar = 200 μm; c: Enlarged view of b, scale bar = 100 μm; d: IHC for GFP, scale bar = 100 μm.

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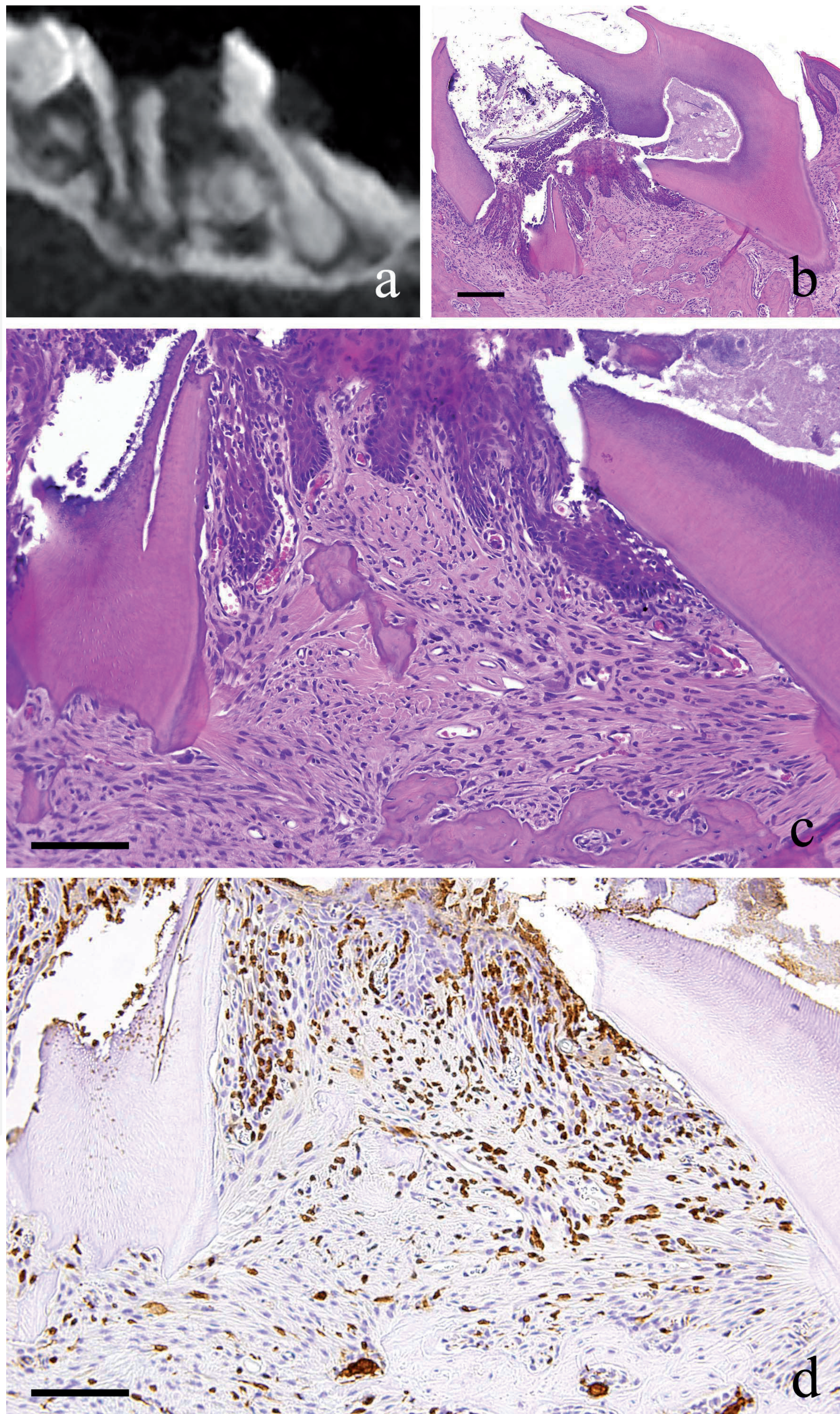

Figure 4.
6-month-specimen. A: m_CT image; b: Histopathological view of the same part of a, scale bar = 200 μm; c: Enlarged view of b, scale bar = 100 μm; d: IHC for GFP, scale bar = 100 μm.

and small oval cells decreased (**Figure 4**). The number of GFP-positive cells was reduced compared to that in the tissue at 3 months. However, many GFP-positive reactions appeared in the cells of mature granulation tissue.

A large number of GFP-positive cells appeared in periodontal polyps. Most of the positive cells were small oval cells. Over time, the number of GFP-positive fibroblasts increased. Therefore, these GFP-positive cells were derived from transplanted bone marrow cells. These GFP-positive cells were undifferentiated mesenchymal cells that migrated from the bone marrow to the pulp. From this, it was shown that the pulp has the potential to supply a large amount of BMDCs.

3. Cell differentiation of BMDCs recruited to the dental pulp

It is known that dental pulp tissue receives various stimuli even under physiological conditions. As a result, degenerative changes, such as atrophy are induced in the dental pulp cells. Moreover, activation of odontoblasts occurs because of stimulation, and new dentin is formed [28]. Histopathological investigations have been performed for a long time on the changes in dental pulp cells caused by stimulation [29]. Since external mechanical stress is applied to the pulp tissue even during orthodontic tooth movement, tissue changes in the pulp have been investigated experimentally [30–32]. Some studies have been performed using electron microscopy [33]. However, previous studies of dental pulp cell differentiation have not considered the presence of BMDCs in the pulp. Recently, many findings have been clarified regarding the factors that control cell differentiation regulation, and factors related to the differentiation of hard tissue cells, such as osteoblasts, have also been investigated. Sigehara et al. reported the gene expression status of osteocalcin, osteopontin, and HSPs [34]. Nakano et al. [35] investigated cell differentiation in the pulp caused by orthodontic mechanical stress using immunohistochemical techniques. The results of the study showed that the expression of Runx2 increased in the odontoblasts in the pulp, and the activity of odontoblasts may be increased. Runx2 is a transcriptional regulator belonging to the Runx family, including Runx1, Runx2, and Runx3 [30]. The Runx family is involved in cell differentiation and cell cycle progression [31] and is an essential transcriptional regulator of osteoblast differentiation and development [32]. Runx2 is a master regulator of odontoblast differentiation as well as osteoblasts [28]. No bone tissue was produced in the pulp. Therefore, the expression of Runx2 in the dental pulp strongly suggests odontoblast differentiation. Additionally, ALP expressed in the early stage of calcification associated with odontoblast differentiation is an index of odonoblastic activity [33]. In our previous studies, we induced the differentiation of dental pulp cells by adding injurious stimulation to the periodontium and pulp [10, 11]. Nabeyama et al. have shown that there is a weak positive reaction for Runx2 in the pulp tissue that has not been artificially stimulated [10]. This is similar to the results of the study conducted by Nakano et al. [35, 36], and it is presumed that it is a reaction to physiological stimuli, such as mastication and occlusion. Furthermore, Nabeyama et al. observed tissue changes in the pulp by stimulating the pulp with the immediate teeth separation method used for dental treatment for 30 min and observed the expression of Runx2 in odontoblasts, vascular endothelial cells of the pulp, and pulp-specific cells [10]. Additionally, its expression reached the maximum levels after 24 h, then gradually disappeared, and decreased to the same level as that of the control group at 1 week. It is considered that this is because BMDCs are recruited to the pulp and differentiated into pulp cells slightly after the local stimulation.

Regarding tooth separation in conservative dental treatment, the mechanical stress generated by the operation puts a stress load on the periodontal tissue,

especially on the periodontal ligament [37]. However, at the same time, it is easy to understand that it also acts as an injury stimulus on the pulp tissue of the tooth. That is, compression of the periodontal ligament in the alveolar region causes strong injurious stimulation of the vessels and nerves. Because these vessels and nerves are connected to the pulp, the damaging stimulus propagates into the pulp. However, there has been little research on the type of damage that actually occurs in dental pulp cells, and within the scope of our literature, we mention the expression of HSPs in dental pulp tissue. Only a few papers have been published regarding this issue. Sens et al. immunohistochemically examined the expression of HSPs in the pulp of human third molars and examined odontoblasts, dentin cell projections, dental fibroblasts, pulp vascular endothelial cells, and some blood vessels. HSPs are expressed in membranous smooth muscle cells [38]. Additionally, Tate et al. and Suzuki et al. experimentally verified the expression of HSPs in odontoblasts of the pulp after laser cavity formation in dental conservative treatment [39, 40]. Furthermore, Matsuzaki et al. reported a study at the mRNA level that HSP27 increased in the pulp with aging [41]. There are also forecast papers that investigated the expression of HSP27 and Runx2 in the pulp under orthodontic mechanical stress loading [35, 36]. The results of these studies indicated that HSP27 expression is associated with the differentiation of dental pulp cells into odontoblasts. It is widely known that HSPs are expressed in response to injurious stimuli, and they work to maintain homeostasis of the injured tissue. HSPs are proteins acquired for survival in a harsh environment in which cells are placed. They are strongly induced by non-physiological stimuli and have anti-apoptotic functions as molecular chaperones [42]. In addition, it is known that most HSPs are expressed as a cell response to stress, suppress protein denaturation, and repair denatured proteins. In fact, HSPs are constitutively expressed even under non-stress conditions and are essential proteins for various cell activities, such as cell differentiation, proliferation, survival, and function maintenance, *in vitro* and *in vivo* [42]. HSPs, such as HSP70 and HSP90, are known to act as molecular chaperones that temporarily bind to immature proteins, mediate the folding and association of polypeptides, and assist in protein maturation [43]. It is speculated that low molecular weight HSPs also function as molecular chaperones in cells, but the details have not been clarified [44]. HSP27 was initially discovered as an inhibitor of actin polymerization [45]. Since then, it is known that HSP27 is present in high concentrations in cells, such as skeletal muscle cells and vascular smooth muscle cells, even in the non-stimulated state. Thus, it is considered that HSP27 plays a physiological role in the vascular system [46].

Saito et al. stimulated the pulp by the same experimental method as Nabeyama et al. and examined the expression of HSP27 [11]. The pulp showed a weak positive reaction for HSP27 from the immunohistochemical reaction of the control group. These results are similar to the experimental results by Nakano et al. [35], and it is inferred that this may be a reaction to the constant load of physiological mechanical stress, such as mastication and tongue pressure on the teeth. The HSP27-positive reaction observed in the pulp of the control group was weakly expressed in the pulp cells but was mainly expressed in the vascular endothelial cells. This indicates that HSP27 plays a physiological role in the vascular system [46]. Next, HSP27 was strongly expressed in the vascular endothelial cells of the dental pulp 30 min after the teeth separation treatment and was also observed in some odontoblasts. Additionally, this expression reached its maximum after 24 h, then gradually disappeared, and its expression decreased to the same level as that of the control group at 1 week. It is considered that this is because dental pulp cells are stimulated by the stress of teeth separation, and HSP27 is expressed as a vascular reaction to it and induction of odontoblast differentiation.

Since all cells derived from GFP transgenic mice express GFP, all bone marrow cells of GFP bone marrow transplanted mice also express GFP. BMDCs are recruited to various places and transformed into various cells. However, we can track BMDCs using GFP as a marker [26, 27]. We used an experimental system using GFP mice to perforate the pulp cavity of the maxillary left first molar. The growth of granulation tissue in the pulp was examined to clarify the origin of the cellular components involved in the formation of periodontal polyps. In other words, a large number of BMDCs were recruited to the pulp that has been stimulated by inflammation. BMDCs are thought to differentiate into various cells in the pulp. BMDCs appear not only in the pulp but also in the periodontal ligament. Cells with spindle-shape cells, blood vessels, and polynuclear giant cells that make up the periodontal ligament were positive for GFP. There were no GFP-positive cells among the epithelial cells, but it was found that the GFP-positive cells infiltrated the epithelial tissue. Therefore, GFP-positive cells are considered dendritic cells.

The cells in the capillaries of the periodontal polyp prepared by Osuka's experimental model can be identified by immunohistochemical examination of CD31 as a marker of vascular endothelial cells. The highest number of capillaries was observed at 3 months. Immunofluorescence double staining of GFP and S100A4 in tissues over 2 weeks to 6 months detected green fluorescence (**Figure 5-a**) showing GFP-positive cells and red fluorescence showing S100A4 positive fibroblast-like spindle-shaped cells. (**Figure 5-b**). The region with orange fluorescence included cells with both proteins (**Figure 5-c**). As a result of marking the nucleus with DAPI, orange fluorescence was observed around the nucleus that usually emits blue fluorescence (**Figure 5d**).

Immunofluorescent double staining for GFP and Runx2 detected both oval and spindle-shaped cells positive for GFP green fluorescence (**Figure 6-a**) and Runx2

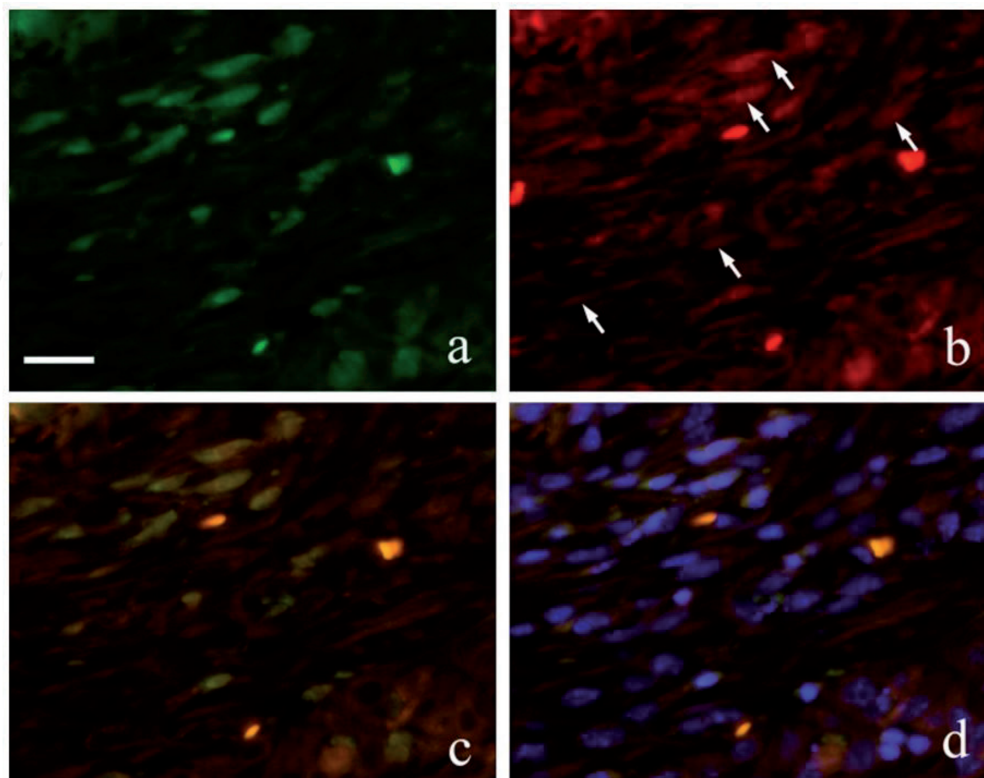

Figure 5.
FIHC images of periodontal polyp (a: GFP; b: S100A4; c: Merged image of GFP and S100A4; and d: Merged image of S100A4, GFP and DAPI; 2 week specimen; scale bar = 20 μm).

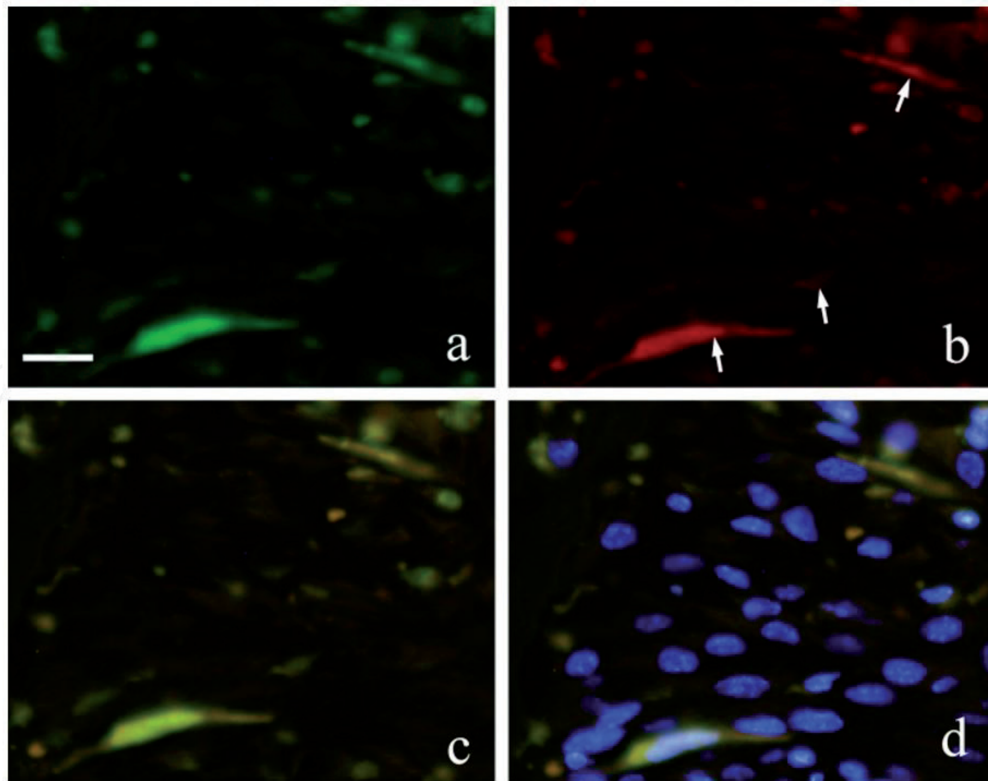

Figure 6.
FIHC images of periodontal polyp (a: GFP; b: Runx2; c: Merged image of GFP and Runx2; and d: Merged image of Runx2, GFP and DAPI; 2 week specimen; scale bar = 20 μm).

red fluorescence (**Figure 6-b**). In the superimposed image, cells showing orange fluorescence coexpressed two proteins (**Figure 6-c**). When the image was overlaid with DAPI, which showed blue fluorescence in the nucleus, orange fluorescence was seen in the cytoplasm (**Figure 6-d**). Runx2-positive and GFP-positive cells that showed orange fluorescence were observed at 2 weeks to 1 month of periodontal polyp formation and then decreased at 3 months. Even at 6 months, both Runx2- and GFP-positive cells appeared. However, there were many cells that were individually stained with GFP. Normally, no bone tissue is formed in the pulp. Runx2-positive cells in the pulp mean odontoblasts. This result indicates that the cells that proliferate in the pulp and differentiate into Runx2-positive odontoblasts are GFP-positive BMDCs.

4. Induction of differentiation of dental pulp stem cells into odontoblasts

Recently, the use of stem cells in molecular and cell biology has led to new therapeutic strategies for regenerating damaged oral tissue. It is well known that the pulp is rich in adult mesenchymal stem cells (MSCs), and the stem cells isolated from the pulp have high proliferative potential and may be able to differentiate into hard tissue-forming cells. Additionally, dental pulp stem cells play an important role in regenerative medicine for both oral and non-oral areas because of their high proliferation rate, pluripotency, and ease of collection [47]. Therefore, pulp stem cells are a promising source of MSCs used in a variety of clinical applications, such as bone formation, tooth tissue engineering, and nerve tissue regeneration [48]. We established a stable dental pulp cell line derived from GFP transgenic rats. It has the characteristics of dental pulp stem cells and exhibits stable odontoblast differentiation both *in vitro* and *in vivo*. To date, there are no reports of established cells showing stable osteoblastic

and stem cell-like properties over time, both *in vitro* and *in vivo*. However, this dental pulp cell line forms dentin-like hard tissue *in vivo* but does not lead to the induction of polar odontoblasts. A scaffold is an integral part of tissue engineering. Various artificial biomaterials have been developed as scaffolds and are widely applied clinically. In recent years, some studies have focused on the geometry of biomaterials. This is because scaffold composition and optimal geometry are believed to be important for inducing cell proliferation and differentiation. Focusing on this, we have already succeeded in developing a new biomaterial, honeycomb tricalcium phosphate (TCP), which contains holes of various diameters. Previous studies have reported that the surface properties of TCP due to different sintering temperatures affect hard tissue inducibility and biocompatibility [49]. Furthermore, cartilage and bone formation can be controlled by changing the diameter of the through holes in the honeycomb TCP. In a skull defect rat model, active bone tissue formation was observed in honeycomb TCP containing a through hole with a diameter of 300 μm, suggesting its clinical applicability [50]. These findings indicate that this honeycomb TCP can potentially act as a bioactive carrier and reproduce the interaction between progenitor cells and the extracellular matrix microenvironment. Additionally, we successfully differentiated polar odontoblasts from dental pulp stem cells using honeycomb TCP.

Gronthos et al. reported the isolation and characterization of pulp stem cells from wisdom tooth pulp tissue of impacted teeth, and reported that pulp stem cells have higher cell proliferation and tissue regeneration capacity than bone marrow-derived mesenchymal stem cells [51]. Since then, many researchers have reported that dental pulp stem cells differentiate into a variety of cells, including nerve cells, adipocytes, chondrocytes, and bone [52, 53].

The TGC we created is a pulp-derived stem cell that can differentiate into functional odontoblast-like cells both *in vitro* and *in vivo*. *In vitro*, the bone-forming medium resulted in increased ALP activity of TGC and formation of calcium deposits (**Figure 7**).

Since transforming growth factor (TGF)-β is involved in dentin repair and dentin formation [54], we also investigated the effect of TGF-β on TGC.

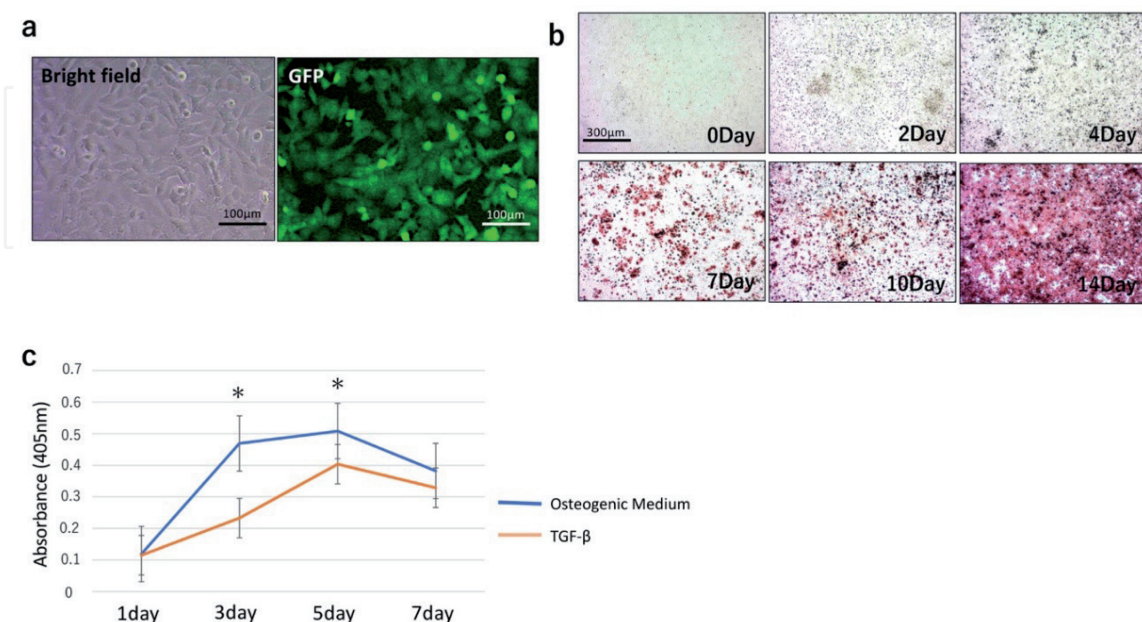

Figure 7.
(a) TGC showed a fibroblast-like shape (left), expression of green fluorescent protein (GFP) (right). (b) Alizarin red staining of TGC exposed to osteogenic medium from 0 to 14 days. (c) Alkaline phosphatase (ALP) activity of TGC cultivated with osteogenic medium or TGF-β. the ALP activity from osteogenic medium became significantly higher than that from TGF-β. $^{*}p < 0.05$.

Addition of TGF-β to TGC yielded results similar to those obtained with bone-forming medium. However, the increase in ALP activity by TGF-β was weaker than that of the bone-forming medium. One hypothesis that explains this weak stimulus is that TGC is already exposed to TGF-β because it can express the TGF family of proteins.

Regeneration using dental pulp cells and scaffolds has been reported. Ceramics, such as polylactic acid, poly (α-hydroxyl) acids, polylactic-co-glycolic acid, and TCP or hydroxyapatite have been used as scaffolds for dentin regeneration [55]. These artificial biomaterials have already been confirmed to be highly biocompatible and are used as scaffolds for odontoblast differentiation and bone induction. To date, there have been several reports of dentin-like hard tissue formation in experiments combining various scaffolds with dental pulp stem cells [56]. Among these artificial biomaterials, TCP has been reported to be highly biocompatible, and when transplanted into a living body, it is absorbed over time and self-assembled. However, previous studies using TCP and pulp cells to induce odontoblast differentiation have not led to the regeneration of polar dentin [6].

Many studies have used artificial biomaterials that are suitable for inducing differentiation into odontoblasts. However, no studies have effectively induced the differentiation into odontoblasts by changing the geometric structure of artificial biomaterials. We have shown by histological observation that changing the pore diameter of honeycomb TCP with multiple through-holes changes the type and amount of hard tissue formed in the pores. *In vivo* TGC transplantation experiments showed bone-like hard tissue formation at a pore diameter of 75 μm TCP and 500 μm TCP. However, for 300 μm TCP, hard tissue formation was observed to be added to the TCP surface, and the induced cells were dentin sialoprotein (DSP)-positive odontoblasts (**Figure 8**). In addition, these cells had a polar sequence and exhibited an odontoblast-like structure, which was present in the pulp cavity. Since the pore diameter of 300 μm resembles the width of the pulp cavity [57], it is considered that the 300 μm honeycomb TCP reproduces the dental pulp environment in the living body and differentiates polar odontoblasts to form dentin.

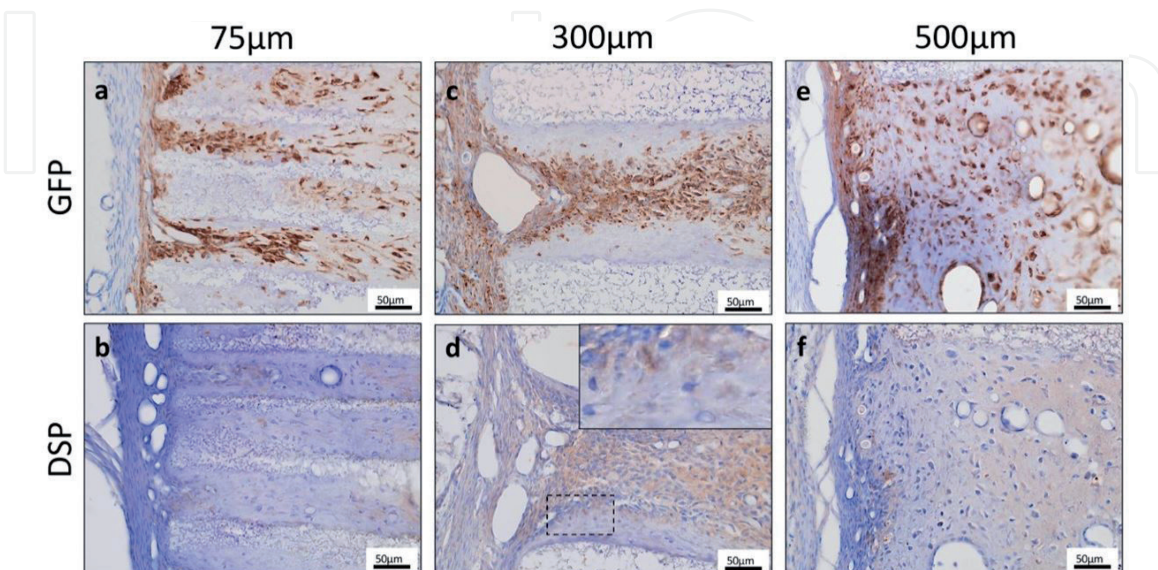

Figure 8.
The cells forming hard tissues in the TCP pores were GFP positive. Dentin Sialprotein (DSP) was not expressed in the cytoplasm of cells forming hard tissues in 75TCP and 500TCP. In contrast, in 300TCP, DSP was expressed in the cytoplasm of cells that were arranged with polarity on the TCP wall.

5. Conclusions

Maintaining tooth function is an important factor in ensuring health and quality of life. Unfortunately, illness, injury, or aging causes many people to lose their teeth and have a poor quality of life. As a result, it is necessary to develop an appropriate method for the restoration of tooth function. Tissue engineering provides an attractive perspective with the potential to regenerate fully functional organs to replace damaged or lost organs. This method required the integration of three key elements: progenitor or stem cells, extracellular matrix scaffold, and morphogens, which induce morphogenetic signals. Therefore, proper stem cell isolation and stable establishment are one of the points that must be achieved to perform regenerative therapy. Within the pulp, it is known that the pulp contains progenitor cells/stem cells, which proliferate and differentiate into various cells. Pulp stem cells can generate a new population of odontoblasts to repair damaged hard tissue. Induction of stem cell proliferation and differentiation is triggered by the release of morphogens from the pulp and periodontal tissue. Such releases occur in response to caries, therapeutic irritation, and injury. We established a cell line from the pulp of GFP transgenic rats, named it TGC, and used it for dentin regeneration. These rat pulp-derived cells were maintained in cultures for more than 80 passages without showing any changes in morphology or ability. Using this TGC and pore diameter 300 μm TCP as a scaffold, odontoblasts could be differentiated to develop polar dentin and create a structure similar to physiological dentin.

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Conflict of interest

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

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