Abstract: An inferior alveolar nerve (IAN) injury is a common clinical problem that can affect a patients’ quality of life. Cellular therapy has been proposed as a promising treatment for this injury. However, the current experimental models for IAN injury require surgery to create bone windows that expose the nerve, and these models do not accurately mimic human IAN injuries. Therefore, in this study, a novel experimental model for IAN injury has been established in rats. Using this model, the effects of Schwann cells and their role in the recovery from IAN injuries were investigated. Schwann cells were isolated from rat sciatic nerves and cultured. The first molar in the mandible was extracted and the IAN was immediately injured for 30 min by inserting an insect pin. Then, the Schwann cells or culture medium were transplanted into the extracted sockets of the cell and injury groups, respectively. After the surgery, the cell group displayed significantly increased sensory reflexes in response to mechanical stimulation when compared with the injury group. In conclusion, a novel animal experimental model for IAN injury has been developed that does not require the creation of a bone window to evaluate the impacts of cell transplantation and demonstrates that Schwann cell transplantation facilitates the regeneration of injured IANs.

Keywords: cellular therapy, extracted socket, inferior alveolar nerve injury, novel inferior alveolar nerve injury model, regeneration, Schwann cells

Introduction

The inferior alveolar nerve (IAN), a division of the mandibular branch of the trigeminal nerve, travels beneath the lateral pterygoid muscle before entering the mandibular canal through the mandibular foramen. The IAN spans a long intraosseous pathway [1,2], and because of the close anatomic relationship between the mandibular canal and the roots of the mandibular third molars, their removal has been implicated as the main cause of temporary or permanent IAN sensory deficiency [3-5]. IAN injuries are associated with various symptoms, including local edema, limited ability to open the mouth, and pain, all of which reduce the quality of life because of their effect on eating, speech, and social interaction [6-8]. Partial spontaneous healing of the IAN can occur through endogenous regeneration, and the nerve’s position inside the mandibular canal favors spontaneous regeneration of the proximal nerve stump towards the distal stump [9,10]. However, spontaneous regeneration is often insufficient for complete functional recovery, and if associated neuropathic pain is present, immediate treatment is particularly important to prevent chronic and irreversible IAN damage [1,11]. Partial IAN regeneration is possible 4-8 weeks after injury; however, injuries that last longer than 6 months often result in chronic pain [3,12].

The development of treatments that encourage IAN repair is currently an important research focus in the field of oral and maxillofacial surgery [13]. Surgical repair, including nerve grafting, is usually required to treat nerve transections, whereas drug therapy or physical rehabilitation is required to treat moderately severe crush injuries [14-16]. Although these types of repairs are frequently performed in humans, the absence of an experimental model that allows for the repeated study of intraosseous IAN repairs has made it difficult to scientifically evaluate the reliability of IAN repair methods in such situations.

Over the past few decades, cell transplantation has emerged as a promising strategy for peripheral nerve repair, and a variety of cell types, including Schwann cells (SCs), neural stem cells, bone marrow stromal cells, and umbilical cord-derived mesenchymal stromal cells have been used to create environments that are conducive to nerve regeneration through neurotrrophic factor production and SC differentiation [17-19]. However, the type of cell that would be most effective to graft into the injured IAN remains to be determined.

SCs are glial cells of the peripheral nervous system, which surround the axons and facilitate impulse conduction [18]. They also participate in peripheral nerve regeneration after an injury. In Wallerian axonal degeneration, SCs along with macrophages, mediate initial myelin removal [20,21]. The SCs proliferate, migrate to form Büngner bands, and secrete neurotrophic factors that aid axonal guidance and establish a favorable microenvironment for precise target innervation [22,23]. These functions suggest that SC-based therapy has great potential to support and promote peripheral nerve regeneration by secreting a wide variety of growth factors and integrating into the distal nerve microenvironment, guiding the reconnection of severed axons [24]. However, no reports exist on the effects of SC transplantation in IAN injuries.

To evaluate the extent of IAN regeneration after an injury, several experimental models have been developed using IAN ligation, crushing, and transection injuries [25-27]. However, these models require an incision on the lower edge of the mandible to expose the nerve and form buccal plate bone windows through surgical exposure of intraosseous IAN segments in the exposed mandible. This approach is inconsistent with nerve injuries that occur after tooth extraction and implant placement procedures, and there is no established experimental animal model that can accurately evaluate the effects of transplanted cells on an IAN injury.

Therefore, this study had two purposes. The first purpose was to develop a novel and clinically consistent experimental model of an IAN injury, and the second purpose was to evaluate the effects of SC transplantation on IAN regeneration using this model.

Materials and Methods

All of the experimental procedures involving animals were performed in accordance with institutional animal care guidelines, and ethical approval was obtained from the Animal Care and Use Committee for School of Dentistry at Aichi Gakuin University (approval number: AGUD392). The recombinant DNA experiments for the transgenic Sprague-Dawley (SD) rats expressing enhanced green fluorescent protein (GFP, henceforth referred to as GFP rats) were approved by the Safety Committee of Aichi Gakuin University (approval number: 17-5).
A novel rat experimental model of IAN injury

Four-week-old male SD rats weighing 80-100 g (Japan SLC, Hamamatsu, Japan) were anesthetized with an intraperitoneal injection of 7.4% medetomidine hydrochloride (Meiji Seika Pharma, Tokyo, Japan), 8% midazolam (Astellas Pharma Inc., Tokyo, Japan), and 10% butorphanol (Meiji Seika Pharma). Initially, the position of the distal root of the first molar and the mandibular canal in the mandible were confirmed using micro-computed tomography (micro-CT). In all of the rats, the mandibular canal was located under the distal root of the first molar (Fig. 1A, B). Before the insect pins (0.7 × 26 mm; Yahata, Kitanagoya, Japan) were inserted into the extracted sockets, a micro-CT confirmed that there was no remaining tooth root in the extracted sockets. Further, no damage to the mandibular canal was observed under a microscope after the extraction of the first molar on the right side (Fig. 1C, D). Thereafter, in the injury group (n = 5), the IAN was immediately subjected to injury for 30 min by inserting an insect pin into the extracted socket until it reached the lower border of the mandible (Fig. 2A-C). To confirm whether the inserted insect pin was correctly positioned, a micro-CT analysis (Fig. 2D, E) and histological analysis (Fig. 2F, G) were performed using undecalcified sections as described in the following sections. Bleeding from the extraction socket was controlled by gauze compression. In the control rats (n = 5), the first molar in the mandible was extracted, but the IAN was not injured.

Isolation and cultivation of SCs from rat sciatic nerves

The SCs were isolated from rat sciatic nerves and cultured as previously described with some modifications [28-30]. GFP or SD rats were purchased from Japan SLC or Chubu Kagaku Shizai (Nagoya, Japan), respectively. The four-week-old GFP rats were anaesthetized using inhalation of 2.5% isoflurane (DS Pharma Animal Health, Osaka, Japan) in a Narcobit-E Anesthesia Unit (Natsume Seikakusho, Tokyo, Japan). The sciatic nerves were exposed, ligated with 4-0 silk thread at one position, and transected distally to the knot to induce Wallerian degeneration and enhance SCs. The skin incisions were sutured and the rats were housed to allow degeneration. After 2 weeks, the rats were euthanized with CO2, and the distal segments of the degenerated sciatic nerves (10 mm in length) were resected and removed. After the epineuria were removed, the nerve segments were rinsed with phosphate-buffered saline (10 mM PBS; Fujifilm Wako, Osaka, Japan), cut into 1-mm pieces, and digested with a mixture of 0.2% collagenase NB4 (Serva Electrophoresis GmbH, Heidelberg, Germany) and 0.2% Dispase II (Fujifilm Wako) at 37°C for 50 min. The digested materials were centrifuged at 1,500 rpm for 5 min and the supernatants were discarded. The cell pellets were resuspended in SC culture medium (SCM; composed of Dulbecco’s modified Eagle’s medium high glucose, Fujifilm Wako) and supplemented with 2 µM forskolin (Sigma-Aldrich, St. Louis, MO, USA), 10 ng/mL heregulin-β1 (PeproTech, Rocky Hill, NJ, USA), 50 ng/mL basic fibroblast growth factor (R&D systems, Minneapolis, MN, USA), and 1% penicillin-streptomycin solution (Fujifilm Wako). The cells were cultured in SCM that contained 20% fetal bovine serum (FBS) in culture dishes (TPP, Trasadingen, Switzerland) at 37°C under 5% CO2 (Fig. 3A). After the cells reached 70% confluency, they were cultured in SCM for 8 days as passage 0 (P0) cells (Fig. 3B). The cells (GFP-SCs or SD-SCs) were expanded up to P4 in SCM with 10% FBS and used in the following experiments.

In order to evaluate whether the expanded cells displayed the characteristics of SCs, immunocytochemistry was performed as previously described [31]. Briefly, the cells were fixed with 4% paraformaldehyde...
and permeabilized with 0.1% Triton X-100 (Sigma-Aldrich). After blocking, the cells were incubated with a mouse monoclonal anti S100 calcium binding protein B (S100B) antibody (ab40666, 1:50; Abcam, Cambridge, UK), and then incubated with Alexa Fluor 594 goat anti-mouse IgG (A11032, 1:100; Thermo Fisher Scientific, Tokyo, Japan). The cells were then counterstained with 1 μg/mL 4',6-diamidino-2-phenylindole (DAPI; Dojindo, Kumamoto, Japan) and observed with a BZ-X710 fluorescence microscope (Keyence, Osaka, Japan) (Fig. 3C).

In order to determine the percentage of S100B-positive cells, the number of S100B-positive and the number of total cells were counted in three randomly selected fields of view to obtain the average values. DAPI staining was used to count the total number of cells. The SC purity was then calculated according to the following equation: SC purity (%) = S100B positive cell/total cells × 100.

**SC transplantation**

After removing the insect pin from the extracted sockets, the tip of a MS-NE05 micro syringe (ITO Corp., Fuji, Japan) was inserted into the extracted sockets to reach the damaged mandibular canal, and the SCs with or without GFP expression (4.0 × 10^5 cells in 4 μL of SCM) were slowly injected into the cell group (n = 5). In the injury group, 4 μL of SCM without SCs was injected into the extracted socket. After the injections, the rats were left to rest for 1 h, facing upward.

**Von Frey testing**

Before behavioral testing, the rats were trained daily for approximately 1 week. They were gently guided into a dark plastic cage by hand and encouraged to keep their snout and mouth protruding through a hole in the cage wall during mechanical stimulation of the right facial skin above the mental foramen using Von Frey filaments (Muromachi Kikai, Tokyo, Japan) in ascending order of mechanical intensity. The head-withdrawal threshold (HWT) was determined as the minimum mechanical intensity that evoked withdrawal in response to three or more of five stimuli. In each trial, the filament was applied at 4-s intervals. Three measurements were performed for each rat and the results at each time point were averaged. The maximum intensity of mechanical stimulation was 60 g. The HWT was determined every day for 2 weeks after the surgery in the cell, injury, and control groups (n = 5 in each).

**Micro CT imaging and analysis**

Before the SC transplantation, the location of the inserted insect pin in the extracted socket was evaluated using micro-CT imaging (Fig. 2D, E, Cosmo Scan GX; BMD., Tokyo, Japan) with exposure parameters of 2 min, 90 kV, and 100 mA, and an isotropic voxel size of 25 mm, as previously described [17-19]. Also, the destruction of the mandibular canal before the SC transplantation and the repair of the mandibular canal after the SC transplantation were evaluated using micro-CT imaging (Cosmo Scan GX; Rigaku Corp.). Micro-CT images were obtained from each rat prior to surgery and 1 and 2 weeks after surgery, and the bone volume and bone mineral density (BMD) were measured in 1.5 × 1.5 × 1.0 mm regions of interest that contained the extracted sockets of the distal root of the mandibular first molar. The radio-opacity of each extracted socket was measured using voxel images in the bone volume measuring software 3 by 4 viewer 2011 (Kitasenju Radist Dental Clinic i-View Image Center, Tokyo, Japan). Increases in the bone volume and BMD in individual rats were calculated by subtracting the presurgical value from the values that were obtained 1 and 2 weeks after surgery.

**Histological analysis**

In order to evaluate whether the insect pin in the extracted socket reached the lower border of the mandible to injure the IAN, a histological observation was performed using undecalcified sections (Fig. 2F, G). Briefly, the mandibles with insect pins were harvested and fixed in 70% ethanol for 3 days. The undecalcified samples were dehydrated with an ethanol gradient from 70% to 100%, stained with Villanueva Osteochrome Bone stain, and then embedded in methyl methacrylate (Fujifilm Wako). The samples were sectioned to 20 μm thickness in the coronal and sagittal planes (Ito Bone Histomorphometry Institute, Niigata, Japan).

To confirm axonal regeneration and examine the extent of bone repair in the damaged mandibular canals, a histological analysis was performed using paraffin-embedded sections. To prepare the paraffin sections, mandibles from the cell, injury, and control groups were harvested at 1 or 2 weeks after surgery and fixed in 4% paraformaldehyde. Then, the samples were decalcified in 10% EDTA-2Na (Muto Pure Chemicals, Tokyo, Japan) for 3 weeks. Next, the paraffin-embedded tissues were sectioned to a thickness of 5 μm in the coronal plane and stained with hematoxylin and eosin (H&E).

The area of the newly formed bone in the extracted sockets was measured using ImageJ software at 1 and 2 weeks after the surgery (National Institutes of Health, Bethesda, MD, USA) in five randomly selected fields of view per standardized field (1.4 × 1.4 mm) at low magnification (4×). In addition, the extent of IAN regeneration was analyzed by measuring the maximum diameter of the regenerated IAN in the mandibular canal using five randomly selected coronal specimens per group.

**Immunohistochemical analysis**

Immunohistochemical analysis was performed on 5 μm-paraffin sections of the SD rat mandibles 1 and 2 weeks after the surgery. A rabbit polyclonal...
anti-myelin basic protein (MBP) antibody (ab40390, 1:500; Abcam) was used to label the myelin sheaths, and a mouse monoclonal anti-S100B antibody (ab4066, 1:50; Abcam) was used to observe the distribution of the SCs, and the rabbit polyclonal anti-GFP antibody (ab290, 1:1000; Abcam) was used to identify the transplanted SCs. The antibodies were applied overnight at 4°C after blocking endogenous peroxidase activity with 3% hydrogen peroxide in methanol for 20 min at room temperature and antigen retrieval in HistoVT One (Nacalai Tesque, Kyoto, Japan) for 20 min at 90°C. After incubation with the primary antibodies, an ImmPRESS HRP Anti-Rabbit IgG (Peroxidase) Polymer Detection kit (Vector Laboratories) was applied for 30 min at room temperature. The sections were visualized with a 3,3′-diaminobenzidine substrate (Vector Laboratories). Then, the number of MBP-positive myelin sheaths and the areas of the S100B-positive regions at the regenerated nerve fibers in a standardized field (128 × 96 µm) at high magnification (100×) were counted and measured, respectively, using ImageJ software (National Institutes of Health). Seven slides were randomly selected in each group.

Statistical analysis
The data are shown as the mean ± standard deviation (SD). The statistical significance of the differences between the groups was determined using Ekuseru-Toukei software (Version 1.04; Social Survey Research Information, Tokyo, Japan). A two-way repeated-measures analysis of variance (ANOVA) with a post hoc Bonferroni test was used to examine the differences between the injury and cell groups for the Von Frey test. A Student’s t-test was also used for intergroup comparisons for the other experiments. P < 0.05 was considered statistically significant.

Results
Clinical findings
The surgical procedures were well tolerated by all of the rats. All of the extracted sockets healed uneventfully, with no severe inflammation or swelling during the experimental period. The rats also showed no weight reduction after the surgery.

A novel rat experimental model of IAN injury
A novel experimental model of IAN injury was evaluated using radiological findings, histological observation, and a mechanical sensitivity test. After the removal of the inserted insect pin from the extracted sockets, micro-CT images showed that the mandibular canal and lower border of the mandible were destroyed in the injury group (Fig. 4A). Furthermore, H&E staining images showed that both the upper and lower borders of the mandibular canal were destroyed (Fig. 4B). In addition, the image showed that the IAN was completely absent within the mandibular canal in the injury group. Finally, the Von Frey test showed that the sensory reflexes in the injury group were lost until postoperative day 6, and began to improve on postoperative day 7 (Fig. 5). The results demonstrated that the IAN was clearly damaged by the insertion of the insect pin into the extracted socket.

Characterization of rat SCs
After the expanded cells were stained with the anti-S100B antibody, the average rate of the S100B-positivity in bipolar and tripolar cells was 94.9% (Fig. 3C).

Mechanical sensitivity after SC transplantation
The sensory reflexes in both the cell and injury groups began to improve on postoperative day 7. Importantly, all of the animals that received the SC transplantation exhibited behavioral improvements. The HWT in response to the mechanical stimulation of the facial skin at the mental foramen was significantly lower in the cell group than in the injury group at postoperative day 8; however, this difference disappeared by postoperative day 10, at which point both groups displayed similar responses to the sham group (Fig. 5).

Micro-CT analysis
Representative processed micro-CT images (coronal plane) from the injury and cell groups are shown in Fig. 6A-F. The micro-CT images in the immediate postoperative period (0 week) displayed damage to the mandibular canal under the distal root socket of the mandibular first molar in both groups. The coronal plane images that were acquired during postopera-
tive week 2 displayed an increased radio-opacity at the superior wall of the mandibular canal in the cell group. However, the superior wall of the mandibular canal in the injury group had still not formed 2 weeks after the surgery.

With regard to the healing of the extracted sockets, 1 week after surgery the coronal plane images displayed an increase in radiopaque regions in the socket of the cell group when compared with the injury group. Therefore, the bone volume in the extracted sockets of both groups was calculated. However, the bone volume of the distal root socket was not significantly different between the cell group and the injury group after 1 week and 2 weeks (Fig. 6G). The BMD in the radiopaque area was also calculated after 1 and 2 weeks, and displayed no significant differences at either timepoint (Fig. 6H).

Osseous repair of extracted sockets

In order to observe the healing process of the extracted sockets, a histological analysis was performed 1 and 2 weeks after surgery. Representative H&E-stained sections (Fig. 7A-D) showed that the extracted sockets were filled with newly formed tissues, and bone had formed to reach the alveolar crest after 1 week. Bone marrow was observed in the cell group after 1 week, but not in the injury group. The trabecular bone area was measured in both groups, and was significantly higher in the cell group than in the injury group both 1 and 2 weeks after the surgery (Fig. 7E).

At high magnification, the newly formed bone still displayed the morphology of the woven bones in the injury group after 2 weeks (Fig. 8B), and cuboidal osteoblasts were observed on the bone surface. The bones in the cell group displayed organized lamellar bone after 1 week when compared with the bone in the injury group (Fig. 8A, C). In addition, the lamellar bones displayed concentric cylindrical osteon after 2 weeks, and the osteoblasts along the bone surface were flattened. Furthermore, the osteocyes that were trapped in the lamellar bone were concentrically arranged after 2 weeks (Fig. 8D). In order to detect the presence of the transplanted SCs, an immunohistochemical analysis using anti-GFP antibody was performed. GFP-positive SCs were easily visualized within the newly formed connective tissues and on the surface of the newly formed bone, which suggested that the transplanted SCs had survived in the extraction socket until this time (Fig. 9A, B).

Bone repair of mandibular canal

In the injury group, the external shape of the mandibular canal was not circular after 1 week (Fig. 7A), but was after 2 weeks (Fig. 7B). In the cell group, the external shape of the mandibular canal was already circular after 1 week (Fig. 7C).

At high magnification, the mandibular canal in both groups was still surrounded by trabecular bone after 1 week (Fig. 10A, E), and in the injury group, the superior wall of the mandibular canal was still not closed after 2 weeks (Fig. 10C). In the cell group, the superior wall was not closed after 1 week, but was almost completely regenerated after 2 weeks (Fig. 10E, G).

Regeneration of IAN

IAN regeneration in the cell and the injury groups was histologically analyzed 1 and 2 weeks after the surgery at low (Fig. 7A-D) and high (Fig. 10) magnification. After 1 week, the regenerated tissues that had formed inside the mandibular canals were observed at low magnification in both groups. At high magnification, the peripheral nerve-like tissues were observed (Fig. 10). The thickness of these tissues in the cell group was significantly increased when compared with their thicknesses in the injury group (P < 0.05) (Fig. 7F).
Next, an immunohistochemical analysis using anti-MBP and anti-S100B antibodies was performed to detect the presence of nerve fibers and SCs. After 2 weeks, the numbers of MBP-positive myelin sheaths in the regenerated tissues of the cell group (Fig. 11D) were on average significantly higher when compared with the injury group (Fig. 11B); however, there was a small number of MBP-positive myelin sheaths in the cell and injury groups at 1 week after the surgery (Figs. 11A, C, 13A; \( P < 0.05 \)).

The S100B-positive area was also measured in both groups (Fig. 12), and was significantly higher in the cell group than in the injury group both 1 and 2 weeks after the surgery (Fig. 13B; \( P < 0.05 \)). In order to determine the localization of the transplanted SCs, immunohistochemical staining of GFP was performed. The GFP-positive cells were mainly located within the newly formed myelin sheaths after 1 and 2 weeks (Fig. 9C, D).

**Discussion**

Recently, there has been growing enthusiasm for the use of cell therapy to facilitate the regeneration of injured peripheral nerves. However, no studies have examined the prospect of using cell therapy for IAN injury, partly due to a lack of effective animal models. IAN ligation, crushing, and transection injury models have been developed to evaluate IAN regeneration; however, these procedures require the surgical creation of a bone window to expose the IAN since it lies within the mandibular canal [32]. In addition, these models are not consistent with how IAN injuries occur in the human clinical situation [33], and an experimental model that better mimics a human IAN injury after a tooth extraction is needed [1,2]. In this study, a novel rat model of IAN injury has been developed and used to determine whether cell therapy is a plausible approach to IAN regeneration.

Mechanosensitivity measurement is a key method in the study of sensitive functions. The up-down method for mechanosensitivity testing using Von Frey filaments is one of the most widely used methods for measuring pain in animals, and was used in this study to evaluate IAN damage after the insertion of an insect pin. Behavior data confirmed the onset of mechanosensitivity changes 1 days after the IAN injury, as shown by the increased mechanical thresholds in all of the rats in the injury group, and mechanosensitivity recovery began 7 days after the surgery. These results confirm the existence of IAN injury due to the pin insertion. Interestingly, mechanosensitivity recovery also began 7 days after surgery in a rat transection model of IAN injury [34], but only 2 days after injury in an IAN crush model [26]. These results suggest that the injury model used in this study should be considered a transection model. After crush injuries, the majority of the basal lamina scaffolds remain intact, and axons and SCs can extend and migrate along them. However, after transection injuries, the continuity of the basal lamina scaffolds is completely destroyed, resulting in slow axonal growth. The basal lamina of the IAN may have been completely transected by the insect pin, although destruction of the basal lamina was not observed in this study. This is the first report describing an IAN injury model that is similar to human IAN injuries.

The SCs were evaluated for effects on the recovery of the injured IAN since they play an important role in peripheral nerve regeneration.
Two important findings were observed about the role of SCs in IAN regeneration in this study. The behavioral results showed that mechanosensitivity was significantly recovered in the cell group when compared with the injury group at day 8. Although functional recovery is the primary goal when evaluating nerve regeneration [36,37], the recovery of IAN injuries can be also measured using a histological analysis [38,39].

H&E staining on the regenerated IAN fibers showed many small and large myelinated fibers 14 days after cell transplantation. Interestingly, the thickness of the regenerated IAN was increased in the cell group when compared with the injury group, and was almost identical to the thickness of the regenerated IAN in a healthy rat. These results suggest that there is a correlation between sensory sensitivity and the thickness of the regenerated IAN. Nerve fiber myelination determines the intensity of nerve regeneration. MBP is a major constituent of the myelin sheath, which is predominantly expressed in SCs [24,25]. After nerve crush, MBP-positive myelin sheaths were detected in the cell group, but not in the injury group. Furthermore, the MBP staining pattern was remarkably similar to the S100B staining, which was used to detect the SCs. These results indicate that cell therapy increased the number of axons and the thickness of bundles in the cell group compared with the injury group. The regenerating axons were closely associated with the SCs [8,11]. GFP-positive cells were observed in the regenerated IAN, which indicates that donor SCs can be integrated into the nerve and facilitate its myelination. This is the first report that demonstrates that SC transplantation enhances the regeneration and reinnervation of the injured IAN and contributes to the myelination of regenerated nerve fibers.

Sensory nerves regulate bone formation and remodeling through the synthesis and secretion of neurotrophic factors [40-48], and IAN transection significantly impairs mandibular bone healing and aggravates periodontal tissue destruction by inhibiting osteoblast function and promoting osteoclast formation [42,47,49]. The repaired bones in the mandibular canal and the extracted socket were evaluated. The results indicate that both healing processes were similar and that the regenerated bone in the extracted socket also showed similar maturity in the injury and cell groups. Interestingly, GFP-expressing cells were also observed in the extracted sockets after 1 week and 2 weeks. These observations may implicate the SCs in bone healing after tooth extraction. However, whether the SCs differentiated into osteoblasts was not determined in this study, and further studies will be necessary to examine these complex processes.

This novel animal model provides an ideal setting to investigate the influence of peripheral sensory nerves on bone remodeling, as the IAN is a sensory nerve that is surrounded by the mandibular cortex and trabeculae [50].

Previous experimental animal models of IAN injury have concentrated on extracranial repair rather than involving the intracranial segment where human IAN injuries most commonly occur [51]. In this study, a novel, less invasive, and more representative animal model of IAN injury has been developed and studied to demonstrate the potential of SC transplantation to promote IAN regeneration.

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Conflict of interest

The authors declare no conflict of interest.

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