Immunohistochemical analysis of stem cells from human exfoliated deciduous teeth seeded in carbonate apatite scaffold for the alveolar bone defect in Wistar rats (Rattus novergicus)

by Tania Saskianti

Submission date: 08-Dec-2021 11:30AM (UTC+0800)
Submission ID: 1724021045
File name: r_the_alveolar_bone_defect_in_Wistar_rats_Rattus_novergicus.pdf (2.83M)
Word count: 9001
Character count: 49341

Check for updates

RESEARCH ARTICLE

REVISED Immunohistochemical analysis of stem cells from human exfoliated deciduous teeth seeded in carbonate apatite scaffold for the alveolar bone defect in Wistar rats (*Rattus novergicus*) [version 2; peer review: 2 approved]

Tania Saskianti[1], Alexander Patera Nugraha[2], Chiquita Prahasanti[3], Diah Savitri Ernawati[4], Ketut Suardita[5], Wibi Riawan[6]

[1]Pediatric Dentistry Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia
[2]Orthodontics Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia
[3]Periodontology Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia
[4]Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia
[5]Conservative Dentistry Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, 60132, Indonesia
[6]Biomolecular Biochemistry, Faculty of Medicine, Brawijaya University, Malang, Indonesia

v2 **First published:** 22 Sep 2020, **9**:1164
https://doi.org/10.12688/f1000research.25009.1

Latest published: 02 Dec 2020, **9**:1164
https://doi.org/10.12688/f1000research.25009.2

Abstract

Background: Stem cells from human exfoliated deciduous teeth (SHED) seeded in carbonate apatite scaffold (CAS) may have multiple functions that could be used to regenerate the alveolar bone defects. The purpose of this study is to examine the ability of SHED and CAS in alveolar bone defects using an immunohistochemical analysis.

Methods: ten three-month-old healthy male Wistar rats *(R. novergicus*) that weighed between 150–250 grams (g) were used as animal models. A simple blind random sampling method was used to select the sample that was assigned to the study group for CAS and SHED seeded in CAS (n=5). The animal study model of the alveolar bone was established by extracting the anterior mandible teeth. Rodent anesthesia was applied to relieve the pain during the procedure for all test animals. Immunohistochemistry was performed after seven days to facilitate the examination of the receptor activator of NF-κβ ligand (RANKL), osteoprotegrin (OPG), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), runt-related transcription factor 2 (RUNX2), alkaline phosphatase (ALP), osteocalcin, and osteopontin expression. The data was analyzed using the unpaired t-test ($p<0.01$) and Pearson's correlation test ($p<0.05$).

Results: The OPG, RUNX2, TGF-β, VEGF, ALP, osteocalcin, and ostepontin expressions were higher in SHED seeded in CAS than CAS only with a significant difference between the groups ($p<0.01$). Furthermore, the RANKL expression was lower in SHED seeded in CAS compared to CAS only. There was a strong reverse significant

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	2
version 2 (revision) 02 Dec 2020	✓ report	
version 1 22 Sep 2020	? report	✓ report

1. **Niswati Fatmah Rosyida**, Universitas Gadjah Mada, Yogyakarta, Indonesia
2. **Lidia Audrey Rocha Valadas**, Federal University of Ceará, Fortaleza, Brazil

Any reports and responses or comments on the article can be found at the end of the article.

correlation between OPG and RANKL expression ($p<0.05$).
Conclusions: The number of osteogenic marker expressing cells, such as OPG, RUNX2, TGF-β, VEGF, ALP, osteocalcin, and ostepontin, increased. However, RANKL expression in the alveolar bone defects that were implanted with SHED seeded in CAS did not increase after seven days.

Keywords
Bone Defect Socket, Carbonate Apatite Scaffold, Medicine, Osteogenic Ability, Stem Cell from Human Exfoliated Deciduous Teeth

Corresponding author: Chiquita Prahasanti (chiquita-p-s@fkg.unair.ac.id)

Author roles: Saskianti T: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Nugraha AP**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Prahasanti C**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Ernawati DS**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Suardita K**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Riawan W**: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by a Hibah Mandat grant from the Universitas Airlangga
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2020 Saskianti T *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Saskianti T, Nugraha AP, Prahasanti C *et al.* **Immunohistochemical analysis of stem cells from human exfoliated deciduous teeth seeded in carbonate apatite scaffold for the alveolar bone defect in Wistar rats (*Rattus novergicus*) [version 2; peer review: 2 approved]** F1000Research 2020, **9**:1164 https://doi.org/10.12688/f1000research.25009.2

First published: 22 Sep 2020, **9**:1164 https://doi.org/10.12688/f1000research.25009.1

REVISED **Amendments from Version 1**

We have reduced the explanation about periodontitis as a causative factor on the bone defect as we used the tooth extraction induce alveolar bone defect model in rats. We have also added information and explanation regarding experimental animal grouping, control group without treatment SHED in CAS, or SHED group only, CAS role in defective alveolar bone and the study limitation.

Any further responses from the reviewers can be found at the end of the article

Introduction

Periodontitis is the second most prevalent oral disease after dental caries[1]. Approximately 743 million people globally suffer from periodontitis, and this figure has increased by 57.3% over the last ten years[2,3]. Globally, the losses that are due to reduced productivity caused by severe periodontitis are estimated to be 53.99 million United States (US) dollars annually[3,4]. Periodontitis is common in Indonesia[5]. A previous study that was conducted by the Health Ministry of Republic of Indonesia in Basic Health Research (*Riset Kesehatan Dasar* or RISKESDA) in 2018 showed that there was a 74.1% prevalence of periodontitis[6]. The rate of periodontitis varies in each country, but together with dental caries, periodontitis is the main reason for tooth loss in adults[1,7]. Low socio-economic conditions in certain populations will increase the prevalence and extent of the tooth loss, which can result in an alveolar bone defect due to the limited access to dental treatment[8]. Tooth extraction can lead to alveolar bone resorption and the destruction of the alveolar bone components[9]. The resorption of the alveolar bone or a reduction in the jawbone dimensions might occur[9–11]. The presence of periodontal disease, irrational or traumatic dental extraction, periapical root fractures or alveolectomies during dental extractions are considered risk factors for or the etiology of an alveolar bone defect[12]. An alveolar bone defect can be problematic for dental rehabilitation due to the placement of dental prosthethics[13]. Osseointegrated of dental implants with sufficient initial stability requires adequate bone quality and quantity. Moreover, it is suggested that socket preservation is performed to enhance the success of the osseointegrated dental implants[14].

In the dental medicine field, the management and rehabilitation of alveolar bone defects has long been viewed as a challange[15]. To overcome alveolar bone defects, dentists must consider bone grafting surgeries for socket preservation to obtain an adequate bone density, volume, quality, and geometry for the implant placement. This will enable osseointegration of the dental implant[15].

There have been many attempts to overcome alveolar bone defects, such as bone grafts, platelet rich fibrin (PRF), mesenchymal stem cells, hematopoetic stem cells, and herbal medicine[16–23]. Bone grafts are still not effective; therefore, alternative tissue engineering approaches are required[24].

The current most promising treatment for an alveolar bone defect is through regenerative medicine, which uses tissue engineering. This tissue engineering involves three components, and is therefore referred to as triad tissue engineering: growth factors, stem cells, and a scaffold[25,26]. Mesenchymal stem cells (MSCs) can differentiate into various cells, such as osteogenic, adipogenic and chondrogenic differentiations[27]. The oral cavity provides a rich source of MSCs. MSCs, such as gingiva mesenchymal stem cells (GMSCs), dental pulp stem cells (DPSCs), and stem cells from human exfoliated deciduous teeth (SHED), can be easily isolated and obtained from the oral cavity tissue using minimally invasive procedures comparted to those needed for bone marrow mesenchymal stem cells (BM-MSCs)[28–31].

SHED is one of the MSCs from the oral cavity that can be used to regenerate damaged tissue, such as an alveolar bone defect[24]. SHED is capable of differentiating and proliferating. Moreover, to optimally facilitate SHED proliferation, cell growth, and differentiation, a biocompatible cell carrier or scaffold is necessary[32]. Carbonate apatite is a biomaterial that is commonly used as a scaffold. Carbonate apatite has been clinically proven to be a good bone scaffold for the regenerative medicine[33]. The study about combination of SHED and CAS ameliorate alveolar bone defect post tooth extraction is still limited. The hypothesis of this study is that the number of osteogenic markers expressing cells, such as OPG, RUNX2, TGF-β, VEGF, ALP, osteocalcin, and ostepontin, would increase in the alveolar bone defects seven days after being implanted with SHED seeded in carbonate apatite scaffold (CAS), with the exception of the receptor activator of NF-κβ ligand (RANKL) expression. Osteocalcin, osteopontin, ALP, RUNX are the osteogenic differentiation markers of SHED. CAS can facilitate the osteogenic differentiation of SHED *in vitro*. Meanwhile, RANKL / OPG ratio are well-known as markers that can be used to predict the success of bone remodeling. Some growth factors are secreted by SHED, such as TGF-β and VEGF, which have an important role in supporting bone formation and controlling the inflammation process[17–24,32–34]. Wistar rats (*Rattus novergicus*) were selected as the animal models because many studies have used this animal to study the effect of medication on the alveolar bone defects[21–24]. Additionally, these rats are not aggressive, and they are easy to handle and observe. This made them suitable animal models to induce the response of the human tissue system. Furthermore, the purpose of this study is to examine the ability of SHED and CAS in the alveolar bone defects using an immunohistochemical analysis.

Methods

Ethical clearance

All experimental procedures involving animals were carried out in accordance with the guidelines from the National Health Institute on the care and use of laboratory animals to ameliorate any suffering for the animals.

Study design

This study was an experimental laboratory design. A post-test-only control group study design was conducted. The formulation that used to calculate the sample size in this study was sample size = $2SD^2(Z^{\alpha/2} + Z\beta)^2/d^2$ where the standard deviation (SD) = 1.1; $Z^{\alpha/2} = Z_{0.05/2} = Z_{0.025} = 1.96$ (from Z table) at type 1 error of 5%; $Z\beta = Z_{0.20 = 0.842}$ (from Z table) at 80% power; d = effect size = 1.94. The number of samples, which was five trial animals in each group. The sample in each group was randomly chosen by giving each trial animal a tag number. Following that, the researcher randomly chose the tag numbers.

SHED Isolation, Culture, and Sub-Culture

The SHED were obtained from deciduous teeth using the following criteria: #83, #73 deciduous tooth, free of caries, no root resorption, and a vital and intact pulp was obtained through tooth extraction from a healthy, 7–10 years-old pediatric patient who underwent orthodontics treatment. The healthy deciduous tooth was extracted from healthy pediatric patients undergoing orthodontics treatment performed at the Dental Hospital, Universitas Airlangga, Surabaya, Indonesia. Patient anonymity was maintained and written informed consent was obtained from the patient's parents. Ethical approval was obtained from the Universitas Airlangga, Faculty of Dental Medicine ethics committee (171/HRECC.FODM/VIII/2017) that covered for both human sampling and the animal procedures.

The dental pulp cavity was opened using drills under aseptic condition. The dental pulp was isolated with a broach then washed three times with phosphate-buffered saline (PBS). Dental pulp tissue was minced into small pieces (≤0.5 mm) in 10-cm culture dishes digested in a solution of 3 mg/mL collagenase type I (no cat. CLS-01, Worthington Biochem, Freehold, NJ) and 4 mg/mL Dispase® II (cat no. 42613-33-2, Sigma Aldrich, USA) for 1 h at 37°C. Dulbecco's Modified Eagle's Medium (cat no. D5030, Merck, US), was utilized to culture the dental pulp from the deciduous tooth. Fetal bovine serum (FBS, catcat no. F2442, Merck, US) with 20% concentration, five milimeter L-glutamine (cat no. 5030081, Gibco Invitrogen®, 25, USA), 100 U/ ml penicillin-G, 100 ug/ml streptomycin, and 100 ug/ml kanamycin (cat no. 15140163, Gibco Invitrogen®, 25, USA) was added[34].

Every four days, the medium was changed to eliminate the unattached cell on the culture plate and the cells were maintained up to four passages. Phosphate Buffer Saline was used to wash the cells to eliminate debris. Trypsin-EDTA 0.05% was applied to detach the cells and transfer them onto a bigger culture plate. After the cells reached 70–80% confluence was obtained, the SHED cells in the 4 passaged were then prepared for the next step of the study[24,32,34].

The alveolar bone defect in animal models

Ten three-month-old healthy male Wistar rats *(R. novergicus)* that weighed between 150–250 grams (g) were used as animal models and were obtained from the Research Center of Faculty of Dental Medicine, Universitas Airlangga (UNAIR) Surabaya, Indonesia. Ten wistar rats were assigned into two groups respectively; CAS group and CAS+SHED group.

All experimental procedures involving animals were carried out in keeping with guidelines from the National Institutes of Health Guide for the Care and Use of Laboratory Animals to ameliorate any suffering of animals[35]. The animal models were acclimatized for a week at a temperature of 21–23 °C with controlled humidity (50 ± 5%) in a 12-hour artificial light cycle (8 am to 8 pm) to help them to adapt to the same conditions, as they had various origins. All the rats were located individually in polycarbonate cages (0.90 × 0.60 × 0.60 m). Furthermore, every animal model was fed with standard pellet, and water was provided *ad libitum* with the husk replaced every three days. All animal models were routinely inspected and observed regarding their food consumption and fecal characteristics[20].

Rodent anesthesia of 0.1 mL/10 grams body weight (BW) (160095, Kepro™, Netherlands), and xylazine (160096, Xyla™, Netherlands) (ketamine dose 35 mg/kg body weight and xylazine five mg/kg body weight) was administered intramuscularly on the gluteus muscle to ameliorate the pain during the procedure of inducing the alveolar bone defects on the animal models. Sterile needle holder clamps were used to extract the anterior teeth of the mandibular to induce the alveolar bone defects in the animal models[36].

The Transplantation of Stem Cells from Human Exfoliated Deciduous Teeth Seeded in Carbonate Apatite Scaffold

After the alveolar bone defect was induced, the transplantation of the SHED seeded in CAS or CAS only was performed in the afflicted area. Before being placed in a 24-well tissue culture plate and prepared for the experimental group, a 20 ml suspension of SHED at passage four to five with a density of 10^6 cells was seeded into CAS (no cat AKD 20602410125, GAMACHA, Swayasa Prakarsa Company, Indonesia). The dose was determined based on the evidence from a previous *in vivo* study, which was 10^6 cells per sample[34]. To perform the interrupted suture to fix the wound after transplantation, a 5.0 suture monofilament was used[24,32,34].

Seven days post transplantation, all the animal models were terminated using an overdosed rodent anesthesia with an intravenous injection of 100 mg/kg BW (Pentobarbital, 1507002, Pubchem, USA). We used this euthanasia method to ameliorate animal suffering that arises from the termination process. After the termination of animal study, we collected the afflicted alveolar bone samples for further histological analysis. The animal model's head was cut from the back by sterile sharp surgical scissors (metzenbaum scissors fine tips, no cat. 3565, Medesy, Maniago, Italy) and tweezer (Tweezer de bakey mini, no cat. 1007/10-TO, Medesy, Maniago, Italy), exposing the anterior of the mandible allowing the afflicted alveolar bone sample to be obtained. Before sample collection, all the animals were observed for any general toxicity probability, including edema or death, and measured had their body weight (using a digital scale, ZB22-P, Zieis®, USA). All these measurements were done by a single blinded observer. The afflicted tissue was then extracted and immersed in 10% neutral buffer formalin for fixation.

Tissue Processing, Embedding and Sectioning

The sample was decalcified and immersed in 10% EDTA (cat no. 17892, Ajax Finechem, Thermo Fisher Scientific; Taren Point, Australia). Following that, the samples were underwent tissue processing overnight (Leica TP1020, USA), prior to embedding in molten paraffin wax (HistoCore Arcadia H - Heated Paraffin Embedding Station, Leica, USA). Sections were cut at 5 µm rotary microtome (RM2235, Leica, USA). Paraffin ribbons were flattened in a water bath at 40°C and collected onto polysine microscope slides (Thermo Scientific) prior to drying at 60°C for 16 hr (Sakura Heater, Tokyo, Japan)[37].

Immunohistochemistry staining

Immunohistochemistry staining was conducted using a 3.3'-diaminobenzidine stain kit (DAB) (cat noD7304-1SET, Sigma Aldrich, US). Antibody monoclonal (AbMo) of RANKL 1:500 dilution (cat. no sc-377079), osteoprotegrin (OPG) 1:500 dilution (cat. no sc-390518), runt-related transcription factor 2 (RUNX2) 1:500 dilution (cat. no sc-390351), transforming growth factor-β (TGF-β) 1:500 dilution (cat. no sc-130348), vascular endothelial growth factor (VEGF) 1:500 dilution (cat. no sc-7269), alkaline phosphatase (ALP) 1:500 dilution (cat. no sc-271431), osteocalcin (cat. no sc-365797)) 1:500 dilution, and ostepontin (cat. no sc-21742) 1:500 dilution were used in this study (Santa Cruz Biotechnology™, US). The observation and examination of the number of the RANKL, OPG, RUNX2, TGF-β, VEGF, ALP, osteocalcin, and ostepontin expressions in the periodontal tissue were performed manually by two observers in five perspective fields of view by utilizing Nikon H600L light microscope (Japan) at 400x magnification. We also provide 200x and 1000x magnification of each marker for context (Nikon, Japan)[37].

Statistical analysis

The Statistical Package for Social Science (SPSS) 20.0 version (IBM corporation, Illinois, Chicago, United State) software was used in this study to analyze the data. To compare the significant differences between the groups in the RANKL, OPG, RUNX2, TGF-β, VEGF, ALP, osteocalcin, and ostepontin expressions, a t-test was employed ($p<0.01$). The OPG and RANKL expressions' association was examined using Pearson's correlation test ($p<0.05$).

Results

The transplantation of SHED seeded in CAS or CAS only at selected doses did not lead to any general toxicity, edema, death or changes in body weight of the rats (see underlying data[38]). The expressions of OPG, RUNX2, TGF- β, VEGF, ALP, osteocalcin, and osteopontin in SHED seeded in CAS were greater than in the CAS only group. In comparison, the RANKL expression was lower in SHED seeded in CAS compared to CAS only (see Figure 1–Figure 4[39–46]). There was a significant increase in OPG, RUNX2, TGF-β VEGF, ALP, osteocalcin, and osteopontin expressions and decreased RANKL expression in SHED seeded in CAS compared to CAS only ($p<0.01$). There was a significant strong reverse correlation between the OPG and RANKL expressions ($p<0.01$) (Table 1).

Discussion

Severe alveolar defect has become a problem for both the patients and clinicians, especially regarding dental implant placement and ossteointegration[15].

This experimental study confirms the hypothesis that the transplantation of SHED seeded in CAS could increase the number of osteogenic markers expressing cells, such as OPG, RUNX2, TGF-β, VEGF, ALP, osteocalcin, and ostepontin, but not the RANKL expression in the bone defects after seven days in comparison to the CAS group[17–20,22,32].

This result supports the theory that SHED possess functions that can enhance OPG to bind to RANKL, which results in the inhibited osteoclastogenesis[34]. There is a strong reverse significant correlation between OPG and RANKL expressions in this study. The SHED with the scaffold increases the OPG expression meanwhile, decreases the RANKL expression, which is supported by the previous study by Prahasanti *et al.*[34]

CAS plays an important role in supporting SHED proliferation and differentiation[24,32]. RUNX2, ALP, osteocalcin, and osteopontin are osteogenic differentiation markers of MSCs. These markers are essential and important for the analysis of osteoblastogenesis and bone regeneration[17,18,20]. ALP expression increases due to the signaling bone morphogenic protein (BMP), RUNX2, osterix system, and Wnt cascade interacting with each other. The increased expression of RUNX can enhance ALP expression[17,18]. Several growth factors also stimulate the activation of the MSCs' osteogenic differentiation, such as VEGF and TGF- β. TGF-β significantly increases the expression of the early-phase osteogenic differentiation marker genes[47]. VEGF is associated with all the bone formation steps, including mesenchymal condensation[48]. VEGF has a direct influence on the MSC osteogenic differentiation through the regulation of osteoprogenitors using the angiocrine function. VEGF recruits immune cells to the osteogenic niche[49].

The osteogenic microenvironment in defective alveolar bone can induce SHED to differentiate into bone cells, especially osteoblast[24,32,34]. The activation of the osteorix and RUNX2 systems can stimulate the expression of osteocalcin and osteopontin[17]. OSC is a secreted protein that is dependent on Vitamin K, a macromolecule with a role in bone mineralization[18]. Osteopontin plays a pivotal role in bone remodeling, regulating osteoclastogenesis, osteoclast activity, and differentiation. Osteopontin maintains the bone mineral matrix inorganic components of bone, such as hydroxyapatite, Ca(PO4)(OH)2. Osteopontin, which is expressed in osteoblasts, is responsible for bone remodeling in bone homeostasis[20]. Both osteocalcin and osteopontin are important for bone maturation because they are major non-collagenous proteins that are involved in bone matrix organization and deposition.

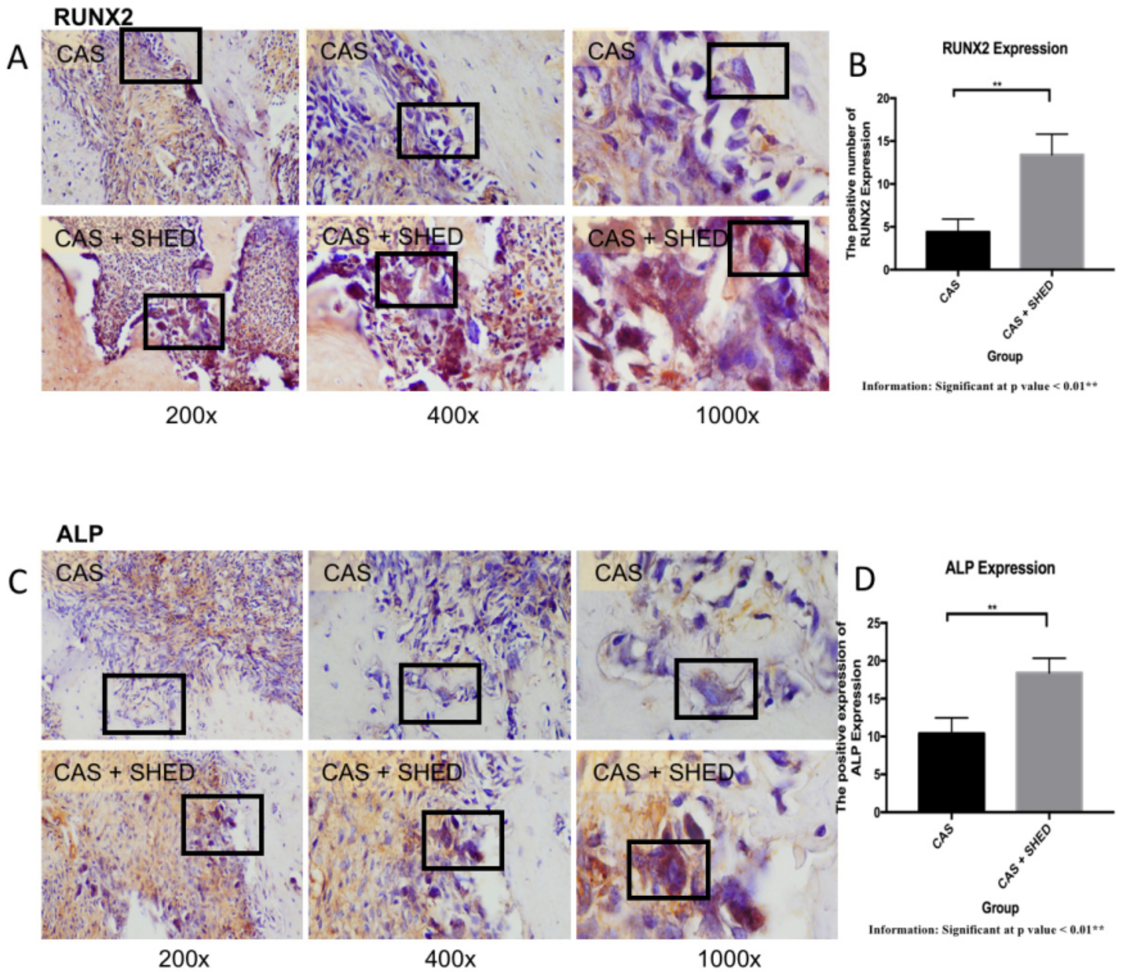

Figure 1. Histological sections of the Wistar rats' (*R. Novergicus*) afflicted periodontal tissues. Immunohistochemistry with antibody monoclonal (AbMo) and DAB were performed to examine the (**A**) RANKL and (**B**) OPG expressions. The positive cells were stained brown (black box) with a 200x, 400x, and 1000x magnification using a light microscope. The number of osteoblasts expressing (**C**) RANKL and (**D**) OPG in the alveolar bone of the rats.

Osteocalcin and osteopontin are produced during bone formation[49]. Both of them control—either directly and/or indirectly–the mass, mineral size, and orientation[50–52]. Both proteins also play structural roles in the bone and determine the bone's propensity to fracture[53]. This is in accordance with our findings, as it states that there is a significant enhancement of the OPG, RUNX2, TGF- β, VEGF, ALP, osteocalcin, and osteopontin expressions, and the decreased RANKL expression is more significant in Group II than Group I. Bone regeneration is a complex process that requires highly orchestrated interactions between different cells and signals to form the new mineralized tissue[54]. MSCs have the ability to differentiate into osteoprogenitors and osteoblasts, as well as to form the calcified bone matrix[55].

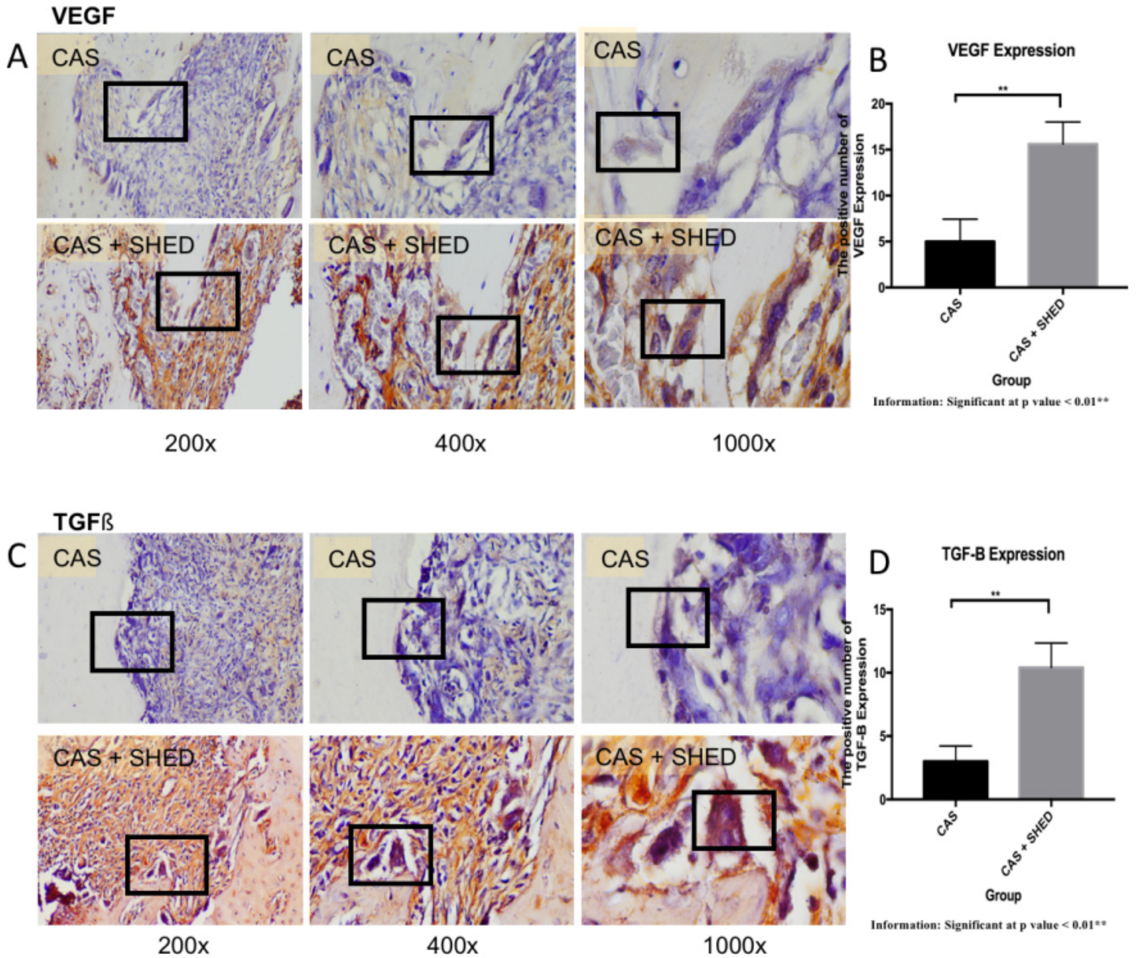

Figure 2. Histological sections of the Wistar rats' (*R. Novergicus*) afflicted periodontal tissues. Immunohistochemistry with antibody monoclonal (AbMo) and DAB were performed to examine the (**A**) VEGF and (**B**) TGF-β expressions. The positive cells were stained brown (black box) with 200x, 400x, and 1000x magnification using the light microscope. The number of osteoblasts expressing (**C**) VEGF and (**D**) TGF- β in the alveolar bone of the rats.

SHED have the potential to play a significant role in tissue engineering and regenerative medicine. A previous study by Nakajima *et al.* declared that SHED, in comparison to the hDPSCs or hBMSCs group, produce the largest osteoid and collagen fibers. Furthermore, SHED transplantation possess a potential and sufficient ability for bone regeneration to repair the bone defect[56,57].

The limitations of this study were that the observation and evaluation were performed seven days post transplantation of SHED seeded in CAS on the animal model, and only an immunohistochemical examination was performed. Further studies will be necessary to evaluate the changes in the alveolar bone and periodontal tissue post transplantation of SHED seeded in CAS in the alveolar bone defect animal models.

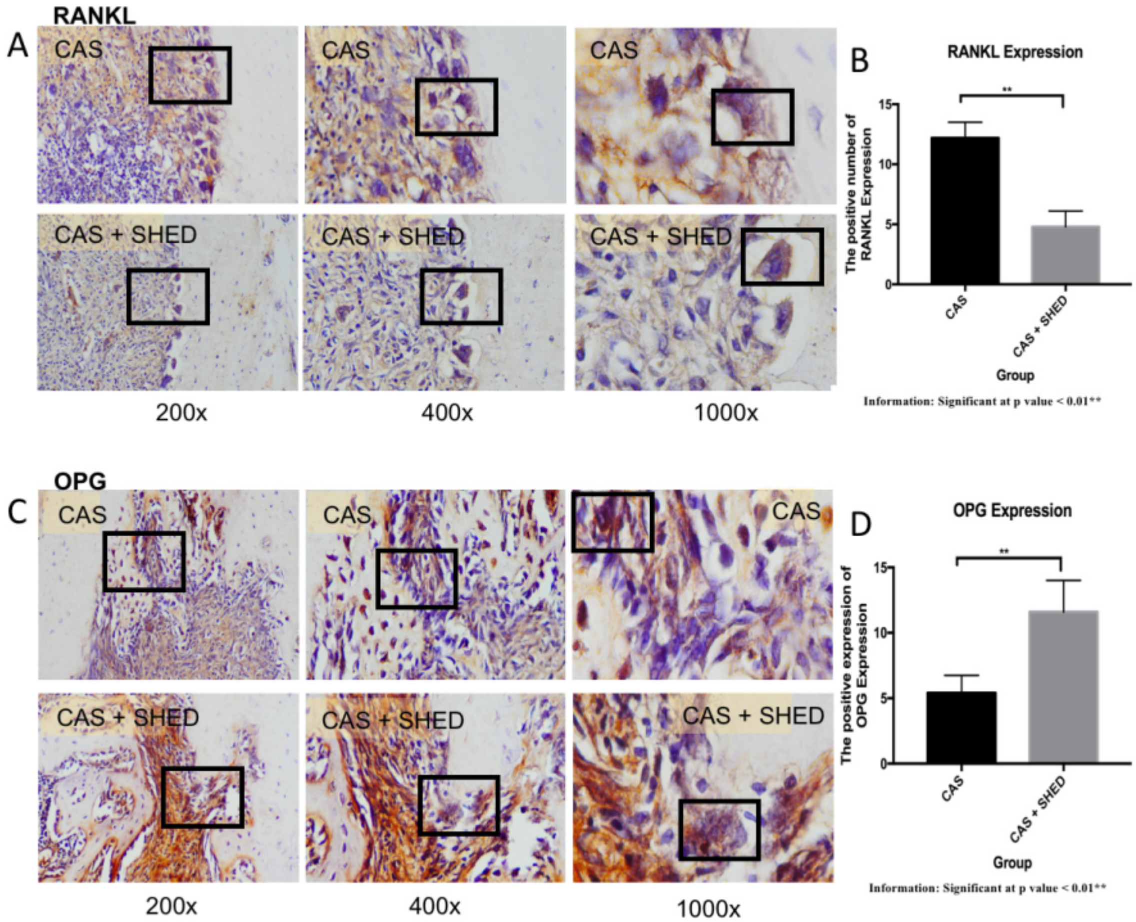

Figure 3. Histological sections of the Wistar rat's (*R. Novergicus*) afflicted periodontal tissues. Immunohistochemistry with antibody monoclonal (AbMo) and DAB were performed to examine the (**A**) RUNX2 and (**B**) ALP expressions. The positive cells were stained brown (black box) with 200x, 400x, and 1000x magnification using the light microscope. The number of osteoblasts expressing (**C**) RUNX2 and (**D**) ALP in the alveolar bone of the rats.

With a longer observation time, further studies using methods, such as qRT-PCR and/or the western blot analysis, could be conducted to estimate the expression of bone molecular markers. Future studies are also required to confirm the effective dose of the used biomaterials when it is ready to be applied in the clinical study of humans.

Conclusion

In conclusion, the expression of OPG, RUNX2, TGF-β, VEGF, ALP, osteocalcin, and ostepontin increases significantly with treatment with SHED seeded in CAS. Moreover, the RANKL expression in the alveolar bone defect did not increase in SHED seeded in CAS as documented immunohistochemically.

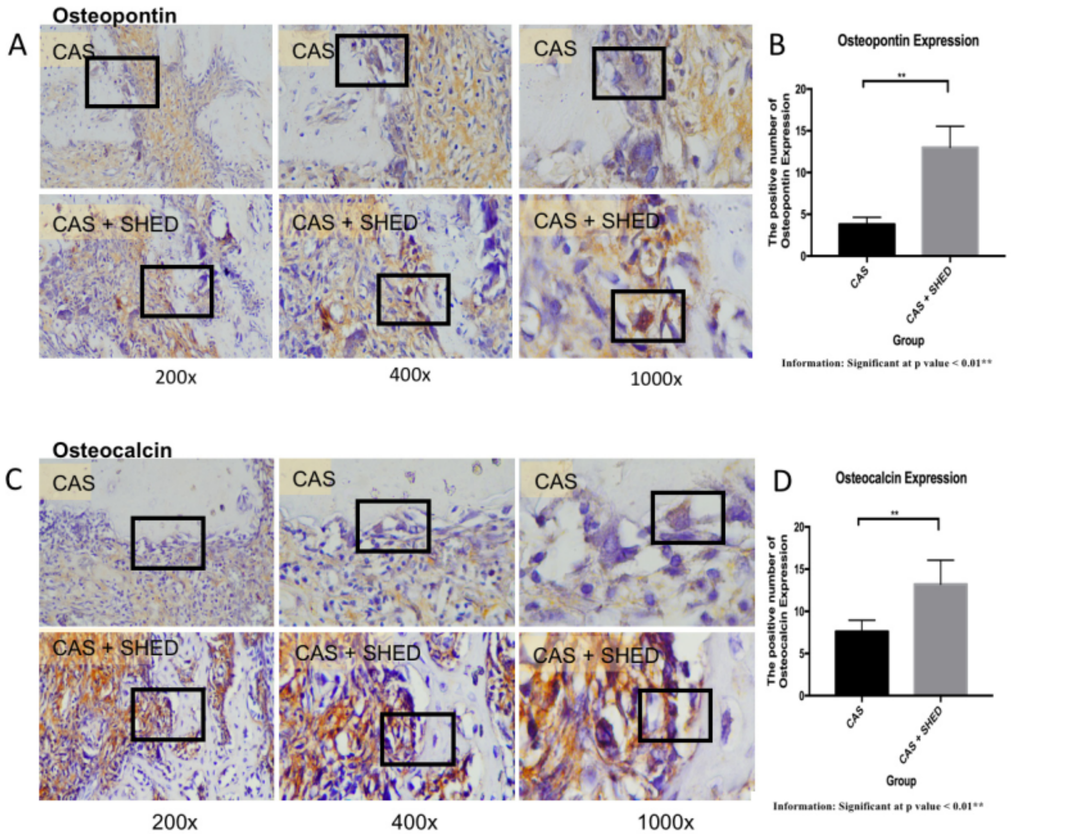

Figure 4. Histological sections of the Wistar rat's (*R. Novergicus*) afflicted periodontal tissues. Immunohistochemistry with antibody monoclonal (AbMo) and DAB were performed to examine the (**A**) osteocalcin and (**B**) osteopontin expressions. Positive cells were stained brown (black box) in 200x, 400x, 1000x magnification by using the light microscope. The number of osteoblasts expressing (**C**) osteocalcin and (**D**) osteopontin in the alveolar bone of the rats.

Table 1. The mean ± standard deviation, the result of the normality test and the t-test of each marker between the groups (n=5).

Group	Molecular Marker							
	Mean ± Standard Deviation							
	OPG	RANKL	TGF- β	VEGF	RUNX2	ALP	OSC	OSP
CHA	5.4 ± 0.6	4.8 ± 0.5831	3 ± 0.5477	5 ± 1.095	4.4 ± 0.6782	10.4 ± 2.073	7.64 ± 0.6	3.8 ± 0.3742
*Normality	0.21	0.421	0.146	0.146	0.86	0.23	0.201	0.314
CHA+SHED	11.6 ± 1.077	12.2 ± 0.5831	10.4 ± 0.8718	15.6 ± 1.077	13.4 ± 1.077	18.4 ± 1.949	13.2 ± 1.281	13 ± 1.14
*Normality	0.787	0.21	0.758	0.787	0.787	0.758	0.823	0.207
**Sig	0.001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0042	0.0001
Pearson Correlation	-0.0801							
**Sig. Correlation	0.005							

Information: *significant at p value > 0.05; **significant at p value < 0.01. RANKL - receptor activator of NF-κβ ligand, OPG - osteoprotegrin, TGF- β - transforming growth factor-β, VEGF - vascular endothelial growth factor, RUNX2 - runt-related, transcription factor 2, ALP - alkaline phosphatase, OSC - osteocalcin, OSP osteopontin, SHED- stem cells from human exfoliated deciduous teeth, CHA - carbonate hydroxyapatite

Data availability

Underlying data

Figshare: RANKL. https://doi.org/10.6084/m9.figshare.12609986.v1[39]

This project contains the following underlying data:

- CAS RANKL 200x.jpg (Expression of RANKL at 200x magnification in the CAS group)
- CAS RANKL 400x.jpg (Expression of RANKL at 400x magnification in the CAS group)
- CAS RANKL 1000x.jpg (Expression of RANKL at 1000x magnification in the CAS group)
- CAS SHED RANKL 200x.jpg (Expression of RANKL at 200x magnification in the CAS SHED group)
- CAS SHED RANKL 400x.jpg (Expression of RANKL at 400x magnification in the CAS SHED group)
- CAS SHED RANKL 1000x.jpg (Expression of RANKL at 1000x magnification in the CAS SHED group)

Figshare: OPG. https://doi.org/10.6084/m9.figshare.12609983.v1[40]

This project contains the following underlying data:

- CAS OPG 200x.jpg (Expression of OPG at 200x magnification in the CAS group)
- CAS OPG 400x.jpg (Expression of OPG at 400x magnification in the CAS group)
- CAS OPG 1000x.jpg (Expression of OPG at 1000x magnification in the CAS group)
- CAS SHED OPG 200x.jpg (Expression of OPG at 200x magnification in the CAS SHED group)
- CAS SHED OPG 400x.jpg (Expression of OPG at 400x magnification in the CAS SHED group)
- CAS SHED OPG 1000x.jpg (Expression of OPG at 1000x magnification in the CAS SHED group)

Figshare: RUNX2. https://doi.org/10.6084/m9.figshare.12610478.v1[41]

This project contains the following underlying data:

- CAS RUNX2 200x.jpg (Expression of RUNX2 at 200x magnification in the CAS group)
- CAS RUNX2 400x.jpg (Expression of RUNX2 at 400x magnification in the CAS group)
- CAS RUNX2 1000x.jpg (Expression of RUNX2 at 1000x magnification in the CAS group)
- CAS SHED RUNX2 200x.jpg (Expression of RUNX2 at 200x magnification in the CAS SHED group)
- CAS SHED RUNX2 400x.jpg (Expression of RUNX2 at 400x magnification in the CAS SHED group)
- CAS SHED RUNX2 1000x.jpg (Expression of RUNX2 at 1000x magnification in the CAS SHED group)

Figshare: TGF-Beta. https://doi.org/10.6084/m9.figshare.12610487.v1[42]

This project contains the following underlying data:

- CAS TGF-β 200x.jpg (Expression of TGF-β at 200x magnification in the CAS group)
- CAS TGF-β 400x.jpg (Expression of TGF-β at 400x magnification in the CAS group)
- CAS TGF-β 1000x.jpg (Expression of TGF-β at 1000x magnification in the CAS group)
- CAS SHED TGF-β 200x.jpg (Expression of TGF-β at 200x magnification in the CAS SHED group)
- CAS SHED TGF-β 400x.jpg (Expression of TGF-β at 400x magnification in the CAS SHED group)
- CAS SHED TGF-β 1000x.jpg (Expression of TGF-β at 1000x magnification in the CAS SHED group)

Figshare: VEGF. https://doi.org/10.6084/m9.figshare.12610484.v1[43]

This project contains the following underlying data:

- CAS VEGF 200x.jpg (Expression of VEGF at 200x magnification in the CAS group)
- CAS VEGF 400x.jpg (Expression of VEGF at 400x magnification in the CAS group)
- CAS VEGF 1000x.jpg (Expression of VEGF at 1000x magnification in the CAS group)
- CAS SHED VEGF 200x.jpg (Expression of VEGF at 200x magnification in the CAS SHED group)
- CAS SHED VEGF 400x.jpg (Expression of VEGF at 400x magnification in the CAS SHED group)
- CAS SHED VEGF 1000x.jpg (Expression of VEGF at 1000x magnification in the CAS SHED group)

Figshare: ALP. https://doi.org/10.6084/m9.figshare.12610493.v1[44]

This project contains the following underlying data:

- CAS ALP 200x.jpg (Expression of ALP at 200x magnification in the CAS group)
- CAS ALP 400x.jpg (Expression of ALP at 400x magnification in the CAS group)
- CAS ALP 1000x.jpg (Expression of ALP at 1000x magnification in the CAS group)

- CAS SHED ALP 200x.jpg (Expression of ALP at 200x magnification in the CAS SHED group)
- CAS SHED ALP 400x.jpg (Expression of ALP at 400x magnification in the CAS SHED group)
- CAS SHED ALP1000x.jpg (Expression of ALP at 1000x magnification in the CAS SHED group)

Figshare: Osteocalcin. https://doi.org/10.6084/m9.figshare.12610481.v1[45]

This project contains the following underlying data:
- CAS osteocalcin 200x.jpg (Expression of osteocalcin at 200x magnification in the CAS group)
- CAS osteocalcin 400x.jpg (Expression of osteocalcin at 400x magnification in the CAS group)
- CAS osteocalcin 1000x.jpg (Expression of osteocalcin at 1000x magnification in the CAS group)
- CAS SHED osteocalcin 200x.jpg (Expression of osteocalcin at 200x magnification in the CAS SHED group)
- CAS SHED osteocalcin 400x.jpg (Expression of osteocalcin at 400x magnification in the CAS SHED group)
- CAS SHED osteocalcin 1000x.jpg (Expression of osteocalcin at 1000x magnification in the CAS SHED group)

Figshare: Ostepontin. https://doi.org/10.6084/m9.figshare.12610490.v1[46]

This project contains the following underlying data:
- CAS osteopontin 200x.jpg (Expression of osteopontin at 200x magnification in the CAS group)
- CAS osteopontin 400x.jpg (Expression of osteopontin at 400x magnification in the CAS group)
- CAS osteopontin 1000x.jpg (Expression of osteopontin at 1000x magnification in the CAS group)
- CAS SHED osteopontin 200x.jpg (Expression of osteopontin at 200x magnification in the CAS SHED group)
- CAS SHED osteopontin 400x.jpg (Expression of osteopontin at 400x magnification in the CAS SHED group)
- CAS SHED osteopontin 1000x.jpg (Expression of osteopontin at 1000x magnification in the CAS SHED group)

Figshare: Raw Data Bone Molecular Markers. https://doi.org/10.6084/m9.figshare.12610499.v1[58]

This project contains the following underlying data:
- Raw Data Molecular Marker.xlsx (The raw data of molecular markers examined by means of IHC analysis)

Figshare: Animal Body Weight. https://doi.org/10.6084/m9.figshare.12610502.v1[38]

This project contains the following underlying data:
- Animal Body Weight.xlsx (Animal Body Weight, pre and post test)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

References

1. Edman K, Öhrn K, NordstrÖm B, *et al.*: **Trends over 30 years in the prevalence and severity of alveolar bone loss and the influence of smoking and socio-economic factors-based on epidemiological surveys in Sweden 1983 – 2013.** *Int J Dent Hyg.* 2015; **13**(4): 283–291.
PubMed Abstract | Publisher Full Text
2. Frencken JE, Sharma P, Stenhouse L, *et al.*: **Global epidemiology of dental caries and severe periodontitis–a comprehensive review.** *J Clin Periodontol.* 2017; **44 Suppl 18**: S94–S105.
PubMed Abstract | Publisher Full Text
3. Tonetti MS, Jepsen S, Corgel JO: **Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action.** *J Clin Periodontol.* 2017; **44**(5): 456–462.
PubMed Abstract | Publisher Full Text
4. Listl S, Galloway JS, Mossey PA, *et al.*: **Global economic impact of dental diseases.** *J Dent Res.* 2015; **94**(10): 1355–61.
PubMed Abstract | Publisher Full Text
5. Ministry of Health Republic of Indonesia: **Annual Report of Basic Health Research.** 2018; 207.
Reference Source
6. Wijaksana IKE: **Periodontal chart and periodontal risk assessment as education and evaluation patient with periodontal disease.** *Jurnal Kesehatan Gigi.* 2019; **6**: 19–25.
Reference Source
7. Shaddox LM, Walker CB: **Treating chronic periodontitis: Current status, challenges, and future directions.** *Clin Cosmet Investig Dent.* 2010; **2**: 79–91.
PubMed Abstract | Free Full Text
8. Susin C, Oppermann RV, Haugejorden O, *et al.*: **Tooth loss and associated risk indicators in an adult urban population from south Brazil.** *Acta Odontol Scand.* 2005; **63**(2): 85–93.
PubMed Abstract | Publisher Full Text
9. Lande R, Kepel BJ, Siagian KV: **Profile of risk factor and complication of tooth extraction at RSGM PSPDG-FK Unsrat.** *Jurnal E-Gigi (Eg).* 2015; **3**(2): 1–6.
10. Hamzah Z, Kartikasari N: **Irrational tooth extraction increasing structural and functional bone resorption.** *Stomatognatic (J. K. G Unej).* 2015; **12**(2): 61–6.
11. Schropp L, Wenzel A, Kostopoulos L, *et al.*: **Bone healing and soft tissue contour changes following single-tooth extraction: A clinical and radiographic 12-month prospective study.** *Int J Periodontics Restorative Dent.* 2003; **23**(4): 313–323.
PubMed Abstract
12. Coradazzi LF, Garcia IR Jr, Manfrin TM: **Evaluation of autogenous bone grafts, particulate or collected during osteotomy with implant burs: Histologic and histomorphometric analysis in rabbits.** *Int J Oral Maxillofac Implants.* 2007; **22**(2): 201–207.
PubMed Abstract
13. de Freitas R1, Kaizer OB, Hamata MM, *et al.*: **Prosthetic rehabilitation of a bone defect with a teeth-implant supported, removable partial denture.** *Implant Dent.* 2006; **15**(3): 241–247.
PubMed Abstract | Publisher Full Text
14. de Carvalho PS, de Carvalho MC, Ponzoni D: **Reconstruction of alveolar bone defect with autogenous bone particles and osseointegrated implants: Histologic analysis and 10 years monitoring.** *Ann Maxillofac Surg.* 2015; **5**(1): 135–139.
PubMed Abstract | Publisher Full Text | Free Full Text

15. Duan Y, Chandran R, Cherry D: **Influence of alveolar bone defects on the stress distribution in quad zygomatic implant-supported maxillary prosthesis.** *Int J Oral Maxillofac Implants.* 2018; **33**(3): 693–700.
PubMed Abstract | Publisher Full Text

16. Ari MDA, Yuliati A, Rahayu RP, *et al.*: **The differences scaffold composition in pore size and hydrophobicity properties as bone regeneration biomaterial.** *J Inter Dent Med Res.* 2018; **11**(1): 318–322.
Reference Source

17. Nugraha AP, Narmada IB, Ernawati DS, *et al.*: **Osteogenic potential of gingival stromal progenitor cells cultured in platelet rich fibrin is predicted by core-binding factor subunit-α1/Sox9 expression ratio (*in vitro*) [version 1; peer review: 1 approved, 2 approved with reservations]** *F1000Res.* 2018; **7**: 1134.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Nugraha AP, Narmada IB, Ernawati DS, *et al.*: **Bone alkaline phosphatase and osteocalcin expression of rat's gingival mesenchymal stem cells cultured in platelet-rich fibrin for bone remodeling (*in vitro* study).** *Eur J Dent.* 2018; **12**(4): 566–73.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Nugraha AP, Narmada IB, Ernawati DS, *et al.*: **Somatic cells acceleration by platelet rich fibrin.** *Indian Vet J.* 2019; **94**(04): 30–34.
Reference Source

20. Nugraha AP, Narmada IB, Ernawati DS, *et al.*: ***In vitro* bone sialoprotein-I expression in combined gingival stromal progenitor cells and platelet rich fibrin during osteogenic differentiation.** *Trop J Pharm Res.* 2018; **17**(12): 2341–2345.
Publisher Full Text

21. Rahmawati D, Roestamadji RI, Yuliati A, *et al.*: **Osteogenic ability of combined hematopoetic stem cell, hydroxyapatite graft and platelet rich fibrin on rats (*Rattus novergicus*).** *J Krishna Instit Med Sci Univ.* 2017; **6**: 88–95.
Reference Source

22. Kresnoadi U, Rahmania PN, Caesar HU, *et al.*: **The role of the combination of *Moringa oleifera* leaf extract and demineralized freeze-dried bovine bone xenograft (xenograft) as tooth extraction socket preservation materials on osteocalcin and transforming growth factor-beta 1 expressions in alveolar bone of *Cavia cobaya*.** *J Indian Prosthodont Soc.* 2019; **19**(2): 120–125.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Kresnoadi U, Ariani MD, Djulaeha E, *et al.*: **The potential of mangosteen (*Garcinia mangostana*) peel extract, combined with demineralized freeze-dried bovine bone xenograft, to reduce ridge resorption and alveolar bone regeneration in preserving the tooth extraction socket.** *J Indian Prosthodont Soc.* 2017; **17**(3): 282–288.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Saskianti T, Yualiartanti W, Ernawati DS, *et al.*: **BMP4 expression following stem cells from human exfoliated deciduous and carbonate apatite transplantation on Rattus norvegicus.** *J Khrishna Institute Med Sci Uni.* 2018; **7**(2): 56–61.
Reference Source

25. Egusa H, Sonoyama W, Nishimura M, *et al.*: **Stem cells in dentistry--part I: Stem cell sources.** *J Prosthodont Res.* 2012; **56**(3): 151–165.
PubMed Abstract | Publisher Full Text

26. Egusa H, Sonoyama W, Nishimura M, *et al.*: **Stem cells in dentistry--Part II: Clinical applications.** *J Prosthodont Res.* 2012; **56**(4): 229–248.
PubMed Abstract | Publisher Full Text

27. Rantam FA, Ferdiansyah P: **Stem cell mesenchymal, hematopoetic stem cells and application model.** 2nd Ed. Surabaya: Airlangga University Press. 2014; **2**: 38.

28. Nugraha AP, Narmada IB, Ernawati DS, *et al.*: **The aggrecan expression post platelet rich fibrin administration in gingival medicinal signaling cells in Wistar rats (Rattus novergicus) during the early osteogenic differentiation (*in vitro*).** *Kafkas Univ Vet Fak Derg.* 2019; **25**(3): 421–425.
Reference Source

29. Nugraha AP, Narmada IB, Ernawati DS, *et al.*: **Gingival mesenchymal stem cells from Wistar rat's gingiva (Rattus novergicus) - Isolation and characterization (*in vitro* study).** *J Int Med Res.* 2018; **11**(2): 694–699.
Reference Source

30. Suciadi SP, Nugraha AP, Ernawati DS, *et al.*: **The efficacy of human dental pulp stem cells in regenerating submandibular gland defects in diabetic Wistar rats (*Rattus novergicus*).** *Research J Pharm and Tech.* 2019; **12**(4): 1573–1579.
Publisher Full Text

31. Miran S, Mitsiadis TA, Pagella P: **Innovative dental stem cell-based research approaches: The future of dentistry.** *Stem Cells Int.* 2016; **2016**: 7231038.
PubMed Abstract | Publisher Full Text | Free Full Text

32. Saskianti T, Ramadhani R, Budipramana ES, *et al.*: **Potential proliferation of stem cell from human exfoliated deciduous teeth (SHED) in carbonate apatite and hydroxyapatite scaffold.** *J Int Dent Med Res.* 2017; **10**(2): 350–353.
Reference Source

33. Alhasyimi AA, Pudyani PP, Asmara W, *et al.*: **Enhancement of post-orthodontic tooth stability by carbonated hydroxyapatite-incorporated advanced platelet-rich fibrin in rabbits.** *Orthod Craniofac Res.* 2018; **21**(2): 112–118.
PubMed Abstract | Publisher Full Text

34. Prahasanti C, Subrata LH, Saskianti T, *et al.*: **Combined hydroxyapatite scaffold and stem cell from human exfoliated deciduous teeth modulating alveolar bone regeneration via regulating receptor activator of nuclear factor-Kb and osteoprotegerin system.** *Iran J Med Sci.* 2019; **44**(5): 415–421.
PubMed Abstract | Publisher Full Text | Free Full Text

35. Tan B: **Guidelines on the care and use of animals for scientific purposes.** *National Advisory Committee for Laboratory Animal Research.* 2004.
Reference Source

36. Khoswanto C: **A new technique for research on wound healing through extraction of mandibular lower incisors in Wistar rats.** *Euro Dent J.* 2019; **13**(2): 235–237.
PubMed Abstract | Publisher Full Text | Free Full Text

37. Savi FM, Brierly GI, Baldwin J, *et al.*: **Comparison of Different Decalcification Methods Using Rat Mandibles as a Model.** *J Histochem Cytochem.* 2017; **65**(12): 705–722.
PubMed Abstract | Publisher Full Text | Free Full Text

38. Nugraha AP: **Animal Body Weight.** *figshare.* Dataset. 2020.
http://www.doi.org/10.6084/m9.figshare.12610502.v1

39. Nugraha AP: **RANKL.** *figshare.* Figure. 2020.
http://www.doi.org/10.6084/m9.figshare.12609986.v1

40. Nugraha AP: **OPG.** *figshare.* Figure. 2020.
http://www.doi.org/10.6084/m9.figshare.12609983.v1

41. Nugraha AP: **RUNX2.** *figshare.* Figure. 2020.
http://www.doi.org/10.6084/m9.figshare.12610478.v1

42. Nugraha AP: **TGF-Beta.** *figshare.* Figure. 2020.
http://www.doi.org/10.6084/m9.figshare.12610487.v1

43. Nugraha AP: **VEGF.** *figshare.* Figure. 2020.
http://www.doi.org/10.6084/m9.figshare.12610484.v1

44. Nugraha AP: **ALP.** *figshare.* Figure. 2020.
http://www.doi.org/10.6084/m9.figshare.12610493.v1

45. Nugraha AP: **Osteocalcin.** *figshare.* Figure. 2020.
http://www.doi.org/10.6084/m9.figshare.12610481.v1

46. Nugraha AP: **Osteopontin.** *figshare.* Figure. 2020.
http://www.doi.org/10.6084/m9.figshare.12610490.v1

47. Igarashi Y, Chosa N, Sawada S, *et al.*: **VEGF-C and TGF-β reciprocally regulate mesenchymal stem cell commitment to differentiation into lymphatic endothelial or osteoblastic phenotypes.** *Int J Mol Med.* 2016; **37**(4): 1005–13.
PubMed Abstract | Publisher Full Text | Free Full Text

48. Ge Q, Zhang H, Hou J: **VEGF secreted by mesenchymal stem cells mediates the differentiation of endothelial progenitor cells into endothelial cells via paracrine mechanisms.** *Mol Med Rep.* 2018; **17**(1): 1667–1675.
PubMed Abstract | Publisher Full Text | Free Full Text

49. Bailey S, Karsenty G, Gundberg C, *et al.*: **Osteocalcin and osteopontin influence bone morphology and mechanical properties.** *Ann N Y Acad Sci.* 2017; **1409**(1): 79–84.
PubMed Abstract | Publisher Full Text | Free Full Text

50. Stein GS, Lian JB, Owen TA: **Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation.** *FASEB J.* 1990; **4**(13): 3111–23.
PubMed Abstract | Publisher Full Text

51. Boskey A, Gadaleta S, Gundberg C, *et al.*: **Fourier transform infrared microspectroscopic analysis of bones of osteocalcin-deficient mice provides insight into the function of osteocalcin.** *Bone.* 1998; **23**(3): 187–96.
PubMed Abstract | Publisher Full Text

52. Thurner PJ, Chen CG, Ionova-Martin S, *et al.*: **Osteopontin deficiency increases bone fragility but preserves bone mass.** *Bone.* 2010; **46**(6): 1564–73.
PubMed Abstract | Publisher Full Text | Free Full Text

53. Poundarik A, Diab T, Sroga G, *et al.*: **Dilatational band formation in bone.** *Proc Natl Acad Sci U S A.* 2012; **109**(47): 19178–83.
PubMed Abstract | Publisher Full Text | Free Full Text

54. Grosso A, Burger MG, Lunger A, *et al.*: **It takes two to tango: Coupling of angiogenesis and osteogenesis for bone regeneration.** *Front Bioeng Biotechnol.* 2017; **5**: 68.
PubMed Abstract | Publisher Full Text | Free Full Text

55. Tsao YT, Huang YJ, Wu HH, *et al.*: **Osteocalcin mediates biomineralization during osteogenic maturation in human mesenchymal stromal cells.** *Int J Mol Sci.* 2017; **18**(1): 159.
PubMed Abstract | Publisher Full Text | Free Full Text

56. Nakajima K, Kunimatsu R, Ando K, *et al.*: **Comparison of the bone regeneration ability between stem cells from human exfoliated deciduous teeth, human dental pulp stem cells and human bone marrow mesenchymal stem cells.** *Biochem Biophys Res Commun.* 2018; **497**(3): 876–882.
PubMed Abstract | Publisher Full Text

57. Leyendecker JA, Gomes PCC, Lazzaretti FT, *et al.*: **The use of human dental pulp stem cells for in vivo bone tissue engineering: A systematic review.** *J Tissue Eng.* 2018; **9**: 2041731417752766.
PubMed Abstract | Publisher Full Text | Free Full Text

58. Nugraha AP: **Raw Data Bone Molecular Markers.** *figshare.* Dataset. 2020.
http://www.doi.org/10.6084/m9.figshare.12610499.v1

Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 03 December 2020

https://doi.org/10.5256/f1000research.31029.r75620

© **2020 Rosyida N.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The author(s) is/are employees of the US Government and therefore domestic copyright protection in USA does not apply to this work. The work may be protected under the copyright laws of other jurisdictions when used in those jurisdictions.

✔

Niswati Fatmah Rosyida
Department of Orthodontics, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

The authors have revised the article based on the my comments before. Therefore the article can be approved.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biomaterial in Orthodontic, Biological Tooth Movement

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 03 November 2020

https://doi.org/10.5256/f1000research.27586.r72008

© **2020 Valadas L.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

✔

Lidia Audrey Rocha Valadas
Postgraduate Program in Development and Technological Innovation in Drugs, School of Pharmacy, Federal University of Ceará, Fortaleza, Brazil

In general the main topic of the manuscript is ambitious and interesting. The paper is well designed and supported with updated references. The introduction is written in clear language, covering a large number of relevant issues. Methods show all details of the methodology used to perform the research but I suggest to provide more details about the sample. Please provide the number and place of approval of your project's ethical opinion. Results are precise and Discussion supports them.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 08 October 2020

https://doi.org/10.5256/f1000research.27586.r71798

© **2020 Rosyida N.** This is an open access peer review report distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

?

Niswati Fatmah Rosyida
Department of Orthodontics, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

The authors of this study have investigated the ability of SHED and CAS in alveolar bone using immunohistochemical analysis.
Based on the study results, the authors have concluded that all the variables increased on Day 7, while RANK expression decreased. The results are certainly interesting.
The paper is very well organized, easy to follow, having a novelty and impact.

Minor concerns about the study are listed as follows:

Introduction

1. Please reduce the explanation about periodontitis as a causative factor on the bone defect, moreover this study does not use the periodontitis model.

2. Please add the information about the problem or the success of the SHED and CAS combination from current or past research.

Method

1. There is no information regarding the experimental animal grouping. The authors should mention it.

2. Please explain why there is no control group without treatment SHED in CAS, or SHED group only.

Discussion

1. There was no discussion why the histological analysis only was done only on 7 day ater treatment, what the reason?

2. CAS have proved induce the healing from the previous study. The success of this formulation is caused by the combination of SHED and CAS, but in the discussion, there is lack of CAS role. Please add the explanation.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biomaterial in Orthodontic, Biological Tooth Movement

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 25 Nov 2020

Alexander Patera Nugraha, Faculty of Dental Medicine, Universitas Airlangga, Indonesia

The authors thank the Honorable Reviewer 1 for her excellent review of the paper and in-depth suggestions made to improve the quality of the paper. Followings are the responses to the suggestions of the Honorable Reviewer. The valuable suggestions and corrections are incorporated cautiously, and the paper is made clearer while revising the manuscript.

Introduction
Please reduce the explanation about periodontitis as a causative factor on the bone defect, moreover this study does not use the periodontitis model. * Please kindly check the new version of our paper. We have been reduced the explanation about periodontitis as a causative factor on the bone defect due to we used the tooth extraction induce alveolar bone defect model in rats. Please add the information about the problem or the success of the SHED and CAS combination from current or past research.
*This study novely was the transplantation of SHED seeded in CAS could increase the number of osteogenic markers expressing cells, such as OPG, RUNX2, TGF-β, VEGF, ALP, osteocalcin, and ostepontin, but not the RANKL expression in the bone defects after seven days in comparison to the CAS group. The previous researchs about combination of SHED and CAS ameliorate alveolar bone defect post tooth extraction is still limited.

Method
There is no information regarding the experimental animal grouping. The authors should mention it.
*Ten wistar rats were assigned into two groups respectively; CAS group and CAS+SHED group.

Please explain why there is no control group without treatment SHED in CAS, or SHED group only.
*The limitations of this study were that the observation and evaluation were performed on transplantation of SHED seeded in CAS and CAS only on the animal model. **Discussion**
There was no discussion why the histological analysis only was done only on 7 day after treatment, what the reason?
*The limitations of this study were that the observation and evaluation were performed seven days post transplantation of SHED seeded in CAS on the animal model, and only an immunohistochemical examination was performed. Further studies will be necessary to evaluate the changes in the alveolar bone and periodontal tissue post transplantation of SHED seeded in CAS in the alveolar bone defect animal models. With a longer observation time, further studies using methods, such as qRT-PCR and/or the western blot analysis, could be conducted to estimate the expression of bone molecular markers.

CAS have proved induce the healing from the previous study. The success of this formulation is caused by the combination of SHED and CAS, but in the discussion, there is lack of CAS role. Please add the explanation.
CAS plays an important role in supporting SHED proliferation and differentiation. The osteogenic microenvironment in defective alveolar bone may induce SHED seeded in CAS to differentiate into bone cells, especially osteoblast.

Competing Interests: There is no competing interest

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

Immunohistochemical analysis of stem cells from human exfoliated deciduous teeth seeded in carbonate apatite scaffold for the alveolar bone defect in Wistar rats (Rattus novergicus)

ORIGINALITY REPORT

19%	12%	17%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Margaret E Katz, Kathryn Braunberger, Gauncai Yi, Sarah Cooper, Heather M Nonhebel, Cedric Gondro. "A p53-like transcription factor similar to Ndt80 controls the response to nutrient stress in the filamentous fungus, Aspergillus nidulans", F1000Research, 2013 Publication	2%
2	Celine Young, Olivia M. Chesniak, Denise Drane, Henry Campa III et al. "Improving the design of an online course with virtual focus group feedback", F1000Research, 2020 Publication	1%
3	m.f1000research.com Internet Source	1%
4	Hiruy S. Meharena, Xiaorui Fan, Lalima G. Ahuja, Malik M. Keshwani et al. "Decoding the Interactions Regulating the Active State	1%

Mechanics of Eukaryotic Protein Kinases", PLOS Biology, 2016
Publication

5	researchrepository.murdoch.edu.au Internet Source	1%
6	global.oup.com Internet Source	1%
7	Flavia M. Savi, Gary I. Brierly, Jeremy Baldwin, Christina Theodoropoulos, Maria A. Woodruff. "Comparison of Different Decalcification Methods Using Rat Mandibles as a Model", Journal of Histochemistry & Cytochemistry, 2017 Publication	1%
8	Alida Alida, Ervina Restiwulan Winoto, Ida Bagus Narmada. "Receptor Activator of Nuclear Factor-Kappa Ligand and Osteoprotegerin Expressions on Hyperglycemic Wistar Rats (Rattus Novergicus) During Orthodontic Tooth Movement", Pesquisa Brasileira em Odontopediatria e Clínica Integrada, 2020 Publication	1%
9	Katrina Podsypanina, Katerina Politi, Levi J. Beverly, Harold E. Varmus. " Oncogene cooperation in tumor maintenance and tumor recurrence in mouse mammary tumors induced by and mutant ", Proceedings of the	1%

National Academy of Sciences, 2008
Publication

10	Pedro Miguel Lacal, Maria Grazia Atzori, Federica Ruffini, Manuel Scimeca et al. "Targeting the vascular endothelial growth factor receptor-1 by the monoclonal antibody D16F7 to increase the activity of immune checkpoint inhibitors against cutaneous melanoma", Pharmacological Research, 2020 Publication	1 %
11	Thomas S Rogers, Mark K Fung, Sarah K Harm. "Recent Advances in Preventing Adverse Reactions to Transfusion", F1000Research, 2015 Publication	1 %
12	"Abstracts", Clinical Oral Implants Research, 09/2011 Publication	<1 %
13	Alexander Patera Nugraha, Fedik Abdul Rantam, Ida Bagus Narmada, Diah Savitri Ernawati, Igo Syaiful Ihsan. "Gingival-Derived Mesenchymal Stem Cell from Rabbit (Oryctolagus cuniculus): Isolation, Culture, and Characterization", European Journal of Dentistry, 2020 Publication	<1 %
14	Submitted to Universitas Airlangga Student Paper	<1 %

15	irep.iium.edu.my Internet Source	<1%
16	www.thieme-connect.de Internet Source	<1%
17	www.biorxiv.org Internet Source	<1%
18	link.springer.com Internet Source	<1%
19	Ananto Ali Alhasyimi, Sri Suparwitri, Christnawati Christnawati. "Effect of Carbonate Apatite Hydrogel-Advanced Platelet-Rich Fibrin Injection on Osteoblastogenesis during Orthodontic Relapse in Rabbits", European Journal of Dentistry, 2020 Publication	<1%
20	"Poster", Journal Of Clinical Periodontology, 2015. Publication	<1%
21	Alexander Patera Nugraha, Ida Bagus Narmada, Putri Intan Sitasari, Fidiyah Inayati et al. "High Mobility Group Box 1 and Heat Shock Protein-70 Expression Post (-)-Epigallocatechin-3-Gallate in East Java Green Tea Methanolic Extract Administration During Orthodontic Tooth Movement in Wistar Rats",	<1%

Pesquisa Brasileira em Odontopediatria e Clínica Integrada, 2020
Publication

---

22 Hyun-A Lee, Hyunjoo Jeong, Eun-Young Kim, Mi Young Nam, Yun-Jung Yoo, Jeong-Taeg Seo, Dong Min Shin, Seung-Ho Ohk, Syng-Ill Lee. " Bumetanide, the Specific Inhibitor of Na -K -2Cl Cotransport, Inhibits 1α,25-Dihydroxyvitamin D -Induced Osteoclastogenesis in a Mouse co-culture System ", Experimental Physiology, 2003 <1 %
Publication

---

23 Li Zhang, Lei Mao, Handong Wang. "The neuroprotection effects of exosome in central nervous system injuries: A new target for therapeutic intervention", Research Square Platform LLC, 2021 <1 %
Publication

---

24 www.frontiersin.org <1 %
Internet Source

---

25 www.benthamopen.com <1 %
Internet Source

---

26 Alphy-Alphonsa Sebastian, Thirumulu-Ponnuraj Kannan, Mohd-Nor Norazmi, Asma-Abdullah Nurul. "Interleukin-17A promotes osteogenic differentiation by increasing OPG/RANKL ratio in stem cells from human exfoliated deciduous teeth (SHED)", Journal of <1 %

Tissue Engineering and Regenerative Medicine, 2018
Publication

27 Brian M Wasko, Daniel T Carr, Herman Tung, Ha Doan et al. "Buffering the pH of the culture medium does not extend yeast replicative lifespan", F1000Research, 2013 <1%
Publication

28 Submitted to iGroup <1%
Student Paper

29 www.pubmedcentral.nih.gov <1%
Internet Source

30 Stacyann Bailey, Gerard Karsenty, Caren Gundberg, Deepak Vashishth. "Osteocalcin and osteopontin influence bone morphology and mechanical properties", Annals of the New York Academy of Sciences, 2017 <1%
Publication

31 A. A. Alhasyimi, P. P. Pudyani, W. Asmara, I. D. Ana. "Enhancement of post-orthodontic tooth stability by carbonated hydroxyapatite-incorporated advanced platelet-rich fibrin in rabbits", Orthodontics & Craniofacial Research, 2018 <1%
Publication

32 eprints.usq.edu.au <1%
Internet Source

33	www.ecmconferences.org Internet Source	<1 %
34	annalsmedres.org Internet Source	<1 %
35	dokumen.pub Internet Source	<1 %
36	mafiadoc.com Internet Source	<1 %
37	onlinelibrary.wiley.com Internet Source	<1 %
38	"1-A6: Cell and Molecular Biology 2", Respirology, 2013 Publication	<1 %
39	www.mdpi.com Internet Source	<1 %
40	www.spandidos-publications.com Internet Source	<1 %
41	Arofi Kurniawan, Shintya Rizky Ayu Agitha, Mieke Sylvia Margaretha, Haryono Utomo et al. "The applicability of Willems dental age estimation method for Indonesian children population in Surabaya", Egyptian Journal of Forensic Sciences, 2020 Publication	<1 %

42 Fujio, Masahito, Zhe Xing, Niyaz Sharabi, Ying Xue, Akihito Yamamoto, Hideharu Hibi, Minoru Ueda, Inge Fristad, and Kamal Mustafa. "Conditioned media from hypoxic-cultured human dental pulp cells promotes bone healing during distraction osteogenesis : Hypoxic culture condition increases the angiogenic factors from hDPCs", Journal of Tissue Engineering and Regenerative Medicine, 2015. <1 %

Publication

---

43 Fumihiko Ogasawara, Kazumitsu Ueda. "ABCA1 causes an asymmetric cholesterol distribution to regulate intracellular cholesterol homeostasis", Cold Spring Harbor Laboratory, 2021 <1 %

Publication

---

44 Vicki Slater, Jennie Rose, Ellinor Olander, Karen Matvienko-Sikar, Sarah Redsell. "Barriers and enablers to Caregivers Responsive feeding Behaviour (CRiB): A mixed method systematic review protocol", HRB Open Research, 2020 <1 %

Publication

---

45 www.dovepress.com <1 %

Internet Source

---

46 Ananto Ali Alhasyimi, Niswati Fathmah Rosyida, Mufliha Santi Rihadini. <1 %

"Postorthodontic Relapse Prevention by Administration of Grape Seed (Vitis vinifera) Extract Containing Cyanidine in Rats", European Journal of Dentistry, 2019
Publication

47	Md. Rafat Tahsin, Arifa Sultana, Muhammad Shah Mohtasim Khan, Ishrat Jahan et al. "An evaluation of pharmacological healing potentialities of Terminalia Arjuna against several ailments on experimental rat models with an in-silico approach", Heliyon, 2021 Publication	<1%
48	cdn.intechopen.com Internet Source	<1%
49	worldwidescience.org Internet Source	<1%
50	Julia K. Bar, Anna Lis-Nawara, Piotr Grzegorz Grelewski. "Dental Pulp Stem Cell-Derived Secretome and Its Regenerative Potential", International Journal of Molecular Sciences, 2021 Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

Immunohistochemical analysis of stem cells from human exfoliated deciduous teeth seeded in carbonate apatite scaffold for the alveolar bone defect in Wistar rats (Rattus novergicus)

GRADEMARK REPORT

FINAL GRADE	GENERAL COMMENTS
/100	**Instructor**

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14

PAGE 15

PAGE 16

PAGE 17