PLOS ONE

RESEARCH ARTICLE

Impaired bone healing at tooth extraction sites in CD24-deficient mice: A pilot study

Limor Avivi-Arber[1]*, Doran Avivi[2], Marilena Perez[1], Nadir Arber[2], Shiran Shapira[2]

1 Faculty of Dentistry, University of Toronto, Toronto, Canada, **2** Integrated Cancer Prevention Center, Tel Aviv Sourasky Medical Center, affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

* Limor.avivi.arber@utoronto.ca

Citation: Avivi-Arber L, Avivi D, Perez M, Arber N, Shapira S (2018) Impaired bone healing at tooth extraction sites in CD24-deficient mice: A pilot study. PLoS ONE 13(2): e0191665. https://doi.org/10.1371/journal.pone.0191665

Editor: Dengshun Miao, Nanjing Medical University, CHINA

Received: November 15, 2017

Accepted: January 9, 2018

Published: February 1, 2018

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This research was supported by Tel Aviv Medical Center – research Grant. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abstract

Aim

To use a micro-computed tomography (micro-CT) to quantify bone healing at maxillary first molar extraction sites, and test the hypothesis that bone healing is impaired in *CD24*-knock-out mice as compared with wild-type C57BL/6J mice.

Materials and methods

Under ketamine-xylazine general anaesthesia, mice had either extraction of the right maxillary first molar tooth or sham operation. Mice were sacrificed 1 (n = 12/group), 2 (n = 6/group) or 4 (n = 6/group) weeks postoperatively. The right maxillae was disected. Micro-CT was used to quantify differences in bone microstructural features at extrction sites, between *CD24*-knockout mice and wild-type mice.

Results

CD24-Knockout mice displayed impaired bone healing at extraction sites that was manifested as decreased trabecular bone density, and decreased number and thickness of trabeculae.

Conclusions

This pilot study suggests that CD24 plays an important role in extraction socket bone healing and may be used as a novel biomarker of bone quality and potential therapeutic target to improve bone healing and density following alveolar bone injury.

Introduction

Intraoral surgical procedures, such as tooth extractions, are common clinical procedures that are often associated with alveolar bone loss, resulting in prosthetic and aesthetic rehabilitative challenges. Bone quality and quantity at healed extraction sites are major factors in

determining long-term success of dental implants [1]. A large amount of dental research has focused on ridge preservation procedures aimed to reduce the amount of bone resorption following tooth extraction [2]. Tooth extraction triggers inflammatory and healing responses that involve the immune, vascular, and nervous systems, as well as the activation of various types of bone forming and bone resorptive cells within the bone. This chain of events influences the speed and extent of bone healing and bone loss [3–5].

Despite the clinical significance, the molecular mechanism/s underlying tissue healing following surgical procedures have not been fully elucidated. CD24 is a small, heavily glycosylated, mucin-like cell surface protein, anchored to the membrane via phosphatidylinositol [6]. CD24 plays an important role during embryogenesis and is expressed in most stem cells [7–9]. In addition, CD24 is an important marker of desmosomes and tight junctions [10,11]. While CD24 is expressed on hematopoietic cells, its expression varies. CD24 is highly expressed in progenitor and metabollically active cells, and is expressed to a lesser extent in terminally differentiated cells [12–14]. CD24 is involved in the maturation and activtation of granulocytes and lymphocytes [15,16], the regulation of homeostatic cell renewal, and in the develoment of many infectious diseases [17–20]. Attenuation of CD24 activity by anti-CD24 monoclonal antibodies can reduce tumor volume *in vivo* and inhibit cancerous cell growth *in vitro* [21,22]. We have recently shown that CD24 plays and important role in wound healing, and that increased expression of CD24 enhances wound repair [23].

Limited data is available on the role of CD24 in intraoral inflammatory and healing processes. Studies have shown that CD24 is selectively expressed in epithelial cells of the dental attached gingivae, and increased reactivity of CD24 can be observed in the epithelium lining the gingival pockets produced by chronic periodontitis [24]. On the other hand, increased titers of serum antibodies to CD24 have been correlated with less severe periodontitis, suggesting a protective role of CD24 on the gingivae [10,25]. In addition, CD24 plays an important role in modulating the expression of genes that regulate differentiation of the oral epithelium. While increased expression of CD24 is associated with a more aggressive course of a disease [17], in the oral epithelium, CD24 may play a role in the maintenance of epithelial integrity [10,26]. Thus, the AIM of the present pilot study was to use a micro-computed tomography to quantify bone healing and test the hypothesis that bone healing at molar tooth extraction sockets is impaired in *CD24* knockout mice as compared with wild-type mice. Here we show, for the first time, that CD24 plays an important role in bone healing after molar tooth extraction.

Materials and methods

Animals

All experimental procedures were approved by the Israeli Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and the standard of care of theIsraeli Ministry of Health. This investigation also complied with ARRIVE guidelines for preclinical studies.

The study groups comprised of 7–13 weeks old male wild-type (WT) *C57BL*/6J mice (n = 24) (Harlan Laboratories, Jerusalem) and *CD24* knockout (KO) mice (n = 24) that were bred at the animal facility of the Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. These KO mice are genetically tested on a regular basis by PCR analysis of DNA obtained from tail biopsies at the age of 5 weeks. The expression of CD24 has also been verified by FACS Analysis on heparinized peripheral blood samples that are collected from the orbital sinus of the mice. Mice were housed in an animal room with a 12 h:12 h light/dark cycle and received chow diet and water *ad libitum*. All measures were taken to minimize pain or discomfort, before the procedures, mice were anesthetized by intraperitoneal (i.p.) injection of ketamine (50 mg/kg) and

xylazine (5 mg/kg), and we have used Acamol for pain relief. Animals were monitored every 2–3 days to assess body weight, food consumption, general behavior and any postoperative complications such as bleeding or swelling. Data collection and analyses were carried out in a blinded manner.

Molar tooth extraction

Tooth extraction was carried out under aseptic conditions and general anaesthesia with intraperitoneal administration of 100 mg/ml Ketamine and 20 mg/ml Xylazine prepared in injectable saline (0.1 ml/10gr body weight). The first right maxillary molar tooth (M1) was gently luxated using two 18 gauges needles as elevators under the aid of x3.6 magnifying loups. It has been reported that extraction sites in WT mice normally heal within 21 days following tooth extraction [5]. Thus to test the time course of bone healing, mice were sacrificed one week (n = 12), two weeks (n = 6) or four weeks (n = 6) after tooth extraction (Table 1). Mice were sacrificed under ketamine deep general anesthesia by cervical dislocation. Thereafter, the right maxilla was dissected for subsequent micro-computed tomography (micro-CT). One mouse from the KO-1W group, two mice from the WT-2W group, three mice from KO-2W group and one mouse from the KO-4W group were excluded from the study because of post-extraction complicaitons such as swelling or death.

Micro-computed tomography

The maxillary bone specimens were fixed in 10% neutral formalin. Bone specimens were scanned with a micro-CT scanner equipped with a custom software package (Micro-CT40, Scanco Medical, Basserdorf, Switzerland). Specimens were scanned at 70 kVp and 114 μA, at high resolution (6 μm slice thickness), and in three planes. A region of interest (ROI) was selected distal to the remaining second molar tooth and was highlighted on cross-sectional images from each bone specimen (Fig 1A). The scanned region extended 1 mm distal to the second molar tooth and spanned to include the bone from the alveolar crest to the base of the maxillary sinus. Following the scan, three-dimensional (3-D) images of the ROIs were reconstructed (Fig 1B). The bone volume as a fraction of total tissue volume (BV/TV) within the ROIs was used as a measure of bone density and was calculated for all study groups. In addition, the following morphological parameters were calculated in the 4-week study groups in which extraction sockets were expected to be completely healed [5]: trabecular thickness (Tb.Th, mm), trabecular number (Tb.N, mm), trabecular separation (Tb.Sp), and bone surface area as a fraction of total volume (SA/TV) which was used as a measure of surface roughness [5,27]. The bone surface area and total bone volume were calculated automatically by the microCT software.

Statistical analysis

Based on pilot data and sample size calculation ANOVA ($\alpha = 0.05$, $\beta = 0.8$, 35% effect, and a SD of 15%), at least six mice per group were necessary to detect a statistically significant

Table 1. Study groups and number of animals per group.

	Wild type (WT) *C57BL/6J* mice	*CD24*-KnockOut (KO) mice
1 week following maxillary molar tooth extraction	n = 12 mice	n = 12 mice
2 weeks following maxillary molar tooth extraction	n = 6 mice	n = 6 mice
4 weeks following maxillary molar tooth extraction	n = 6 mice	n = 6 mice

https://doi.org/10.1371/journal.pone.0191665.t001

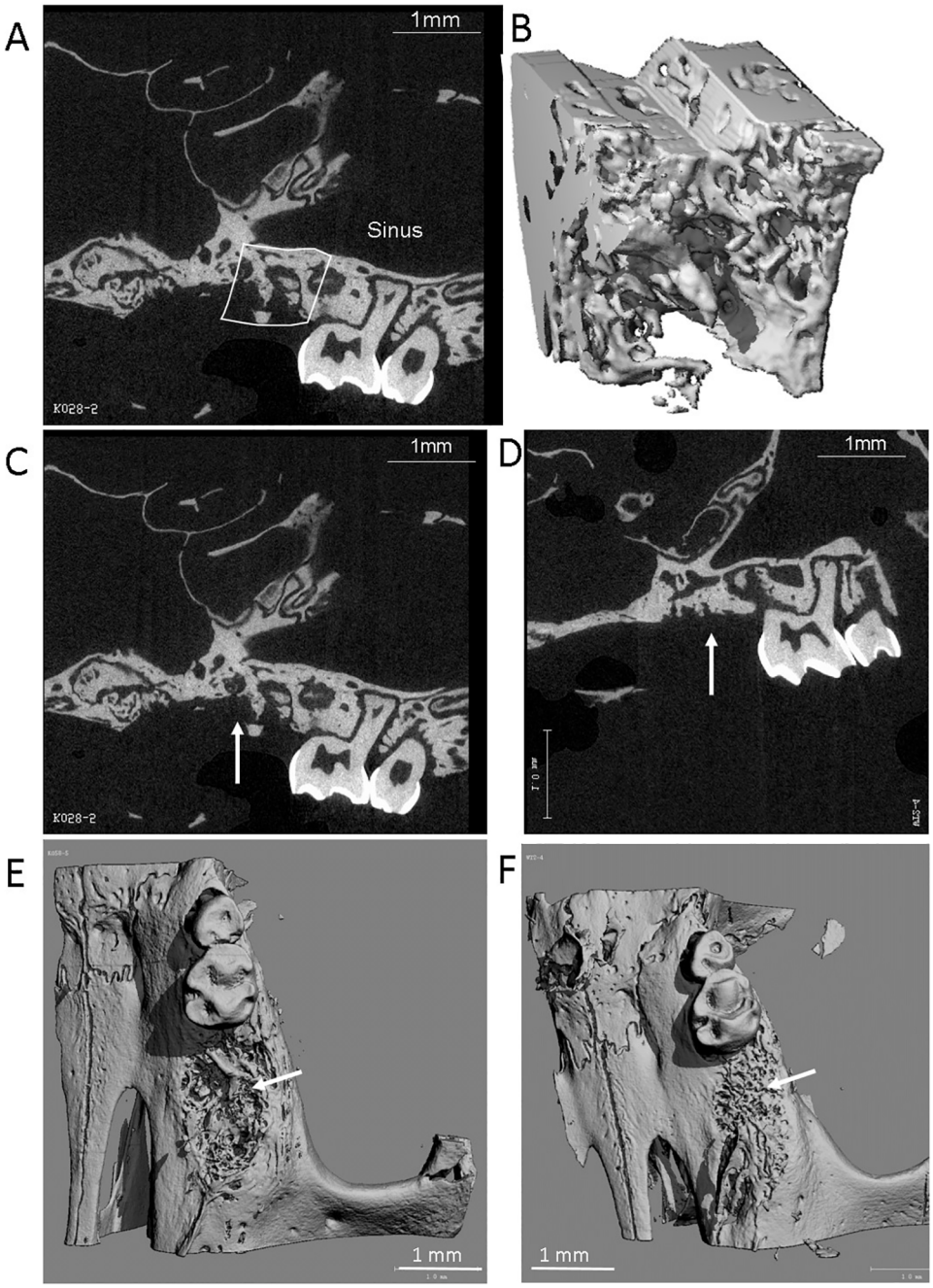

Fig 1. **A.** Micro-CT 2D cross sections through extraction sockets and adjacent molar teeth in representative wild-type (WT) *C57BL/6J* mice (**A and D**) and a *CD24*-knockout (KO) mouse (**C**). White line in A marks the region of interest which includes the complete vertical dimension of the socket within the maxilla and 1 mm of bone length distal to the adjacent molar tooth; **B.** 3D cubic sub-volume of the bone contained in the area marked in A. White arrow in C points to large socket concavities in the KO mouse as compared with a bone-filled socket in the WT mouse in D; **E-F**. Micro-CT surface images in a representative *CD24*-KO mouse (**E**) and a WT mouse (**F**); the images show a more rough surface morphology in the KO mouse as compared to the WT mouse.

https://doi.org/10.1371/journal.pone.0191665.g001

treatment effect in the experimental design of the present study. Data analysis was carried out in a blinded manner. Statistical analysis was performed using SigmaPlot 12.5 software (CA, USA). Two-Way analysis of variance (ANOVA) followed by *post-hoc* Duncan's multiple comparison test was used to test the effects of tooth extraction and post-extraction time on the dependent variables bone density. In addition, independent *t-tests* were used to test the effects of tooth extraction on the following dependent variables measured four weeks following tooth extraction: trabecular thickness, trabecular separation, number of trabeculae, and bone surface/bone volume ratio. Data is presented as Mean ± SD, and $p<0.05$ was considered statistically significant. All relevant data are within the paper and its Supporting Information files [i.e., Tables A and B in S1 File].

Results

During the study, all mice demonstrated normal behaviour and continuous weight gain except during the first 2–3 post-operative days, when weight gain was slower, as expected.

Fig 1C and 1D show cross-sections through extraction sockets of representative mice from the *CD24*-KO and WT groups at four weeks post-extraction. Larger socket concavities were identified in the KO mice (Fig 1C) as compared with the bone-filled sockets in the WT animals (Fig 1D).

Bone density (i.e., BV/TV) measurements at 1, 2 or 4 weeks after tooth extraction are illustrated in Fig 2. Two-way ANOVA revealed a significant post-extraction time effect ($F_{2,35}$ = 10.13, $p<0.001$), as well as a study group by post-extraction time interaction ($F_{2,35}$ = 5.12, p = 0.011). Using Duncan's *post-hoc* multiple comparison analysis, in the WT *C57BL*/6J mice, bone density was significantly higher at 4 weeks post-extraction than at one or two weeks post-extraction ($p<0.001$, p = 0.003, respectively) (Fig 2, Table A in S1 File). Similar changes in bone density were not observed for the *CD24*-KO mice. In addition, *post-hoc* Duncan's test revealed that only at four weeks (but not at one or two weeks) following tooth extraction, WT mice had a significantly greater bone density than *CD24*-KO mice (p = 0.004). *CD24*-KO mice showed no significant differences in bone density across all time points post-extraction ($P>>0.05$).

At four weeks following tooth extraction, various bone healing parameters were measured, such as trabecular thickness (Fig 3A, Table B in S1 File), trabecular separation (Fig 3B), number of trabeculae (Fig 3C, Table B in S1 File), and the bone surface to bone volume ratio (i.e., surface roughness) (Fig 3D, see also 1E-F, Table B in S1 File). Wild type *C57BL/6J* mice had a significantly smoother bone surface (p = 0.014), a significantly greater number of trabeculae (p = 0.05), the trabeculae were significantly thicker (p = 0.035), and there was less bone marrow space between the trabeculae (p = 0.017).

Discussion

The novel findings of this study reveal that CD24 may play an important role in the healing of extraction sockets. Lack of CD24 was strongly associated with delayed healing of extraction sockets, and reduced bone quality and density. *CD24*-KO mice, as compared with WT *C57BL*/6J mice, displayed an increased surface roughness at extraction sites, and a lower trabecular bone volume density. The decreased number of trabeculae and the decrease in trabecular thickeness that were observed in *CD24*-KO, as compared with WT mice, may have contributed to the decreased trabecular volume density observed in *CD24*-KO mice. Furthermore, the structural differences caused by the absence of CD24 may translate biomechanically into a significantly weaker bone in CD24-deficient mice [28].

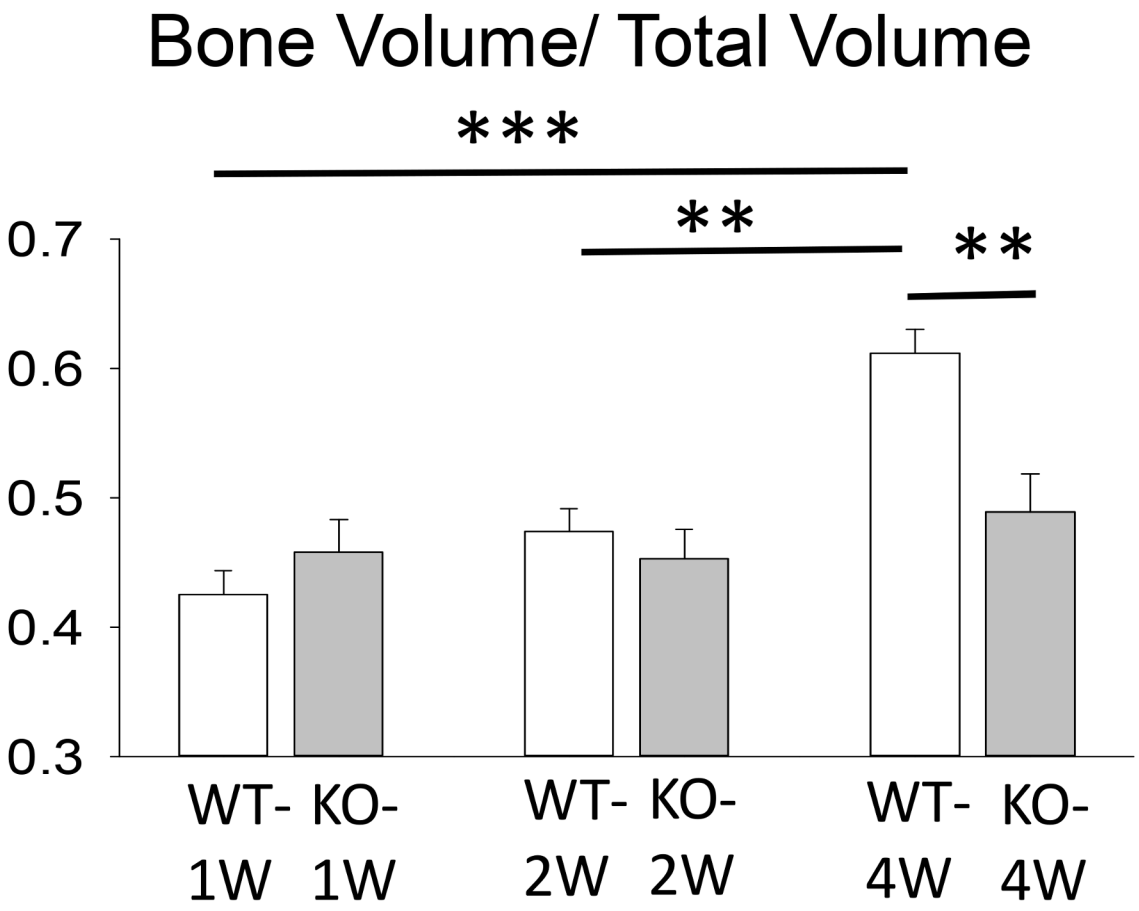

Fig 2. Bone density expressed as the fraction of bone volume out of the total volume. Bone density in wild-type (WT) *C57BL*/6J mice at 4 weeks post-extraction was significantly larger than the bone densities at 1 week and 2 weeks post-extraction (Duncan's $p<0.001$, $p = 0.003$, respectively). At 4 weeks after tooth extraction, WT mice had a significantly larger bone density than the *CD24*-knockout mice ($p = 0.004$).

https://doi.org/10.1371/journal.pone.0191665.g002

These findings are consistent with our recently published findings, whereby we have shown that CD24 plays an important role in skin wound healing [23]. We have shown that in *CD24*-KO mice, as compared with WT *C57BL*/6J mice, large full-thickness skin wounds excised on the back of the mice, demonstrate significant delays in wound healing due to impaired formation of granulation tissue and impaired wound closure. Moreover, the same phenomenon could be achieved following intravenous injections of monoclonal antibodies to CD24. Finally, re-expression of HSA (Heat stable antigen, mCD24) delivered by lentivirus, could restore the normal healing phenotype within 24 hours post-injury, and could also improve wound healing in the WT mice [23]. Thus, the novel findings of the present study holds promise for novel therapies to enhance alveolar bone healing.

CD24 is selectively expressed at high levels by the epithelium associated with the healthy gingival attachment and pocket epithelium of periodontally involved teeth [24]. Highly glycosylated CD24 has recently been described as an important danger associated receptor, protecting tissue from excessive leukocyte activity. CD24 critically mediates a protective effect against tissue injury via CD24-Siglec 10 pathway [29]. CD24 was suggested to play a crucial role in cell differentiation *in vivo*. During tooth development, its mRNA is induced in dental papilla

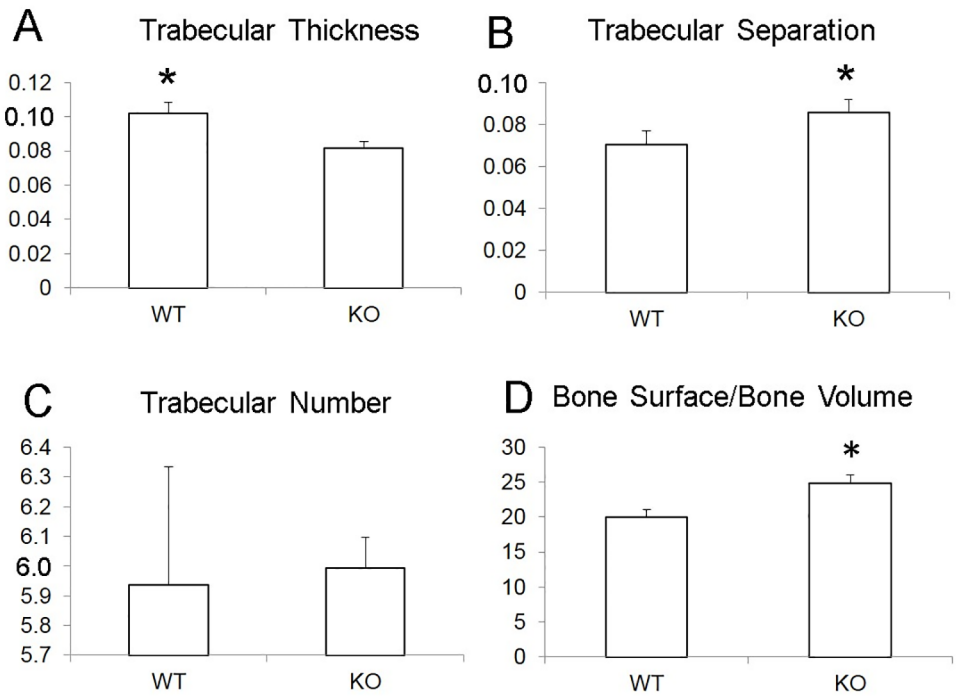

Fig 3. Bone healing parameters at 4-weeks. A. Trabecular thickness; **B**. Trabecular separation; **C**. Trabecular number; **D**. Bone surface/Bone volume. In wild type *C57BL/6J* mice, as compared with *CD24*-knockout mice, the bone surface was significantly smoother ($p = 0.017$); the trabeculae were significantly thicker ($p = 0.035$) and there was less bone marrow space ($p = 0.014$).

https://doi.org/10.1371/journal.pone.0191665.g003

mesenchymal cells that differente into odontoblasts. In addition, it has been shown that the stage of root development can influence the number of CD24 expressing cell [30].

Although there are ample reports on the role of CD24 in tissue healing, regretably, the precise cellular mechanism of CD24 is still unknown, including its function in bone healing. *CD24*-KO mice are viable and display no obvious defects in skeletal or other body tissues. CD24 is normally expressed on hematopoietic cells, including bone marrow lymphocytes, neutrophils, and macrophages, as well as on non-hematopoietic cells such as neural, endothelial, platelets and even dental apical papilla stem cells [12–14]. Since these cells are also involved in bone healing processes, CD24 may play a role in bone healing by interacting with these cell types.

CD24 is a major player in many signal pathways, and is associated with inflamation and cancer. CD24 has a constitutive function of maintaining expression of selected genes (such as zonula occludens-1, zonula occludens-2 and occludin,) encoding for tight junction components associated with a marginal barrier function of epithelial responses, and the regulation of epithelial behavior [10,11,31]. We have already shownn that CD24 involves the catenin pathway [20,32]. It was reported that CD24 inhibits the activation of NF-κB and mediates injury repair via the CD24–SigG/10 pathway [33]. CD24 partners with Siglec-G/10 to negatively regulate the immune response to proteins released by damaged cells. However, because of the highly variable glycosylation of CD24 it has many tissue specific ligands with a variable specificity depening on the cellular context [14].

Tooth extraction typically leads to alveolar bone loss which may impact prosthodontic treatment, including the possibility of placing dental implants. Alveolar (socket) ridge preservation and grafting procedures have received much attention in an attempt to minimize bone

loss following tooth extraction [34]. However, for these procedures to be successful, a better understanding of the molecular aspects of bone healing processes at extraction sockets is required to assist with the development of novel therapeutic strategies. The novel findings of the present pilot study reveal, for the first time, an important role for *CD24* gene in bone healing. Future longitudinal studies will be carried-out to test the effects of tooth extraction at different points of time, and test whether activation of CD24 can improve bone quality and density following tooth extraction, and whether CD24 can be used as a novel biomarker of bone quality prior to skeletal surgical procedures.

Supporting information

S1 File. (Table A) Data for the Bone volume to Total volume ratio for each mouse within each of the study groups: WT-1w, WT-2w and WT-4w; and KO-1w, KO-2w and KO-4w which are, respectively, wild type C57BL/6J mice and CD24 knockout mice at 1, 2 or 4 weeks after unilateral extraction of the maxillary molar teeth. (Table B) Data for the Bone Surface to Bone Volume ratio, Trabecular Number and Trabecular Separation for each mouse within each of the study groups: WT-1w, WT-2w and WT-4w; and KO-1w, KO-2w and KO-4w which are, respectively, wild type C57BL/6J mice and CD24 knockout mice at 1, 2 or 4 weeks after unilateral extraction of the maxillary molar teeth.
(PDF)

Acknowledgments

We would like to thank Nancy Valiquette for assisting with the micro-CT. The authors declare that they have no conflict of interests. This research was supported by Tel Aviv Medical Center —research Grant.

Author Contributions

Conceptualization: Limor Avivi-Arber, Marilena Perez, Nadir Arber, Shiran Shapira.

Data curation: Limor Avivi-Arber, Doran Avivi, Marilena Perez, Nadir Arber, Shiran Shapira.

Formal analysis: Limor Avivi-Arber, Marilena Perez, Shiran Shapira.

Funding acquisition: Limor Avivi-Arber, Nadir Arber.

Investigation: Limor Avivi-Arber, Doran Avivi, Marilena Perez, Nadir Arber, Shiran Shapira.

Methodology: Limor Avivi-Arber, Doran Avivi, Marilena Perez, Nadir Arber, Shiran Shapira.

Project administration: Limor Avivi-Arber, Nadir Arber, Shiran Shapira.

Resources: Limor Avivi-Arber, Nadir Arber.

Software: Limor Avivi-Arber.

Supervision: Limor Avivi-Arber.

Validation: Limor Avivi-Arber, Doran Avivi, Marilena Perez, Nadir Arber, Shiran Shapira.

Visualization: Limor Avivi-Arber, Doran Avivi, Marilena Perez, Nadir Arber, Shiran Shapira.

Writing – original draft: Limor Avivi-Arber, Nadir Arber, Shiran Shapira.

Writing – review & editing: Limor Avivi-Arber, Doran Avivi, Marilena Perez, Nadir Arber, Shiran Shapira.

References

1. Martin W, Lewis E, Nicol A (2009) Local Risk Factors for Implant Therapy. International Journal of Oral & Maxillofacial Implants 24: 28–38.
2. Tomlin EM, Nelson SJ, Rossmann JA (2014) Ridge preservation for implant therapy: a review of the literature. Open Dent J 8: 66–76. https://doi.org/10.2174/1874210601408010066 PMID: 24893595
3. Tsiridis E, Upadhyay N, Giannoudis P (2007) Molecular aspects of fracture healing: which are the important molecules? Injury 38 Suppl 1: S11–25.
4. Stroncek JD, Reichert WM (2008) Overview of Wound Healing in Different Tissue Types. In: Reichert WM, editor. Indwelling Neural Implants: Strategies for Contending with the In Vivo Environment. Boca Raton (FL): CRC Press/Taylor & Francis Group, LLC.
5. Vieira AE, Repeke CE, Ferreira Junior Sde B, Colavite PM, Biguetti CC, Oliveira RC, et al. (2015) Intramembranous bone healing process subsequent to tooth extraction in mice: micro-computed tomography, histomorphometric and molecular characterization. PLoS One 10: e0128021. https://doi.org/10.1371/journal.pone.0128021 PMID: 26023920
6. Kristiansen G, Sammar M, Altevogt P (2004) Tumour biological aspects of CD24, a mucin-like adhesion molecule. J Mol Histol 35: 255–262. PMID: 15339045
7. Salaria SN, Means A, Revetta F, Idrees K, Liu E, Shi C. (2015) Expression of CD24, A Stem Cell Marker, in Pancreatic and Small Intestinal Neuroendocrine Tumors. Am J Clin Pathol 144: 642–648. https://doi.org/10.1309/AJCPMZY5P9TWNJJV PMID: 26386086
8. Jaggupilli A, Elkord E (2012) Significance of CD44 and CD24 as cancer stem cell markers: an enduring ambiguity. Clin Dev Immunol 2012: 708036. https://doi.org/10.1155/2012/708036 PMID: 22693526
9. Gracz AD, Fuller MK, Wang F, Li L, Stelzner M, Dunn JC, et al. (2013) Brief report: CD24 and CD44 mark human intestinal epithelial cell populations with characteristics of active and facultative stem cells. Stem Cells 31: 2024–2030. https://doi.org/10.1002/stem.1391 PMID: 23553902
10. Ye P, Nadkarni MA, Simonian M, Hunter N (2009) CD24 regulated gene expression and distribution of tight junction proteins is associated with altered barrier function in oral epithelial monolayers. Bmc Cell Biology 10: 2. https://doi.org/10.1186/1471-2121-10-2 PMID: 19138432
11. Ye P, Yu H, Simonian M, Hunter N (2011) Ligation of CD24 expressed by oral epithelial cells induces kinase dependent decrease in paracellular permeability mediated by tight junction proteins. Biochem Biophys Res Commun 412: 165–169. https://doi.org/10.1016/j.bbrc.2011.07.067 PMID: 21806966
12. Bakopoulou A, Leyhausen G, Volk J, Koidis P, Geurtsen W (2013) Comparative characterization of STRO-1(neg)/CD146(pos) and STRO-1(pos)/CD146(pos) apical papilla stem cells enriched with flow cytometry. Arch Oral Biol 58: 1556–1568. https://doi.org/10.1016/j.archoralbio.2013.06.018 PMID: 23871383
13. Wu J, Huang GT, He W, Wang P, Tong Z, Jia Q, et al. (2012) Basic fibroblast growth factor enhances stemness of human stem cells from the apical papilla. J Endod 38: 614–622. https://doi.org/10.1016/j.joen.2012.01.014 PMID: 22515889
14. Fang X, Zheng P, Tang J, Liu Y (2010) CD24: from A to Z. Cell Mol Immunol 7: 100–103. https://doi.org/10.1038/cmi.2009.119 PMID: 20154703
15. Li O, Zheng P, Liu Y (2004) CD24 expression on T cells is required for optimal T cell proliferation in lymphopenic host. J Exp Med 200: 1083–1089. https://doi.org/10.1084/jem.20040779 PMID: 15477346
16. Taguchi T, Kiyokawa N, Mimori K, Suzuki T, Sekino T, Nakajima H, et al. (2003) Pre-B cell antigen receptor-mediated signal inhibits CD24-induced apoptosis in human pre-B cells. J Immunol 170: 252–260. PMID: 12496407
17. Sagiv E, Arber N (2008) The novel oncogene CD24 and its arising role in the carcinogenesis of the GI tract: from research to therapy. Expert Rev Gastroenterol Hepatol 2: 125–133. https://doi.org/10.1586/17474124.2.1.125 PMID: 19072375
18. Chappel MS, Hough MR, Mittel A, Takei F, Kay R, Humphries RK. (1996) Cross-linking the murine heat-stable antigen induces apoptosis in B cell precursors and suppresses the anti-CD40-induced proliferation of mature resting B lymphocytes. J Exp Med 184: 1639–1649. PMID: 8920854
19. Sagiv E, Memeo L, Karin A, Kazanov D, Jacob-Hirsch J, Mansukhani M, et al. (2006) CD24 is a new oncogene, early at the multistep process of colorectal cancer carcinogenesis. Gastroenterology 131: 630–639. https://doi.org/10.1053/j.gastro.2006.04.028 PMID: 16890615
20. Naumov I, Zilberberg A, Shapira S, Avivi D, Kazanov D, Rosin-Arbesfeld R, et al. (2014) CD24 knockout prevents colorectal cancer in chemically induced colon carcinogenesis and in APC(Min)/CD24 double knockout transgenic mice. Int J Cancer 135: 1048–1059. https://doi.org/10.1002/ijc.28762 PMID: 24500912

21. Sagiv E, Starr A, Rozovski U, Khosravi R, Altevogt P, Wang T, et al. (2008) Targeting CD24 for treatment of colorectal and pancreatic cancer by monoclonal antibodies or small interfering RNA. Cancer Res 68: 2803–2812. https://doi.org/10.1158/0008-5472.CAN-07-6463 PMID: 18413748

22. Shapira S, Shapira A, Starr A, Kazanov D, Kraus S, Benhar I, et al. (2011) An immunoconjugate of anti-CD24 and Pseudomonas exotoxin selectively kills human colorectal tumors in mice. Gastroenterology 140: 935–946. https://doi.org/10.1053/j.gastro.2010.12.004 PMID: 21147107

23. Shapira S, Ben-Amotz O, Sher O, Kazanov D, Mashiah J, Kraus S, et al. (2015) Delayed Wound Healing in Heat Stable Antigen (HSA/CD24)-Deficient Mice. PLoS One 10: e0139787. https://doi.org/10.1371/journal.pone.0139787 PMID: 26440795

24. Guo W, Ye P, Yu H, Liu Z, Yang P, Hunter N. (2014) CD24 activates the NLRP3 inflammasome through c-Src kinase activity in a model of the lining epithelium of inflamed periodontal tissues. Immun Inflamm Dis 2: 239–253. https://doi.org/10.1002/iid3.40 PMID: 25866631

25. Ye P, Simonian M, Nadkarni MA, DeCarlo AA, Chapple CC, Hunter N. (2005) Identification of epithelial auto-antigens associated with periodontal disease. Clin Exp Immunol 139: 328–337. https://doi.org/10.1111/j.1365-2249.2005.02692.x PMID: 15654832

26. Ye P, Nadkarni MA, Hunter N (2006) Regulation of E-cadherin and TGF-beta 3 expression by CD24 in cultured oral epithelial cells. Biochem Biophys Res Commun 349: 229–235. https://doi.org/10.1016/j.bbrc.2006.08.033 PMID: 16930538

27. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. (2010) Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. J Bone Miner Res 25: 1468–1486. https://doi.org/10.1002/jbmr.141 PMID: 20533309

28. Huber MB, Lancianese SL, Nagarajan MB, Ikpot IZ, Lerner AL, Wismuller A. (2011) Prediction of biomechanical properties of trabecular bone in MR images with geometric features and support vector regression. IEEE Trans Biomed Eng 58: 1820–1826. https://doi.org/10.1109/TBME.2011.2119484 PMID: 21356612

29. Chen GY, Tang J, Zheng P, Liu Y (2009) CD24 and Siglec-10 selectively repress tissue damage-induced immune responses. Science 323: 1722–1725. https://doi.org/10.1126/science.1168988 PMID: 19264983

30. Aguilar P, Lertchirakarn V (2016) Comparison of stem cell behaviors between indigenous high and low-CD24 percentage expressing cells of stem cells from apical papilla (SCAPs). Tissue Cell 48: 397–406. https://doi.org/10.1016/j.tice.2016.08.008 PMID: 27613575

31. Ye P, Yu H, Simonian M, Hunter N (2014) Expression patterns of tight junction components induced by CD24 in an oral epithelial cell-culture model correlated to affected periodontal tissues. J Periodontal Res 49: 253–259. https://doi.org/10.1111/jre.12102 PMID: 23713517

32. Fokra A, Kazanov D, Bedny F, Brazowski E, Varol C, Kraus S, et al. (2016) CD24 Induces the Activation of β-Catenin in Intestinal Tumorigenesis. J Cancer Sci Ther 8: 135.

33. Liu Y, Chen G-Y, Zheng P (2009) CD24-Siglec G/10 discriminates danger- from pathogen-associated molecular patterns. Trends Immunol. 30: 557–561. https://doi.org/10.1016/j.it.2009.09.006 PMID: 19786366

34. Allegrini S Jr., Koening B Jr., Allegrini MR, Yoshimoto M, Gedrange T, Fanghaenel J, et al. (2008) Alveolar ridge sockets preservation with bone grafting—review. Ann Acad Med Stetin 54: 70–81.