Saudi Journal of Biological Sciences 29 (2022) 1789–1795

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# Comparative assessment of the cell-surface antigens and gene expression profiles of the gingival tissue biomarkers in subjects with fixed functional and removable functional orthodontic appliances

Hosam Ali Baeshen [a,*], Fahad F. Alsulaimani [a], Wael Awadh [b], Maram Ahmed Ageeli [c], Shahabe Saquib Abullais [d], Nabeeh Abdullah Alqahtani [e], Mohammad Y. Alshahrani [f], Jagadish Hosmani [g], Shankargouda Patil [h,*]

[a] Department of Orthodontics, Faculty of Dentistry, King Abduaziz University, Jeddah 21589, Saudi Arabia
[b] Division of Orthodontics, Department of Preventive Dental Sciences, College of Dentistry, Jazan University, Jazan, Saudi Arabia
[c] College of Dentistry, Jazan University, Jazan, Saudi Arabia
[d] Periodontics and Community Dental Sciences, College of Dentistry, King Khalid University, Abha 62529, Saudi Arabia
[e] Periodontics and Community Dental Sciences, College of Dentistry, King Khalid University, Abha 62529, Saudi Arabia
[f] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, P.O. Box 61413, Abha 9088, Saudi Arabia
[g] Oral Pathology Division, Department of Dental Sciences, College of Dentistry, King Khalid University, Abha 62529, Saudi Arabia
[h] Department of Maxillofacial Surgery and Diagnostic Sciences, Division of Oral Pathology, College of Dentistry, Jazan University, Jazan, Saudi Arabia

ARTICLE INFO

*Article history:*
Received 29 September 2021
Revised 13 October 2021
Accepted 18 October 2021
Available online 25 October 2021

*Keywords:*
Biomarkers
Cell-surface antigens
Fixed functional appliances
Gene expression
Removable functional appliances

ABSTRACT

*Background:* This study aimed to examine the cellular components of the gingiva during orthodontic treatment with fixed and removable appliances. The cellular and molecular cues of pathologies of the gingival tissue associated with the use of different orthodontic appliances could be studied.
*Materials and methods:* Tissue samples of gingiva were received from healthy patients undergoing gingivectomy for aesthetic purpose and from patients with fixed and removable functional orthodontic appliances undergoing gingivectomy for gingival overgrowth. The collected samples were stored in a sterile container with phosphate-buffered saline and to carry out further processes it was transported to the laboratory.
*Results:* Cells positive for ECAD and NCAD were found to be increased in fixed appliances where as CD90 and CD105 positive cells showed no significant difference in all the three groups. CD24 and CD146 positive cells were increased significantly in removable and fixed than normal whereas CD133 positive cells were decreased in removable and fixed than normal. CD44 positive cells showed no noticeable change in all three groups. The gene expression levels of KRT5, SOX2, NANOG, and CXCL5 were found to be significantly increased in removable and fixed appliance groups. However, KRT8, CXCL10, and TIMP1 were increased only in fixed appliance group but CXCL10 showed decreased expression in removable appliance group. KRT6A, MYC, and MMP9 were decreased in fixed appliance group whereas MYC and MMP9 were increased in removable appliance group. KRT6A, KRT8, and TIMP1 showed no significant difference in removable appliance group.
*Conclusion:* This study demonstrated essential roles of various genes, showing their contribution in regulating cell proliferation and migration in both the removable and fixed functional appliances.


* Corresponding authors at: Department of Maxillofacial Surgery and Diagnostic Sciences, Division of Oral Pathology, College of Dentistry, Jazan University, Jazan, Saudi Arabia (Shankargouda Patil); Department of Orthodontics, Faculty of Dentistry, King Abduaziz University, Jeddah, Saudi Arabia (Hosam Ali Baeshen).
*E-mail addresses:* drbaeshen@me.com (H.A. Baeshen), dr.ravipatil@gmail.com (S. Patil).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.10.047
1319-562X/

## 1. Introduction

Occlusal disorders in Class II malocclusion patients have been treated using various orthodontic and surgical treatments. Class II malocclusions with mandibular retrusion are usually treated with functional orthodontic appliances by applying orthopedic forces that are directed over the mandibular structures. These appliances affect the jaws by causing remodeling of glenoid fossa and mandibular condyles. It autorotates the mandible by repositioning the condyles in the glenoid (Pancherz et al., 1998).

One of the greatest challenges during orthodontic treatment is to maintain healthy gingival tissue. The esthetic and functional satisfaction of the patients gets affected due to the gingival enlargements caused due to orthodontic treatment and this can be managed by timely and correct diagnosis of periodontal diseases (Casian Romero et al., 2011). The orthodontic treatment not only improves the esthetics by correcting the deformities of the dentition and jaws, but also providing conditions to maintain improved gingival health (Wu et al., 2003). Orthodontics is predicated on the application of mechanical forces to move teeth through hard tissues. This movement and remodelling of the surrounding tissues occurs through the transduction of force that activates bone and related cells generating an inflammatory response. Orthodontic appliances, whether they be fixed or removable are in contact with oral tissues and remains in the mouth for the duration of treatment which can extend several years. Therefore, a patient's periodontal health is of concern throughout the treatment period.

Several researchers have analysed the periodontal and oral health of patients undergoing orthodontic corrective treatment and the consequences of treatment to their oral health, their conclusions remain opaque and unclear (Eid et al., 2014; Ghezzi et al., 2008; Kloehn and Pfeifer, 1974; Şurlin et al., 2012; Zachrisson and Zachrisson, 1972; Zanatta et al., 2012) Generally, orthodontic treatment can help improve a patient's oral health status, correct malocclusion and lead to a greatly improved periodontal outcome. Conventional fixed orthodontics can pose a difficulty while cleaning (Marchese et al., 2003). Patients must be cautious and diligent to keep the appliance and their teeth clean to prevent plaque accumulation. Plaque growth can lead to adverse periodontal outcomes and increase patient discomfort. On the other hand, removable orthodontic appliances are less cumbersome and offer an alternative to patients who find it hard to maintain fixed appliances, easier cleaning (Zhou Q, 2014).

There is limited information in the literature regarding the effects of functional appliances on the periodontal health. Hence, this study aimed to examine the cellular components of the gingiva during orthodontic treatment with fixed and removable appliances. Through this study we could understand the cellular and molecular cues of pathologies of the gingival tissue associated with the use of different orthodontic appliances.

## 2. Materials and methods

### 2.1. Sample collection

During small surgery maneuvers involving tooth extraction, wisdom molar surgery or gingivectomy, the gingival tissue samples were obtained under local anesthesia. Gingival tissue samples were collected from healthy patients undergoing gingivectomy for aesthetic purpose (n = 8) (age: 16–25 years), from patients with fixed functional orthodontic appliances undergoing gingivectomy for gingival overgrowth (n = 6) (age: 13–17 years), and from patients with removable functional orthodontic appliances who were scheduled for extraction due to orthodontic purposes (n = 8) (age: 15–18 years). Informed consents were signed and collected accordingly by the guidelines of institutional ethical committee. The collected samples were stored in a sterile container which has phosphate-buffered saline (PBS) (Sigma, St. Louis, MO, USA) and to carry further processes it is transported to the laboratory directly.

### 2.2. Single cell suspension preparation from tissue samples

Sterile PBS with antibiotic antimycotic (Penicillin-Streptomycin-Amphotericin B) solution was used to rinse the tissues thoroughly. Further, sterile blade was used to mince the tissues and it was directly subjected to enzymatic digestion containing enzyme solution of 0.2% dispase II (Roche Diagnostics GmbH, Mannheim, Germany) and 0.4% collagenase I (MP Biomedicals LLC, Santa Ana, CA, USA) and incubated for 20 min at 37 °C. After incubation, to neutralize the enzymatic action fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) was used, the tissue was digested and then passed into a sterile cell strainer with a pore size of 70 μm (Corning, NY, USA) and the mixture was centrifuged for 5 min at 1800 rpm. The pellet of cells thus obtained were directly used for further experimentation.

### 2.3. Analysis of cells for surface markers by flow cytometry

For flow cytometry analysis, the cells were resuspended from the pellet in PBS from each sample and divided into different tubes. Each tube was individually incubated for 30 min at 4 °C with ECAD-APC, NCAD-PE, CD90-FITC, CD105-APC, CD24-APC, CD44-PE, CD133-FITC, and CD146-PE antibodies (all monoclonal) (Miltenyi Biotec, Bergisch Gladbach, Germany). After incubation, PBS was used to wash the cells and pelleted down. The labelled cells were resuspended in sheath fluid and analyzed with a flow cytometer (Attune NxT, Thermo Fisher Scientific, Waltham, MA, USA). At least for each samples 10,000 events are acquired. Percentage comparison was done with the degree of positive staining and the isotype controls. The median fluorescence intensities for each surface protein were also considered.

### 2.4. Quantitative analysis of gene expression using real time quantitative polymerase chain reaction (RT-qPCR)

From the cells, the total RNA was extracted using GeneJet purification columns (Invitrogen, Thermo Scientific, Lithuania). Using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA) 1 μg of total RNA was converted into cDNA. Gene expressions were Quantitative analyses with the SYBRGreen PCR master mix (Applied Biosystems, Austin, TX, USA) on QuantStudio 5 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Appropriate primers (IDT, Coralville, IA, USA) were used for the analysis of KRT5, KRT6A, KRT8, SOX2, NANOG, MYC, CXCL5, CXCL10, MMP9, and TIMP1. The target genes expressions were then normalized to GAPDH by the ΔCt technique with the ΔΔCt method mRNA levels were calculated and were finally quantified by using the $2^{-\Delta\Delta Ct}$ method.

### 2.5. Statistical analysis of data

The result was presented as the mean ± standard deviation of the values from two independent experimental values. Comparison of each experimental group (Removable and Fixed) was individually done with the Normal group and the data were analysed using unpaired *t* test (two-tailed) on GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA), and $p < 0.05$

was considered significant and $p < 0.01$ was considered highly significant.

## 3. Results

### *3.1. Gingival tissue cells from normal removable and fixed showed altered surface antigen expression profiles for epithelial to mesenchymal transition-related cell–cell adhesion proteins (ECAD and NCAD) and mesenchymal stem cell-specific cell surface antigens (CD90 and CD105)*

Cells positive for ECAD were found to be significantly decreased in removable and fixed groups than normal (Fig. 1A-C and M). Also, shift in fluorescence intensities of these proteins were also found to be decreased in both the groups than normal (Fig. 1Q). Whereas cells positive for NCAD were found to be increased in the fixed than normal (Fig. 1D-F and N) and the shift in fluorescence intensity was also found to be increased in both removable and fixed than normal (Fig. 1R). CD90 and CD105 positive cells showed no significant difference in all the three groups (Fig. 1G-I, J-L, O, P) but shift in fluorescence intensity of CD90 was significantly increased in fixed than normal and CD105 shift in fluorescence intensity was found to be significantly decreased in removable and fixed than normal (Fig. 1S and T).

### *3.2. Gingival tissue cells from normal removable and fixed presented distinct surface antigen expression patterns for other stem cell-specific cell surface antigens (CD24, CD44, CD133, and CD146)*

CD24 and CD146 positive cells were to be increased significantly in removable and fixed than normal (Fig. 2A-C, J-L, M, and P) and also the shift in fluorescence intensity as well (Fig. 2Q and T). Whereas CD133 positive cells were decreased in removable and fixed than normal (Fig. 2G-I and O) and also the shift in fluorescence intensity as well (Fig. 2S). CD44 positive cells showed no noticeable change in all three groups (Fig. 2D-F and N) but shift in fluorescence intensity of CD44 were observed significantly increased in fixed than normal (Fig. 2R).

### *3.3. Gingival tissue cells from normal removable and fixed revealed dysregulated gene expressions for epithelial markers (KRT5, KRT6A, and KRT8), stemness-related transcription factors (SOX2, NANOG, and MYC), inflammatory chemokine genes (CXCL5 and CXCL10), and proteins regulating degradation of extracellular matrix proteins (MMP9 and TIMP1)*

The gene expression levels of KRT5, SOX2, NANOG, and CXCL5 were found to be significantly increased in removable and fixed than normal (Fig. 3A, D, E, and G). However, KRT8, CXCL10, and

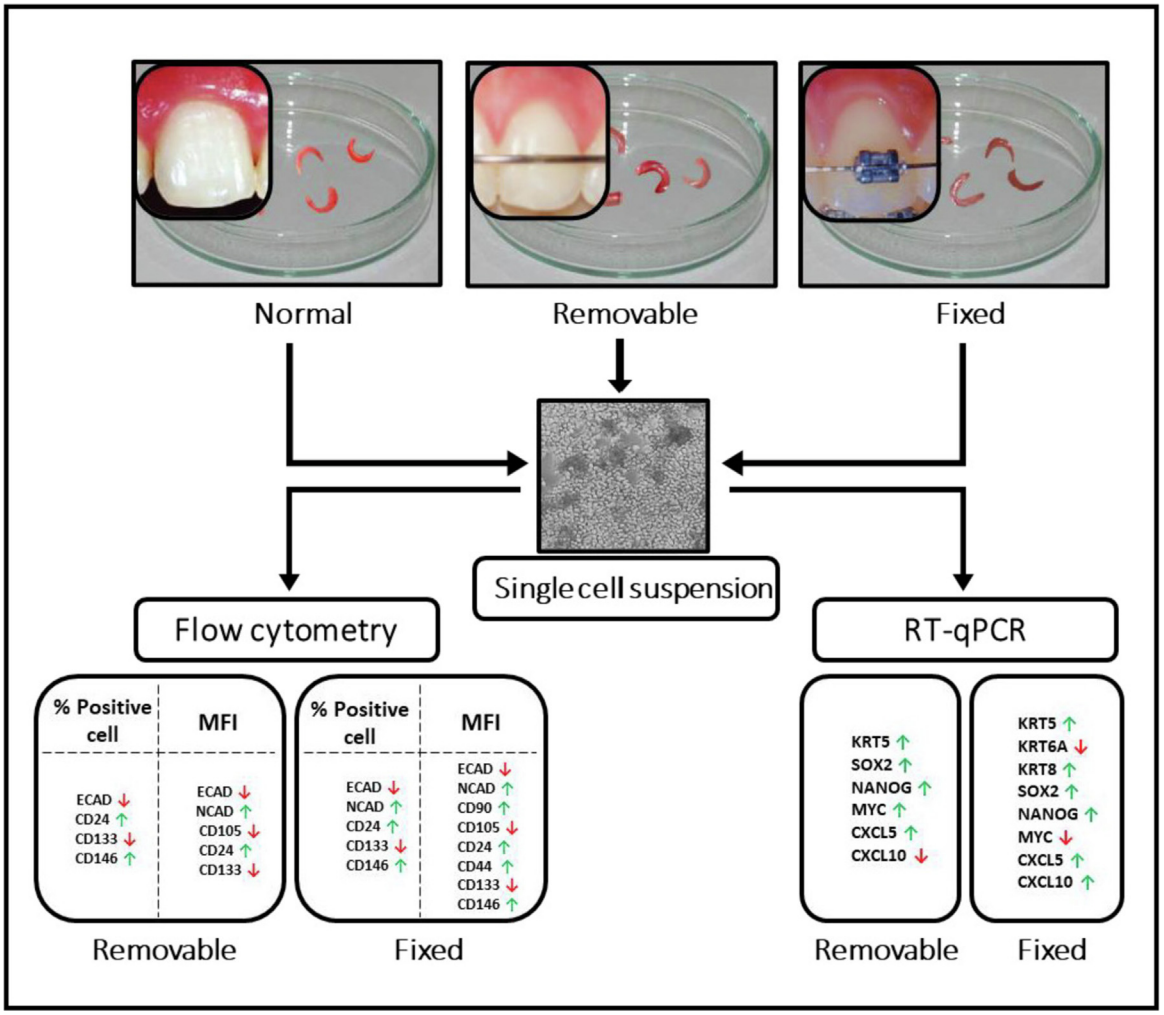


**Fig. 1.** Analysis of cell-surface markers by flow cytometry. (A-L) Analysis of surface marker expression of ECAD, NCAD, CD90, and CD105 in gingival tissue cells. (M-P) Comparative percentage positive cells for ECAD, NCAD, CD90, and CD105 in gingival tissue cells. (Q-T) Comparative median fluorescence intensities for ECAD, NCAD, CD90, and CD105 in gingival tissue cells. ns not significant, *$p < 0.05$, **$p < 0.01$. ECAD: Cadherin-1, NCAD: Cadherin-2, CD90: Thy-1, CD105: Endoglin.

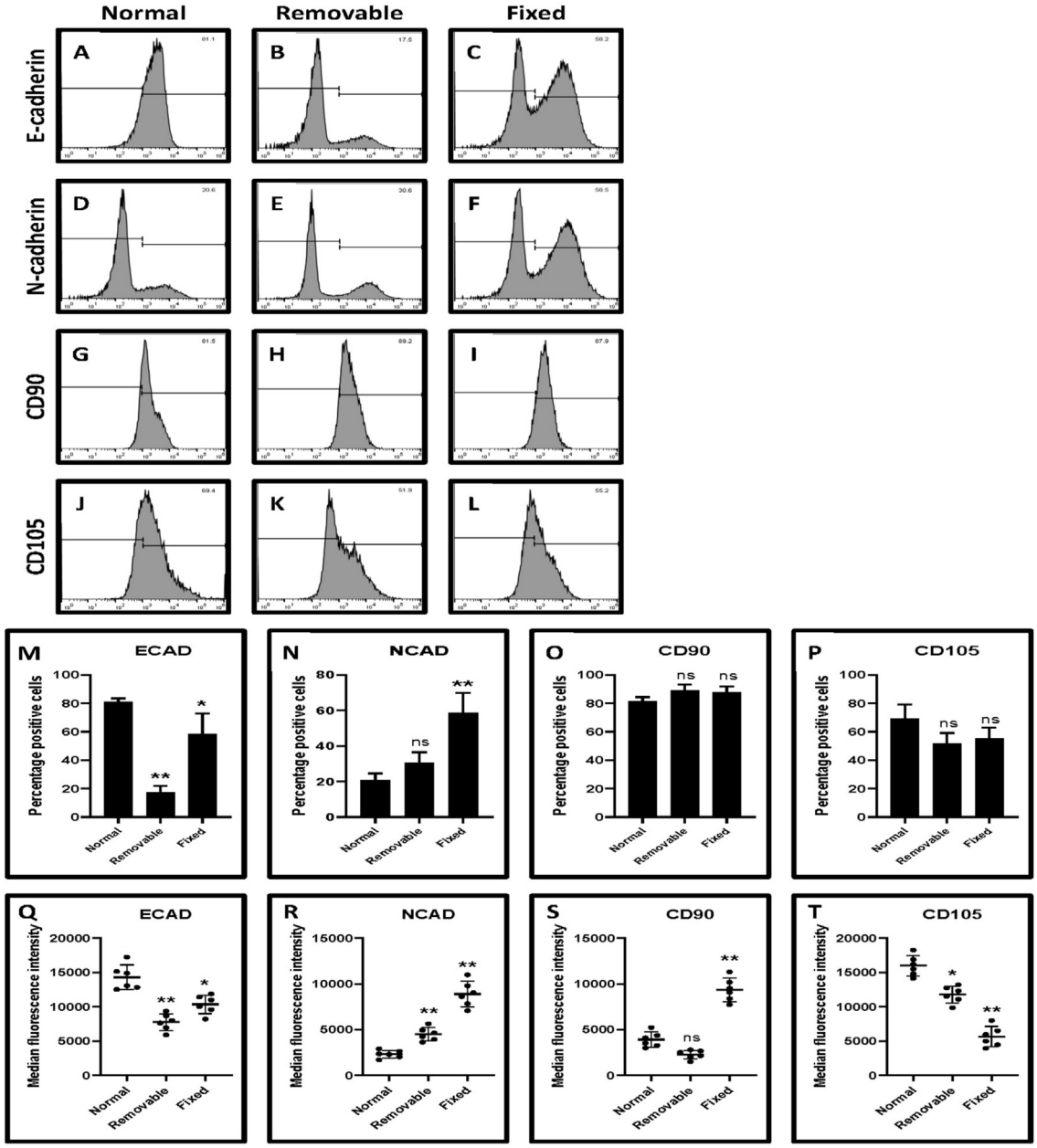


**Fig. 2.** Analysis of cell-surface markers by flow cytometry. (A-L) Analysis of surface marker expression of CD24, CD44, CD133, and CD146 in gingival tissue cells. (M-P) Comparative percentage positive cells for CD24, CD44, CD133, and CD146 in gingival tissue cells. (Q-T) Comparative median fluorescence intensities for CD24, CD44, CD133, and CD146 in gingival tissue cells. ns not significant, *p < 0.05, **p < 0.01. CD24: Heat stable antigen CD24, CD44: Hematopoietic cell E-selectin/L-selectin ligand, CD133: Prominin 1, CD146: Melanoma cell adhesion molecule.

TIMP1 were increased only in fixed than normal (Fig. 3C, H, and J), but CXCL10 showed decreased expression in removable than normal (Fig. 3H). KRT6A, MYC, and MMP9 were decreased in fixed than normal (Fig. 3B, F, and I). Whereas MYC and MMP9 were increased in removable than normal (Fig. 3F and I). KRT6A, KRT8, and TIMP1 showed no significant difference in removable than normal (Fig. 3B, C, and J).

The pictorial illustration of the study is shown in Fig. 4.

## 4. Discussion

Until today, periodontic-orthodontic interrelationship has been an matter of importance and it still is a controversial issue. The periodontal health gets affected by the malocclusion and one of the objective of the treatment through orthodontic appliances is to promote good oral health that prolongs the life of the dentition (Bollen et al., 2008). Although the dental and skeletal problems are improved by the orthodontic treatment, the placement of the orthodontic appliance in can alteration the habits of oral hygiene and periodontal health the in the patient. The orthodontic appliances and their mechanical procedures can evoke soft tissue responses in the gingiva. The proximity in which orthodontic appliance is placed in relation to gingival sulcus, accumulation of plaque further lead to complications of efficient orthodontic care (Boyd, 1983; Willmot, 2008; Zachrisson and Zachrisson, 1972).

Early interception with functional appliances in children with increased overjet has proven to be very effective in improving self-confidence, reducing the risk of trauma and avoiding negative social experiences. 10%–49% is the reported noncompliance rates

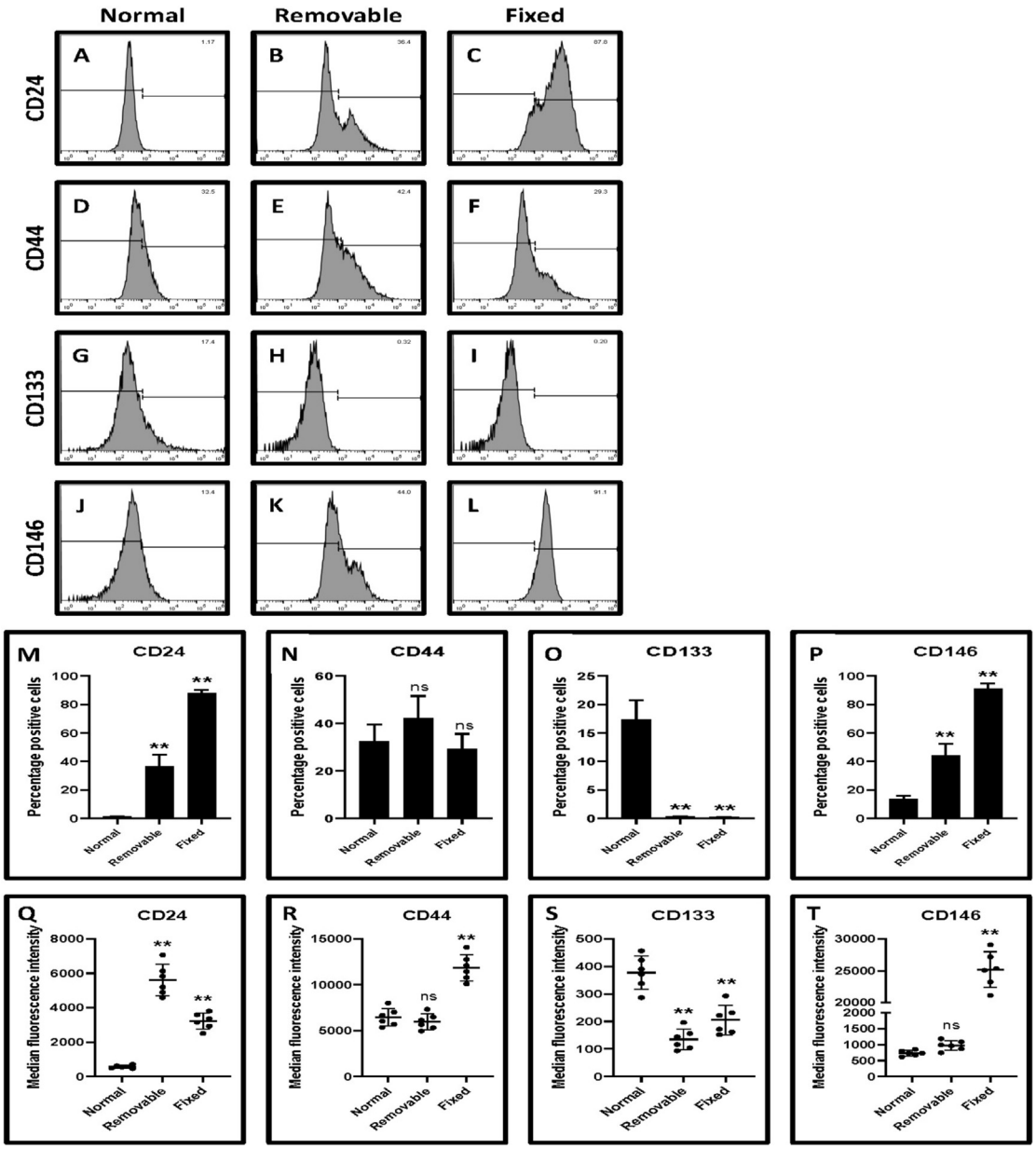


**Fig. 3.** Analysis of gene expression by quantitative RT-PCR. Relative gene expression of analysis by RT-qPCR. (A-J) Comparative gene expression analysis of KRT5, KRT6A, KRT8, SOX2, NANOG, MYC, CXCL5, CXCL10, MMP9, and TIMP1 in gingival tissue cells. ns not significant, *p < 0.05, **p < 0.01. KRT5: Keratin 5, KRT6A: Keratin 6A, KRT8: Keratin 8, SOX2: SRY (sex determining region Y)-box 2, NANOG: Homeobox protein NANOG, MYC: MYC proto-oncogene, bHLH transcription factor, CXCL5: C-X-C motif chemokine 5, CXCL10: C-X-C motif chemokine ligand 10, MMP9: Matrix metallopeptidase 9, TIMP1: TIMP metallopeptidase inhibitor 1.

with removable functional appliances reported in the literature (O'Brien et al., 2003; Seehra et al., 2011; Thiruvenkatachari et al., 2013).

The unspecialized cells that differentiate into any type of cell in an organism retaining the self-renewal capacity are the Stem cells. The regeneration and renewal of many vertebrate organs are enabled by adult stem cells (Alvarez-Buylla et al., 2001). The stem cells resides in a microenvironment called as Niche, which even influences the maintenance of these stem cells in various tissue types. The stem cell behavior is determined by the signals that eminate either from or outside the niche (Kai and Spradling, 2003). The fate of stem cells or progenitor cells are also regulated by the growth factors that are secreted from the tissues surrounding the niche (Ciruna and Rossant, 2001).

A study was done by Chengjuan Qu et al on the growth, stemness and angiogenic properties of dental pulp stem cells where expansion of human dental pulp stem cells in vitro was done using CGMO-defined xeno-free/serum free medium. These cells helped in maintaining their capability for tri-lineage differentiation although very few cells expressed the multipotency marker CD105. The decreased expression of CD105 in the XF medium could be restored when the cells were subsequently cultures in human serum-supplemented medium (Qu et al., 2020).

The transcription factors like Nanog, Sox2, and Oct4 are essential for maintaining the phenotype of pluripotent embryonic stem cell (Rodda et al., 2005). The embryonic stem cells and adult stem cells are maintained by SOX2 in multiple tissues (Arnold et al., 2011). The self renewal of the multiple types of stem cells are regulated by SOX2. It has also been said that SOX2 is oncogenic and plays an active role in forming squamous cell carcinoma in several organs.

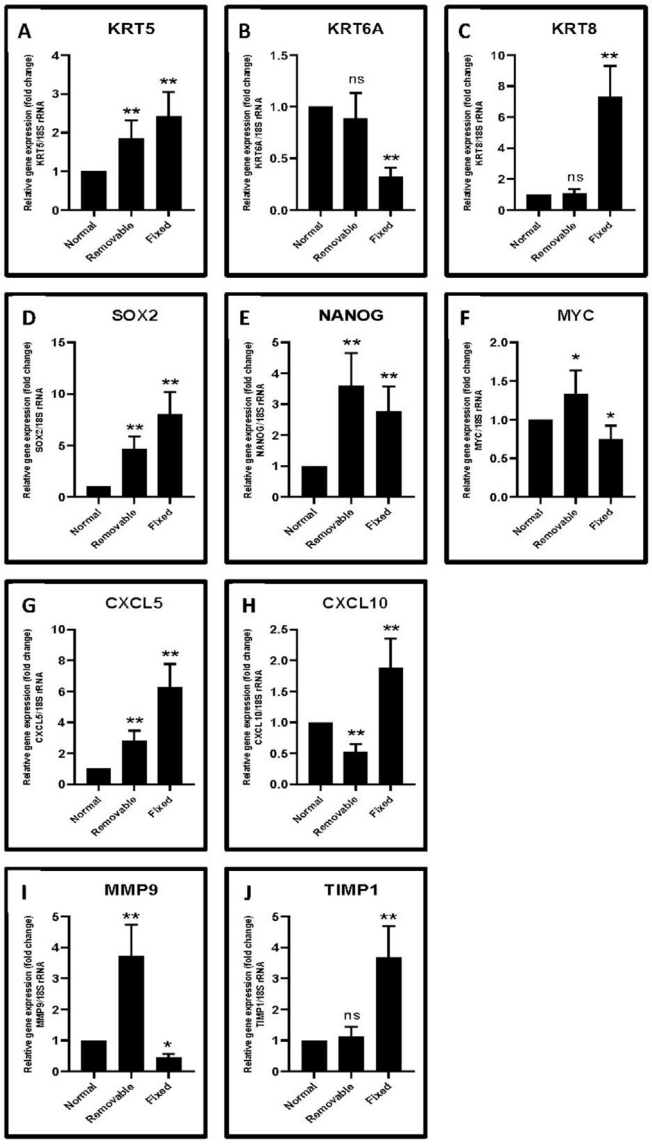


**Fig. 4.** Pictorial illustration of the study. Normal: gingival tissue samples from healthy patients without any treatment, Removable: gingival tissue samples from patients with removable functional orthodontic appliances, Fixed: gingival tissue samples from patients with fixed functional orthodontic appliances, MFI: median fluorescence intensity.

The tissue homeostasis is maintained by the progenitor/stem cells and the irregularity in them contributes to the initiations of tumor and progression of cancer (Barker et al., 2009). Sox 2 acts as signalign molecule and transcription factor and they are important for maintaining stem cells and this needs to be regulated strictly (Bass et al., 2009).

SOX2/OCT4 regulates the target gene NANOG which is expressed in only undifferentiated cells and is another key transcription factor that is a homeobox containing protein. NANOG suppresses or activates OCT4 which is said to maintain the undifferentiated state of the gene (Hepburn et al., 2019; Xu et al., 2020). Furthermore, OCT4/NANOG/SOX2 forms an interconnectexd regulatory circuit gets established as NANOG acts as a direct target for OCT4/SOX2 binding maintaining ESC pluripotency and self-renewal (Verneri et al., 2020).

A study done by Luchian I et al on Salivary MMP-9 was successful in quantifying periodontal inflammation during orthodontic treatment which stated salivary MMP-9 was strongly involved during orthodontic treatment quantifying inflammation in periodontal tissues. MMP-9 could be used to accurately predict the inflammation level associated with the type of malocclusion, making it viable and real diagnostic instrument during orthodontic treatment to monitor the periodontium evolution (Luchian et al., 2021).

Cells positive for ECAD and NCAD were found to be increased in fixed functional appliances where as CD90 and CD105 positive cells showed no significant difference in all the three groups. CD24 and CD146 positive cells were to be increased significantly in removable and fixed functional appliances than normal group whereas CD133 positive cells were decreased in removable and fixed functional appliances than normal group. CD44 positive cells showed no noticeable change in all three groups. The gene expression levels of KRT5, SOX2, NANOG, and CXCL5 were found to be significantly increased in removable and fixed appliance groups. However, KRT8, CXCL10, and TIMP1 were increased only in fixed appliance group but CXCL10 showed decreased expression in removable appliance group. KRT6A, MYC, and MMP9 were decreased in fixed appliance group whereas MYC and MMP9 were increased in removable appliance group. KRT6A, KRT8, and TIMP1 showed no significant difference in removable appliance group.

Very limited information was available in the literature regarding the cell-surface antigens and their genetic expressions in removable and fixed functional appliances in orthodontics. Through this study, we were able to assess the genetic expressions of the mentioned cells and correlate them with the type of functional appliances used.

## 5. Conclusion

This study demonstrated essential roles of various genes, showing their contribution in regulating cell proliferation and migration in both the removable and fixed functional appliances.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University, Abha, Saudi Arabia for funding this work through the Small Research Group Project under grant number (RGP-1/247/1442).

## References

Alvarez-Buylla, A., García-Verdugo, J.M., Tramontin, A.D., 2001. A unified hypothesis on the lineage of neural stem cells. Nat. Rev. Neurosci. 2 (4), 287–293. https://doi.org/10.1038/35067582.

Arnold, K., Sarkar, A., Yram, M., Polo, J., Bronson, R., Sengupta, S., Seandel, M., Geijsen, N., Hochedlinger, K., 2011. Sox2+ Adult Stem and Progenitor Cells Are Important for Tissue Regeneration and Survival of Mice. Cell Stem Cell 9 (4), 317–329. https://doi.org/10.1016/j.stem.2011.09.001.

Barker, N., Ridgway, R.A., van Es, J.H., van de Wetering, M., Begthel, H., van den Born, M., Danenberg, E., Clarke, A.R., Sansom, O.J., Clevers, H., 2009. Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 457 (7229), 608–611. https://doi.org/10.1038/nature07602.

Bass, A.J., Watanabe, H., Mermel, C.H., Yu, S., Perner, S., Verhaak, R.G., Kim, S.Y., Wardwell, L., Tamayo, P., Gat-Viks, I., Ramos, A.H., Woo, M.S., Weir, B.A., Getz, G., Beroukhim, R., O'Kelly, M., Dutt, A., Rozenblatt-Rosen, O., Dziunycz, P., Komisarof, J., Chirieac, L.R., LaFargue, C.J., Scheble, V., Wilbertz, T., Ma, C., Rao, S., Nakagawa, H., Stairs, D.B., Lin, L., Giordano, T.J., Wagner, P., Minna, J.D., Gazdar, A.F., Zhu, C.Q., Brose, M.S., Cecconello, I., Jr, U.R., Marie, S.K., Dahl, O., Shivdasani, R.A., Tsao, M.-S., Rubin, M.A., Wong, K.K., Regev, A., Hahn, W.C., Beer, D.G., Rustgi, A.K., Meyerson, M., 2009. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat. Genet. 41 (11), 1238–1242. https://doi.org/10.1038/ng.465.

Bollen, A.-M., Cunha-Cruz, J., Bakko, D.W., Huang, G.J., Hujoel, P.P., 2008. The effects of orthodontic therapy on periodontal health. J. Am. Dent. Assoc. 139, 413–422. https://doi.org/10.14219/jada.archive.2008.0184.

Boyd, R.L., 1983. Longitudinal evaluation of a system for self-monitoring plaque control effectiveness in orthodontic patients. J. Clin. Periodontol. 10, 380–388. https://doi.org/10.1111/j.1600-051X.1983.tb01287.x.

Casian Romero, A., Trejo Quiroz, P., De León Torres, C., Carmona Ruiz, D., 2011. Hiperplasia Fibrosa Inflamatoria: reporte de un caso. Rev. clínica periodoncia. Implantol. y Rehabil. oral 4, 74–79. https://doi.org/10.4067/S0719-01072011000200007.

Ciruna, B., Rossant, J., 2001. FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. Dev. Cell 1 (1), 37–49. https://doi.org/10.1016/S1534-5807(01)00017-X.

Eid, H.A., Assiri, H.A.M., Kandyala, R., Togoo, R.A., Turakhia, V.S., 2014. Gingival enlargement in different age groups during fixed Orthodontic treatment. J. Int. oral Heal. JIOH 6, 1–4.

Ghezzi, C., Masiero, S., Silvestri, M., Zanotti, G., Rasperini, G., 2008. Orthodontic treatment of periodontally involved teeth after tissue regeneration. Int. J. Periodontics Restorative Dent. 28, 559–567.

Hepburn, A.C., Steele, R.E., Veeratterapillay, R., Wilson, L., Kounatidou, E.E., Barnard, A., Berry, P., Cassidy, J.R., Moad, M., El-Sherif, A., Gaughan, L., Mills, I.G., Robson, C.N., Heer, R., 2019. The induction of core pluripotency master regulators in cancers defines poor clinical outcomes and treatment resistance. Oncogene 38 (22), 4412–4424. https://doi.org/10.1038/s41388-019-0712-y.

Kai, T., Spradling, A., 2003. An empty Drosophila stem cell niche reactivates the proliferation of ectopic cells. Proc. Natl. Acad. Sci. 100 (8), 4633–4638. https://doi.org/10.1073/pnas.0830856100.

Kloehn, J.S., Pfeifer, J.S., 1974. The effect of orthodontic treatment on the periodontium. Angle Orthod. 44, 127–134. https://doi.org/10.1043/0003-3219(1974)044<0127:TEOOTO>2.0.CO;2.

Luchian, I., Moscalu, M., Goriuc, A., Nucci, L., Tatarciuc, M., Martu, I., Covasa, M., 2021. Using Salivary MMP-9 to Successfully Quantify Periodontal Inflammation during Orthodontic Treatment. J. Clin. Med. 10, 379. https://doi.org/10.3390/jcm10030379.

Marchese, C., Visco, V., Aimati, L., Cardinali, G., Kovacs, D., Buttari, B., Bellocci, M., Torrisi, M.R., Picardo, M., 2003. Nickel-induced keratinocyte proliferation and up-modulation of the keratinocyte growth factor receptor expression. Exp. Dermatol. 12, 497–505. https://doi.org/10.1034/j.1600-0625.2002.120419.x.

O'Brien, K., Wright, J., Conboy, F., Chadwick, S., Connolly, I., Cook, P., Birnie, D., Hammond, M., Harradine, N., Lewis, D., McDade, C., Mitchell, L., Murray, A., O'Neill, J., Read, M., Robinson, S., Roberts-Harry, D., Sandler, J., Shaw, I., Berk, N. W., 2003. Effectiveness of early orthodontic treatment with the Twin-block appliance: a multicenter, randomized, controlled trial. Part 2: Psychosocial effects. Am. J. Orthod. Dentofacial Orthop. 124, 488–494. https://doi.org/10.1016/S0889540603006425.

Pancherz, H., Ruf, S., Kohlhas, P., 1998. "Effective condylar growth" and chin position changes in Herbst treatment: A cephalometric roentgenographic long-term study. Am. J. Orthod. Dentofac. Orthop. 114 (4), 437–446. https://doi.org/10.1016/S0889-5406(98)70190-8.

Qu, C., Brohlin, M., Kingham, P.J., Kelk, P., 2020. Evaluation of growth, stemness, and angiogenic properties of dental pulp stem cells cultured in cGMP xeno-/serum-free medium. Cell Tissue Res. 380 (1), 93–105. https://doi.org/10.1007/s00441-019-03160-1.

Rodda, D.J., Chew, J.-L., Lim, L.-H., Loh, Y.-H., Wang, B., Ng, H.-H., Robson, P., 2005. Transcriptional Regulation of Nanog by OCT4 and SOX2. J. Biol. Chem. 280 (26), 24731–24737. https://doi.org/10.1074/jbc.M502573200.

Seehra, J., Fleming, P.S., Newton, T., DiBiase, A.T., 2011. Bullying in orthodontic patients and its relationship to malocclusion, self-esteem and oral health-related quality of life. J. Orthod. 38 (4), 247–256. https://doi.org/10.1179/14653121141641.

Şurlin, P., Rauten, A.M., Pirici, D., Oprea, B., Mogoantă, L., Camen, A., 2012. Collagen IV and MMP-9 expression in hypertrophic gingiva during orthodontic treatment. Rom. J. Morphol. Embryol. 53, 161–165.

Thiruvenkatachari, B., Harrison, J.E., Worthington, H.V., O'Brien, K.D., 2013. Orthodontic treatment for prominent upper front teeth (Class II malocclusion) in children. Cochrane Database Syst. Rev. https://doi.org/10.1002/14651858.CD003452.pub3.

Verneri, P., Vazquez Echegaray, C., Oses, C., Stortz, M., Guberman, A., Levi, V., 2020. Dynamical reorganization of the pluripotency transcription factors Oct4 and Sox2 during early differentiation of embryonic stem cells. Sci. Rep. 10, 5195. https://doi.org/10.1038/s41598-020-62235-0.

Willmot, D., 2008. Orthodontic treatment and the compromised periodontal patient. Eur. J. Dent. 02 (01), 1–2.

Wu, Y.-J., Tu, Y.-K., Huang, S.-M., Chan, C.-P., 2003. The influence of the distance from the contact point to the crest of bone on the presence of the interproximal dental papilla. Chang Gung Med. J. 26, 822–828.

Xu, J., Zheng, Z., Du, X., Shi, B., Wang, J., Gao, D., Zhu, Q., Chen, X., Han, J., 2020. A cytokine screen using CRISPR-Cas9 knock-in reporter pig iPS cells reveals that Activin A regulates NANOG. Stem Cell Res. Ther. 11, 67. https://doi.org/10.1186/s13287-020-1588-z.

Zachrisson, S., Zachrisson, B.U., 1972. Gingival condition associated with orthodontic treatment. Angle Orthod. 42, 26–34. https://doi.org/10.1043/0003-3219(1972)042<0026:GCAWOT>2.0.CO;2.

Zanatta, F.B., Ardenghi, T.M., Antoniazzi, R.P., Pinto, T.M.P., Rösing, C.K., 2012. Association between gingival bleeding and gingival enlargement and oral health-related quality of life (OHRQoL) of subjects under fixed orthodontic treatment: a cross-sectional study. BMC Oral Health 12, 53. https://doi.org/10.1186/1472-6831-12-53.

Zhou, Q., Wang, H., 2014. Comparative study on the effect of fixed appliances and removable aligners. Stomatology 34, 784–786.