Cell culture on human oral mucosa cell in invitro study

Kultur sel pada sel mukosa mulut manusia dalam studi invitro

[1]Faiznur Ridho, [2]Wahyu Hidayat
[1]Oral Medicine Specialist Program, Faculty of Dentistry, Universitas Padjadjaran
[2]Department of Oral Medicine, Faculty of Dentistry, Universitas Padjadjaran
Bandung, Indonesia
Corresponding author: **Faiznur Ridho**, e-mail: **faiznur21001@mail.unpad.ac.id**

ABSTRACT
This systematic review discusses various cell culture techniques, growth media, growth factors and environmental factors that are often used on oral mucosal cells to obtain the most effective oral mucosal cell culture method. Based on the PRISMA statement, the search was conducted using three electronic databases, namely PubMed, Scopus, Ebsco Host for articles published in 2012-2022 using specific keywords. The search was limited to invitro research using cell culture, in English, full text, and human oral mucosal cells. Article quality assessment was conducted using the *Risk of Bias Assessment Tool for Non-randomised Studies* (RoBANS). Eleven articles had good eligibility and met the inclusion criteria for analysis. It was concluded that keratinocytes and fibroblasts for normal tissues, and OSCC for pathogenic tissues were most commonly used for oral mucosal cell culture, while the widely used culture media were F medium and keratinocyte medium.
Keywords: cell culture, invitro, oral mucosa

ABSTRAK
Tinjauan sistematis ini membahas berbagai teknik kultur sel, media tumbuh, faktor pertumbuhan dan faktor lingkungan yang sering digunakan pada sel mukosa mulut untuk mendapatkan metode kultur sel mukosa mulut yang paling efektif. Berdasarkan pernyataan PRISMA, pencarian dilakukan dengan menggunakan tiga database elektronik, yaitu PubMed, Scopus, Ebsco Host untuk artikel terbitan tahun 2012-2022 dengan menggunakan kata kunci tertentu. Pencarian dibatasi pada penelitian invitro menggunakan kultur sel, bahasa Inggris, *fulltext*, dan sel mukosa mulut manusia. Penilaian kualitas artikel dilakukan dengan menggunakan *Risk of Bias Assessment Tool for Non-randomized Studies* (RoBANS). Sebelas artikel memiliki kelayakan yang baik dan sesuai dengan kriteria inklusi untuk dianalisis. Disimpulkan bahwa keratinosit dan fibroblas untuk jaringan normal, dan OSCC untuk jaringan patogen paling sering digunakan untuk kultur sel mukosa mulut, sedangkan media kultur yang banyak digunakan adalah media F dan media keratinosit.
Kata kunci: kultur sel, invitro, mukosa mulut
Received: 10 Ocotober 2024 Accepted: 1 January 2025 Published: 1 April 2025

INTRODUCTION

The cell is the smallest structural and functional unit that supports the processes of human life. The human body consists of thousands to millions of cells that are its constituent components. Cells have an important role in human life. Today, cells have been developed as a medium for therapeutic trials in medical science. Researchers have developed a method of cell culture (CC) so that cells can grow and multiply in certain media and conditions. These growing cells resemble the actual conditions of cells in the human body. This is a good way for testing the new medical treatment for human being.[1-3]

The first scientist that use cell culture for his research is Wilhem Roux in 1885, who proved that chick embryos had the ability to survive for several days in warm saline. Then, Ross G.Harrison in 1907 reported that embryonic nerve fibers can grow in lymph clots. The same thing happened to, Alexis Carell in 1913 who observed the growth of fragmentation of a tissue in plasma tissue after incubation. In 1928, Maitsland demonstrated that the vaccinia virus could reproduce in a medium containing a tissue suspension. Research related to viruses was also carried out by Enders in 1949. He succeeded in publishing his research that the polio virus could be cultured in tissue culture. The first quantitative study of CC was carried out by Abercrombie and Heayssman in 1954 who observed that there was a barrier to contact between fibroblast cells. In 1955, Harry Eagle developed and discovered artificial media, attachment factors and feeder layers. In 1961, Hayflick and Mooehead described the finite lifespan of normal human diploid cells. Furthermore, research continues on tumor cells, Bionassisi and team in 1962 succeeded in publishing a method for defending differentiated cells from tumor cells. CC research on cell differentiation continues. In 1968 David Yaffle conducted an research related to the differentiation of normal myoblasts in vitro.[2]

The CC must be done with the right technique. Currently, there are two kinds of CC techniques that often used. They are primary CC and cell line. These two types of CC techniques have different methods. Primary CC is a CC method that takes cells directly from humans and then placed them in certain media and conditions in order to grow. Meanwhile, the cell line culture technique uses pre-existing CC and then subcultured them again to grow them. Cell line is the first subculture produced from primary culture.[2,4]

Dentistry is one of the health fields that has used CC for testing the use of new drugs and therapies. The aim of the CC in dentistry to verify the effectiveness of a treatment and therapy against the oral mucosa cells. There are so many researcher that used CC for their research. For example Harun et all used the human tongue cancer cell line SP-C1 for investigating the anticancer and antiproliferation activity of ethanol fraction of ant nest plants (*Myrmecodya pendans*).[5] Nugroho et all used the fibroblast BHK-21 CC for investigating the effect of cytotoxicity of 5% ethanol extracts of moringa oleifera leaf as an alternative of root canal irrigant.

There are various types of cells can be found in the oral cavity. Oral mucosal cells consist of keratin and non-

DOI 10.35856/mdj.v14i1.1208

keratinized cells. The various in cell types causes the differences of culture technique that should be used. The researcher should consider any aspect before selecting the apropiate CC technique. The selection of methods and interventions for the components used must be considered carefully so that cells can grow properly and in accordance with the research objectives.

METHODS

Protocol and eligibility criteria

The systematic review was carried out using the *preferred reporting items for systematic reviews and meta-analyses* (PRISMA) method. PICO components used include the population, namely cells in the human oral mucosa, intervention, namely CC both primary and cell line, comparison, namely normal cells and cancer cells, and outcomes that include proliferation of cells after culture.

The inclusion criteria used included research articles published 2012-2022 with access to complete, English-language and indexed manuscripts, research conducted on oral cavity cells in vitro using primary CC techniques and cell lines. The researcher conducted a systematic review because to date there has been no systematic review of CC in the oral mucosa in the last ten years 2012-2022. The exclusion criteria of this systematic review included duplication, irrelevant topics and not performed on the human oral mucosa.

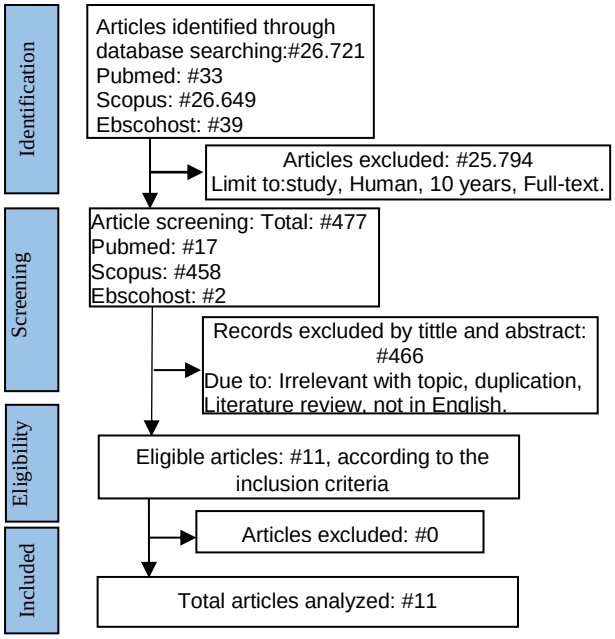

Figure 1 PRISMA flowchart

Information sources and search

The literature search was conducted electronically using Pubmed, Scopus, and Ebsco Host published 2012-2022. The keywords used for this review is *(((((cell culture[MeSH Terms]) OR ("cell culture primary"[MeSH Terms])) OR ("cell line"[MeSH Terms])) OR (in vitro [Me SH Terms])) AND (oral mucosa[MeSH Terms])) OR (oral medicine[MeSH Terms]).*

Risk of bias studies

The bias test in this review uses a non-randomized bias test, *Risk of Bias Assessment of Non-randomized Studies* (RoBANS).[7] This bias test was chosen because it was considered the most appropriate to measure the quality of non-randomized and observational studies. The criteria used in the Robans test consist of six indicators, namely selection of material studies, confounding variables, intervention (exposure) measurement, binding of outcome assessment, incomplete outcome data, selective outcome reporting.

Each indicator has an assessment criterion, i.e. if the indicator is found, it is given a value of 1, but if it is not found then 0 points are given. Then all the total points are added up, if the results are 0-2 then the risk of article bias is considered high and if points 3-5 then the risk of bias is considered low. All articles (Table 2) have a low bias category (good quality).

RESULTS

Figure 1 shows the flowchart of identification, screening and eligibility assessment based on the inclusion criteria. The articles were obtained from the PubMed database, Scopus, and Ebscohost. After screening based on duplication, exclusion, and inclusion criteria as well as review of titles and abstracts, 11 articles were obtained for further analysis.

Table 1 describes the 11 selected articles obtained by the method. The selected articles obtained include CC of the oral mucosa both primary and cell line. The studies include cells found in the oral cavity. They are keratinocyte cells, fibroblasts and also oral squamous cell carcinoma (OSCC). In addition, CC media for oral mucosa that are often used are keratinocyte serum free medium, Dulbecco's modified eagles medium (DMEM) and F medium.

The cells were incubated after given media. Based on the table above, the oral mucosal cells were incubated at room temperature, which is 37^{0}C with 5% of CO2 gas. In addition, the main purpose of CC on the oral mucosa is to verifiy a new therapy or treatment to the proliferation of human oral mucosal cells.

Item 1 = selection of material studies; item 2 = confounding variables; item 3 = intervention (exposure) measurement; item 4 = blinding of outcome assessment; item 5 = incomplete outcome data; item 6 = selective outcome reporting; 1 = Yes; 0 = No; Total point 0-2 = high; total point 3–5 = low.

The results of the bias test showed that two articles had a bias risk value of five (low risk of bias) and the remaining nine articles had a bias risk value of six (low risk of bias). Based on this test, it was found that all articles had a low risk of bias. This shows that all articles are of good quality and deserve further analysis.

DISCUSSION

The CC is a process for placing living cells into a medium that can allow them to reproduce or grow in vitro.[2] By using CC the properties and biological processes of cells which are difficult to study when using whole organisms, can be analyzed. CC is valuable tool for researchers in various fields to develop science related to cells.[8]

Cell culture can be divided into two types, primary CC

Table 1 Characteristics of enrolled studies

No	Author	Kinds of Cell	Medium	Intervention	The Purpose of The Research
1.	Das et al; 2021	Primary human oral keratinocytes	Keratinocyte Serum Free Medium	incubated in 5% CO2 at a 37°C	In vitro study of the effect of growth factor Granulocyte macrophage-colony stimulating factor and keratinocyte growth factor on the stages of differentiation of oral mucosal epithelium
		Primary human normal oral fibroblasts	Minimum Essential Medium Eagle	incubated in 5% CO2 at a 37°C	
2	Marshall et al; 2017	Primary HOK	Keratinocyte Basal Medium-2 Free Serum	incubated in 5% CO2 at a 37°C	In Vitro study to test CD 40 AND CD 82 on OLP patients
3	Cao T et al; 2017	HOK on OLP patitent	Oral Keratinocyte Medium (OKM)	incubated in 5% CO2 at a 37°C	To create an OLP model that resembles clinical conditions in humans so that it can be used for further research .
4	Houacine et al; 2020	Oral Keratinocytes cell line OKF6/TERT1	Keratinocytes Serum-Free Medium (KSFM)	incubated in 5% CO2 at a 37°C	In Vitro Study of P53 Protein in Bechet's Disease Patients
5	Wada et al; 2022	Oral keratinocytes cell line RT-7 cells	Keratinocyte Serum Free Medium - KSFM	incubated in 5% CO2 at a 37°C	Invitro study of the effects of Juncus effusus L. extract on oral keratinocytes
6	Wang et al; 2020	Cell line the HOK	Keratinocyte Serum Free Medium - KSFM	incubated in 5% CO2 at a 37°C	In order to test the effect of disulfiram on OSCC
7	Ardakani et al; 2015	Human gingival fibroblast (HGF) cell line	Dulbecco's Modified Eagles Medium	incubated in 5% CO2 at a 37°C	In Vitro Study of laser effect on fibroblast cell viability
8	Canciani et al; 2021	HGF cell line	Dulbecco's Modified Eagles Medium	incubated in 5% CO2 at a 37°C	In Vitro Study to see the response of fibroblasts when given hyaluronic acid enriched with vitamins and amino acids
9	Kim et al; 2022	HSC2 OSCC cell line	F Medium (Dulbecco's Modified Eagles Medium Dan F-12 Ham)	incubated in 5% CO2 at a 37°C	In vitro study about the effect of Osbeckia octandra extract to induce OSCC apoptosis
		YD OSCC cell line	F Medium (Dulbecco's Modified Eagles Medium Dan F-12 Ham)	incubated in 5% CO2 at a 37°C;	
10	Sakamoto et al; 2017	OSCC cell lines HSC-2, HSC-3, SQUU-A, SQUU-B, SQUUBO, SQUU-BC, dan SAS	F Medium (Dulbecco's Modified Eagles Medium Dan F-12 Ham)	incubated in 5% CO2 at a 37°C.	In vitro study of OSCC
11	Sun et al; 2022	Human OSCC cell line (SCC-25)	F Medium (Dulbecco's Modified Eagles Medium Dan F-12 Ham).	incubated in 5% CO2 at a 37°C	In Vitro study to examine the effect of procyanidine on angiogenesis and growth of OSCC

Note: OLP (Oral Lichen Planus), OSCC (Oral Squamous Cell Carcinoma), SFM (Serum Free Medium)

Table 2 Risk of bias

No.	Author	item 1	item 2	item 3	item 4	item 5	item 6	Total Poin	Risk of bias	Quality
1	Das *et al.,* 2021	1	1	1	0	1	1	5	low	high quality
2	Marshall *et al.,* 2017	1	1	1	1	1	1	6	low	high quality
3	Cao T *et al.,* 2017	1	1	1	1	1	1	6	low	high quality
4	Houacine *et al.,* 2020	1	1	1	0	1	1	5	low	high quality
5	Wada *et al.,* 2022	1	1	1	1	1	1	6	low	high quality
6	Wang *et al.,* 2020	1	1	1	1	1	1	6	low	high quality
7	Ardakani *et al.,* 2015	1	1	1	0	1	1	6	low	high quality
8	Canciani *et al.,* 2021	1	1	1	0	1	1	6	low	high quality
9	Kim *et al.,* 2022	1	1	1	1	1	1	6	low	high quality
10	Sakamoto *et al.,* 2017	1	1	1	1	1	1	6	low	high quality
11	Sun *et al.,* 2022	1	1	1	1	1	1	6	low	high quality

and CC using cell lines. Primary CC is a CC method that takes cells directly from living things and then culture them in a certain media. The cells are isolated from the human tissue and then reproduce under suitable conditions until they occupy all available substrates and reach greater numbers.[3] There are two types of primary CC methods that are often used, they are direct and enzymatic methods. [2]

At this systematic review, there are three articles discussed primary cultures of the human oral mucosa cells. The cells cultured in the study included keratinocytes and fibroblasts. Media selection and intervention media conditions are very important to support cell growth. Oral cell must be cultured on appropriate media. The cell medium and environment should be mimicking the real condition of human cell. There are some factors that must be concern when culturing cell in vivo. All that factors is the key for the successful for oral CC; 1) tonicity, that is important factor for culturing cells. Tonicity is related to a process of entry of cell membrane chemicals which is regulated by osmotic pressure from each side of the cell membrane. The osmotic pressure of mammalian cell membranes is around 7.6 atmospheres with a tolerance limit of 10%,[2] 2) temperature, is one of the most important factors to support cell growth. The optimal cell temperature ranges 36-38.5^{0}C. If the cell exceeds the optimal temperature, then the cells will be destroyed. However, if the cell temperature drops to -180^{0}C, the cell will survive for a long time and can be reproduced again. If the cell is stored at a temperature of 30-33^{0}C, the cell's metabolic processes still occur but do not undergo division,[2,9] 3) potential hidrogen (pH). Every single of cell has different pH for growing in culture medium. In general, the optimal pH for cell growth is 7.2 and for the cell line at pH 7.4. At a pH 6.8-7.6 cells are still able to divide. Some fibroblast cells showed the best growth at pH 7.4-7.7. The cells will stop dividing and lose their viability at pH below 6.5.[2,10] 4) inorganic salt, play important role for culturing cells. The function of inorganic salts is to maintain tonicity. The other function of inorganic salts is needed as components of intracellular enzymes and the respiratory system of cells. The essential ions needed for cell growth are sodium, potassium, calcium, magnesium, iron, carbonate, phosphate, and sulfate,[2,9] 5) amino acids, that are needed by cells for the synthesis of proteins and nucleic acids. Amino acid requirements vary from one cell to another. Essential amino acids that are often needed by cells are cysteine, arginine, glutamine and tyrosine,[9] 6) carbohydrates, are used as an energy source are glucose, galactose and other sugars. CC with higher nutrients will cause cells to divide rapidly,[10] 7) vitamins, act as co-enzymes or catalysts in cell metabolism and essential vitamins that used in CC comes from B-vitamin group,[2] 8) protein, will be broken down by extracellular enzymes before being used by cells to grow. Proteins function to support cloning and maintain cell conditions.[2,9]

Based on the analysis from the selected articles, several CC were obtained on the human mucosa. The oral mucosa cells used on the selected articles are keratinocyte cells, fibroblasts and OSCC cells. Besides that, CC media used include keratinocyte media, DMEM media, and F media.

Oral keratinocyte cells were cultured on keratinocyte serum free medium. Based on the analysis carried out in this study, several articles were found that supported the keratinocyte serum free medium for the growth of oral mucosal keratinocytes cells either primarily or using a cell line. Table 1 describes various studies of oral keratinocyte CC. Research from Das et al, describes the steps for conducting CC on normal keratinocyte cells from the human oral mucosa primarily those were grown on keratinocyte serum free medium.[11] The research of Marshall et al, describes primary cultures derived from human oral cavity keratinocytes. Based on this study, normal oral mucosal tissue was isolated by separating the connective tissue from the normal excised tissue. Keratinocyte CC was also performed in patients with oral lichen planus (OLP) lesions.[12] Cao et al, used oral keratinocyte serum free medium to culture keratinocyte cells in reticular type OLP patients. In this study, the result of confluent cell growth was 80%.[13]

The CC of oral mucosal keratinocytes was also performed using a cell line (Table 1). Houacine et al, cultured oral keratinocytes from the OKF6/TERT1 cell line oral keratinocytes. Cells were maintained in keratinocyte serum free medium routinely.[14] Cell line research using oral keratinocytes was also done by other researchers. Wada et al, using cell line oral keratinocytes RT-7 cells. Cells were grown in keratinocyte serum free medium.[15] Besides that, other researcher also used this media for culturing oral keratinocyte cells. Wang et al, cultured HOK cell line on keratinocyte serum free medium.[16]

The use of keratinocyte serum free medium has been used by various previous studies. The use of serum-free media has been used more than four times when compared with serum-treated media. Serum-free keratinocyte growth media aims to maintain the basal keratinocyte population by regulating calcium levels contained in growth media of keratinocyte cells. The calcium levels that can be used in keratinocyte growth media are less than 0.1 mmol/L. This condition provides an advantage for growth of keratinocyte cells. Low levels of calcium and serum that is not present in the media can cause cells other than keratinocytes not to grow. This will certainly reduce the risk of the growth of contaminants in the keratinocyte cell media.[17,18]

In vitro studies using keratinocyte media are not only used on oral mucosal cells. Research was also done on human body cells. Lamb and Ambler cultured keratinocyte cells derived from neonatal epidermal keratinocytes on serum-free keratinocyte medium. The culture results showed a confluent growth of 80% of keratinocyte cells.[19]

Another type of oral mucosal cell that is often used for CC is fibroblast cells. Based on the selected articles presented in table 1, fibroblast are cultured primary and also using cell line. Ardakani et al, used the HGF cell line to verifiy the effectiveness of laser light on human oral mucosal fibroblast cells. Cells were cultured in DMEM; were

DOI 10.35856/mdj.v14i1.1208

incubated in 5% CO2 in 37°C.[20] The reseach of oral mucosal fibroblast studies were also conducted by Cancianiet al that used HGF cell line. The cell line were cultured on DMEM medium.[21] Dass et al, cultured fibroblast primarily for their reseach. In this study, fibroblast cells from the oral mucosa were grown in the *minimum essential medium eagle medium*, which is a variation of DMEM media or also known as Dulbecco/Vogt modified *eagle's essential medium*.[2,21]

Oral mucosal fibroblast were cultured on DMEM that contains inorganic salts, amino acids, glucose and some vitamins needed by cells to grow. DMEM media contains four times more vitamins and amino acids as well as two to four times more glucose than the original formula. In addition, this media also has additional components. They are iron and phenol red.[2,22]

The CC studies were also conducted on human oral cancer cells. The study was conducted on several cell lines with the aim of testing the effectiveness of a drug or therapy on human oral cancer cells. Kim et al, conducted a study in the form of an application of a Sri Lankan endemic plant, namely Osbeckia which stimulates apoptosis in human oral cancer cells. CC were taken from HSC2 OSCC cells and YD OSCC cell lines, then cells were cultured in F Medium (DMEM and F-12 Ham).[23] Cell line studies for cancer cells were also carried out by other researchers. Sakamoto et al, cultured OSCC cell lines HSC-2, HSC-3, SQUU-A, SQUU-B, SQUUBO, SQUU-BC, and SAS. These cells were also cultured in DMEM/F-12.[24] Another research was also done by Sun et al, who conducted in vitro studies on the human oral cavity cancer cell line, human OSCC cell line (SCC-25) then cells were cultured in modified DMEM/F-12 medium.[25]

Based on the analysis carried out on selected articles, the most widely used medium for culturing human oral mucosal cancer cells was F media, which is a combination of DMEM and F-12 media that will form a new combination of media that has high levels of amino acids, vitamins and other elements so that it can increase the proliferation of cells that will grow. The successful of using this media is also supported Izham et al, used a combination of DMEM and F-12 media for culturing neuroblastoma cell lines compared to DMEM media. Based on this study, it was found that neuroblastoma cells cultured with DMEM and F-12 media was more than DMEM alone. [26,27]

Media F is a combination of DMEM media with F-12 with a 1:1 composition. Another studies have demonstrated the ability of this medium to grow cells. The research of Guo et al, showed that DMEM-F-12 media was the right medium to grow sertoli cells which played an important role in the process of human spermatogenesis. The results show that there are a higher number of cells of sertoli when culture in F medium.[28]

There are so many factors can cause failure in CC. The factors are chemical and biological contaminants. Chemical contaminants include media and components in CC, water, air, plastic storage containers, fluorescent light, incubators, and endotoxins. Meanwhile, biological contaminants can include microorganisms that grow together with the cells to be cultured, namely bacteria, fungi, viruses, protozoa and invertebrates.[2]

It is concluded that oral mucosal CC is widely used to identify the effectiveness of the therapeutic agent to the human oral mucosal cells in vitro. The most commonly used oral mucosal CC for the normal cell were derived from keratinocytes and fibroblasts. For the pathogenic tissue, the oral mucosal CC that usually used were derived from OSCC. The medium that commonly used for oral mucocal CC were keratinocyte serum free medium and F medium.

Acknowledgment

Authors are grateful to Oral Medicine Department, Faculty of Dentistry, Padjadjaran University for the kindly help during the writing process of this systematic review.

Conflict of Interest

The authors report no conflicts of interest.

REFERENCES

1. Sherwood L, Ward C. Human physiology from cells to systems. Ottawa: Nelson Education Ltd; 2019.p.3–22.
2. Ma'at S. Teknik dasar kultur sel. Surabaya: Airlangga University Press; 2011. p.3–27.
3. Scientific TF team. Cell culture basics handbook. Gibsco; 2020.
4. Mitry RR, InstituteHughes RD. Human cell culture protocols, 3rd Ed [Internet]. New York: Humana Press; 2012. Available from: http://books.google.com/books?id=Ku2wPAAACAAJ
5. Achmad H, Supriatno, Marhamah, Rasmidar. Aktivitas antikanker dan antiproliferasi fraksi etanol sarang semut (Myrmecodya pendans) pada sel kanker lidah manusia SP-C1. Dentofasial 2014;13(1):1–6.
6. Nugroho JJ, Trilaksana AC, Natsir N, Rovani CA, Hikmah N. Cytotoxicity of 5% ethanol extracts of moringa oleifera leaf as an alternative of root canal irrigant to fibroblast BHK- 21 cell culture. J Dentomaxillofac Sci 2021;6(1):39–41.
7. Kim SY, Park JE, Lee YJ, Seo HJ, Sheen SS, Hahn S, et al. Testing a tool for assessing the risk of bias for nonrandomized studies showed moderate reliability and promising validity. J Clin Epidemiol 2013;66(4):408–14.
8. Helgason CD. Basic cell culture protocols. 4th Ed. Miller CL, editor. New York: Humana Press; 2013.
9. Rodríguez-Hernández CO, Torres-García SE, Olvera-Sandoval C, Ramírez-Castillo FY, Muro AL, Avelar-Gonzalez FJ, et al. Cell culture: history, development and prospects. IntJCurrResAca 2014;2(12):188–200.
10. Chaudhary MV, Singh P. Cell line: a review. IJARSE 2017;6(4).
11. Das R, Virlan MJR, Xenaki V, Kulasekara KK, Lukandu O, Neppelberg E, et al. Granulocyte macrophage-colony stimulating factor and keratinocyte growth factor control of early stages of differentiation of oral epithelium. Eur J Oral Sci 2022;
12. Marshall A, Celentano A, Cirillo N, Mirams M, McCullough M, Porter S. Immune receptors CD40 and CD86 in oral keratinocytes and implications for oral lichen planus. J Oral Sci 2017;59(3):373–82.
13. Cao T, Zhang H, Zhou L, Wang Y, Du G, Yao H, et al. In vitro cell culture system optimization of keratinocytes from oral lichen planus (OLP) patients. 2017;(March 2016):225–32.
14. Houacine S, Kang A, Parkinson EK, Wan H, Fortune F. Induction of p53 in keratinocyte cultures treated with Behçet's patient sera. J Oral Pathol Med 2020;49(5):435–42.
15. Wada A, Murakami K, Ishikawa Y, Amoh T, Hirao K, Hosokawa Y, et al. Anti-inflammatory and protective effects of Juncus effusus L. water extract on oral keratinocytes. Biomed Res Int 2022;2022.
16. Wang Z, Jiang H, Cai LY, Ji N, Zeng X, Zhou Y. Repurposing disulfiram to induce OSCC cell death by cristae dysfunction promoted auto-

phagy. Oral Dis 2021;27(5):1148-60. doi: 10.1111/odi.13652. Epub 2020 Oct 14. PMID: 32989819.
17. Zare S, Zarei MA, Ghadimi T, Fathi F, Jalili A, Hakhamaneshi MS. Isolation, cultivation and transfection of human keratinocytes. Cell Biol Int 2014;38(4):444–51.
18. Strudwick XL, Lang DL, Smith LE, Cowin AJ. Combination of low calcium with Y-27632 rock inhibitor increases the proliferative capacity, expansion potential and lifespan of primary human keratinocytes while retaining their capacity to differentiate into stratified epidermis in a 3D skin model. PLoS One 2015;10(4):1–12.
19. Lamb R, Ambler CA. Keratinocytes propagated in serum-free, feeder-free culture conditions fail to form stratified epidermis in a reconstituted skin model. PLoS One 2013;8(1):1–8.
20. Talebi-Ardakani MR, Torshabi M, Karami E, Arbabi E, Rezaei Esfahrood Z. In Vitro study of Er:YAG and Er,Cr:YSGG laser irradiation on human gingival fibroblast cell line. Acta Med Iran 2016;54(4):251–5.
21. Canciani E, Sirello R, Pellegrini G, Henin D, Perrotta M, Toma M, et al. Effects of vitamin and amino acid-enriched hyaluronic acid gel on the healing of oral mucosa: In vivo and in vitro study. Med 2021;57(3).
22. Rohanova D, Boccaccini DHAR, Bozdechov, Pavl´ına, Alov ´Castor, Bezdicka P, et al. Is non-buffered DMEM solution a suitable medium for in vitro bioactivity tests? J Mater Chem B 2014; 2:5068-76.
23. Kim JY, Kim J, Bandara BMR, Tilakaratne WM, Kim D. Leaf extract of Osbeckia octandra induces apoptosis in oral squamous cell carcinoma cells. BMC Complement Med Ther 2022;22(1):1–10.
24. Sakamoto T, Kawano S, Matsubara R, Goto Y, Jinno T, Maruse Y, et al. Critical roles of Wnt5a–Ror2 signaling in aggressiveness of tongue squamous cell carcinoma and production of matrix metalloproteinase-2 via ΔNp63β-mediated epithelial–mesenchymal transition. Oral Oncol 2017;69:15–25.
25. Sun Q, Zhang T, Xiao Q, Mei B, Zhang X. Procyanidin B2 inhibits angiogenesis and cell growth in oral squamous cell carcinoma cells through the vascular endothelial growth factor (VEGF)/VEGF receptor 2 (VEGFR2) pathway. Bioengineered 2022;13(3):6500–8.
26. Izham MA, Chia JSM, Vidyadaran S, Sulaiman MR, Bharatham BH. The effect of DMEM and DMEM:F12 culture media on the growth of SH-SY5Y Cells. Life Sci Med Biomed 2018;2(3):3–6.
27. Sakagami H, Suzuki R, Shirataki Y, Iwama S, Nakagawa M, Suzuki H, et al. Re-evaluation of culture condition of PC12 and SH-SY5Y cells based on growth rate and amino acid consumption. In Vivo 2017;31(6):1089–95.
28. Guo Y, Hai Y, Yao C, Chen Z, Hou J, Li Z, et al. Long-term culture and significant expansion of human Sertoli cells whilst maintaining stable global phenotype and AKT and SMAD1/5 activation. Cell Commun Signal 2015;13(1):1–13.