RESEARCH ARTICLE

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Isolation, Culture and Morphological Assessment of Primary Cell Lines from Human Primary Oral Squamous Cell Carcinoma Using Explant Technique

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Abstract

Background: Due to many uses of cell culture in cell biology, biotechnology, and medical research, this technique has evolved into a widely used and accepted methodology. The isolation of primary cells from primary cancer tissue is a crucial step in cell culture technology since it offers a trustworthy source for studying the biology, morphology, and molecular evaluation of cancer cells, just like in the oral cavity tissue of patients. Therefore, the technique used for the isolation, culture, and evaluation of these cells is crucial. **Aim:** The aim of the present study is to isolate and culture the cells from human primary Oral Squamous Cell Carcinoma [OSCC] tissue and evaluate them for morphological variations using an explant method. **Materials and Methods:** The patients with OSCC who were undergoing surgery provided the tissue samples. An explant technique was used to achieve the isolation of cells from tissue samples. Following that, the cells were maintained, subcultured, and stored in accordance with the standard American Type Culture Collection [ATCC] protocol. Routine Hematoxylin & Eosin and crystal violet stains were used. These cells were morphologically studied, and the results were assessed for further studies. **Results:** We were able to successfully isolate and culture cells from 4 different tissue samples using the explant method. Morphological analysis revealed that one tissue had a significantly distinct presentation of epithelial and stromal cells, whereas the other three tissues had only minor morphological differences predominantly stromal cells. Two tissues were discarded after showing contamination. **Conclusion:** Tissue culture should be done very meticulously specially when oral cavity tissue is used as it is house for millions of microorganisms. The technique must also be thoroughly followed and adjusted accordingly. Using common, inexpensive stains like Hematoxylin and Eosin and crystal violet, which are of great help for examining the morphology of cells routinely.

Keywords: Primary oral squamous cell carcinoma- tissue culture- cell lines- primary culture- explants culture

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Introduction

Cell lines are now required for both the investigation of cancer biology and the screening of prospective chemicals for action in the development of anti-cancer therapies. Cell lines yield quick findings, but their applicability to patient outcomes is debatable because they are subjected to several passages of selection that render them unrepresentative of the original tumour. To increase the precision of outcomes during drug development, primary cell cultures, primary tumour cell explants, early passage cell lines, and xenografts are being used more frequently. These techniques have gained popularity over the past few years and are now well-established, with a wide variety of procedures at their disposal. Explants and three-dimensional models, for instance, enable the examination of cell-to-cell interactions in living cells, and endpoints can include the analysis of images and the measurement of gene expression. These techniques should be considered before clinical trials of drugs (Cree et al., 2010).

In recent years, the technique of tissue culture has significantly impacted human society. In numerous domains, including cell biology, medical research, pharmacology to assess the safety and efficacy of novel medications, vaccine production, and assisted reproductive technology, cell culture has emerged as a popular and widely utilised technique (Southgateet al.,

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1987). Researchers can examine the morphological, cellular, and functional behaviour of the cells by isolating primary cell lines from their primary cultures (Southgate et al.,1987). Cell culture issues, such as cell line misidentification, mycoplasma contamination, and genotypic and phenotypic instability, are frequently overlooked by the research community. Scientific data must be retracted or modified on a regular basis due to cell line misidentification. Such issues can arise in any cell culture operation, large or small, academic or commercial (Geraghty et al., 2014).

In recent time period, tissue culture has made extensive use of the direct explant and enzymatic procedures first disclosed by Bernice (1994) and Kedjarune et al., (2001), respectively (Tissue Culture Techniques: An Introduction - Bernice M. Martin - Google Books n.d.) (Kedjarune et al., 2001). In 1996, Daniels et al. provided a thorough procedure for an enzymatic method in which they isolated cells from tissue samples using trypsin or diaspase enzyme (Daniels et al., 1996). The direct explant technique uses fewer stages than the enzymatic method because no enzymes are used in the processing of the tissue samples. (Bayar et al., 2011; Sriram et al., 2015). Additionally, it has been noted that each approach has benefits and drawbacks. In the current study, we modified the explant technique for tissue cultivation. [Supplementary file 1: Protocol]. (Orazizadeh et al., 2015; Keira et al., 2004).

It is usually desirable to research the cell cycle, apoptosis, and cell repair in a controlled environment in order to understand the original properties and functions of the cells for potential therapeutic translation, even though primary cell lines have a finite lifespan. Therefore, it is crucial to create standardised primary culture techniques in the field of cell biology (Daniels et al., 1996).

In tissue culture different types of cells like epithelial cells, stromal cells, immune cells can be studied whereas in cell culture only a particular cell is studied. Among these different types of cells mesenchymal stem cells (MSCs) are approaching beneficial qualities, including their stemness property, absence of alloreactivity, and lack of immediate adverse reaction. (Hendijani and Javanmard, 2015; Hendijani et al., 2015; Hendijani et al., 2015; Lalu et al., 2012). One of the most crucial processes in MSC therapy preparation is tissue isolation, which comes first. A greater focus on method choice is required for MSC separation in clinical applications.

Our study's objectives were to develop a thorough protocol for creating a standard primary cell line from OSSC primary tissue using an explant technique, as well as to research the morphological traits of these cells using routine stains and correlate them with clinical and pathological features, which is crucial for further biological research, such as cell biology, cellular and molecular research and for prognostic purpose.

Materials and Methods

Ethical consideration

Institutional Ethics Committee (DYPV/EC/404/2019) approval was taken. All patients who took part in our study provided informed consent. The confidentiality of the documentation of the detailed case history, clinical details, and patient personnel information was maintained.

Tissue sample collection

Six primary OSCC tissue samples (aged 40 to 80) were collected from Dr. D. Y. Patil Medical College and Hospital and Research Centre. They were labelled as Tissue 1, Tissue 2, Tissue 3, Tissue 4, Tissue 5, Tissue 6. The tissues were processed as mentioned in cell culture technique heading as soon as possible after being transported in a 50 ml/ 15 ml tube containing complete cell culture media DMEM (Dulbecco's Modified Eagle's Medium, Gibco [pH 7.2]) (Figure 1). Within two hours, all 6 tissues were processed from the operating room to the culture lab.

The cell culture technique

Prior to the experiments, all sterilising procedures were followed meticulously while processing the tissue in a biosafety cabinet. Standard tissue culture protocol was followed with small modifications (Lim et al., 2005). Different reagents and media were used to treat tissue samples (Table 1). It was initially removed in petridish using complete DMEM medium (Figure 2a). Then, tissue was put through a variety of chemicals like Betadine-Povidine, PBS, complete DMEM in a 6 well culture plate (Figure 2b). It was initially cleaned and sterilised for about a minute in Betadine-Povidine. It was then washed twice for 2-3 minutes each time in phosphate buffered saline (pH 7.3–7.4) solution, and then in complete DMEM culture medium (Figure 2b). Using a sterile BP blade No. 22 and a sterile, treated Petri plate with culture media (DMEM), tissues were subsequently chopped into 1 mm pieces (Figure 2c). These minced tissue bits were centrifuged at 1,500 RPM for 5 minutes, supernatant was discarded and then the pellet was resuspended with complete DMEM media (Figure 2d). This suspension was seeded in 6 well culture plate (Figure 2e) and was kept in incubator at 37°C in a humid environment with 5% CO_2.

Monitoring of primary cell cultures and contamination

Daily assessments for contamination, explant displacement, and total radial migration of primary cells from the explants were performed under an inverted microscope [OLYMPUS CKX53]. The old medium was changed out for fresh, complete media every three days. Any well whose contents were contaminated were immediately discarded. In the current investigation, where four tissue samples were successfully cultivated, contamination was discovered in two tissue cultures. From day 4 to 6 six of culture, primary cell movement was seen. Between days 8 and 10, cells were shown to be arranged in a monolayer. Until the primary outgrowing cells in the culture flasks achieved 70–80 percent confluency, the culture procedure was repeated.

Establishing secondary cultures/passaging

Upon achieving 70-80% confluency the cells that were surrounding the explant tissue expanded more

evenly. The culture plates' medium was removed, and phosphate buffered saline (PBS) was used to gently wash the cell surfaces three times. The cells were taken out of the cultures by trypsinization. The cells were spherical in shape and started to separate from the culture plate's plastic surface after 1-2 minutes (Figure 3). The mixture was centrifuged at 1500 RPM for 5 minutes and at room temperature of 35°c Using the conventional cell-counting technique, a bright-line hemocytometer, and trypan blue dye, cells were counted to determine viability (Jauregui et al., 1981; Louis and Siegel, 2011). Cells were first routinely subcultured before being trypsinized. Trypan blue solution at 0.4 percent was diluted 1:1 with cell suspension and let to stand for 5 minutes at room temperature. Following the procedure of Louis and Siegel (2011). 20 µL of the cell suspension was then placed between the cover slip and the edge of the hemocytometer chamber and immediately viewed under a light microscope. Blue colour cells were considered as no-viable cells (Figure 4).

Fresh 3mL complete media with cells was introduced to the culture flasks in order to boost the cell count in a new T25 culture flask. Cultures were raised at 37°C in a humid environment with 5% CO_2. The culture medium was replaced every 48 hours.

Cryopreservation of Stromal Cells

Dimethyl sulfoxide (DMSO) was used to cryopreserve the stromal cells in accordance with the accepted cell line cryopreservation technique, which permits the long-term storage of cells in liquid nitrogen (Gupta et al. 2017). The cells from the fourth and fifth passage were chosen once they have reached confluency, and they were trypsinized with 0.25 percent trypsin. The cells were centrifuged at 1500 rpm for 5 minutes. Once the cell count was done the cell suspension was prepared. The Cryopreservative medium [CPM] was prepared by adding 90% FBS to 10% DMSO. Thereafter equal amount of CPM and cell suspension was mixed in cryovials and were frozen in a deep freezer for one hour at 2°C–4°C followed by one hour at 20°C, and one hour at 80°C. After that, they were put in liquid nitrogen. After 15 days of cryopreservation the cells were revived again to check the viability and revival capacity of the cells. We observed that the revival of the cells was around 60% to 70% and the proliferation of the cells were good with proper morphology and confluency occurred after 2 days of passaging.

Morphological analysis of the primary cell lines

Following the establishment of the main cell line, the morphological changes in the cells were studied using an inverted microscope on various time scales, including day 1, day 8, day 15, and day 21. The different cell types were classified according to their morphology as epithelial cells, which are polygonal cells, and stromal cells , which are spindle to stellate or branching cells. All of the tissues used in the experiment were subjected to these parameters, and their morphological assessment was completed.

R

Results

Observations

The findings demonstrated that four out of six primary cultures of OSCC tissues were effectively completed using the explant technique. In our experiment, we were able to harvest the cells after 5-7 days, and after 9–12 days of culture, 70–80 percent of the cells were confluent. The four tissue culture cells displayed various morphological characteristics whereas due to contamination, the remaining 2 tissue samples were discarded. All the 6 tissue cultures were studied under the headings of primary and secondary culture.

Primary culture

All 6 tissue cultures exhibited number of different cells like, Red blood cells (RBC), white blood cells (WBC), desquamated polygonal cells, round/spherical clusters of cells, and other types of cells (Figure 5).

The morphological characteristics of the four tissues varied, and two of them displayed mixed cell morphologies, such as epithelial cells and stromal cells. The other two tissues, however, displayed branching cells and stromal cells.

Secondary culture/passaging

Primary cells surrounding the explant of OSCC tissue continued to grow after achieving confluency. The cultures showed that the majority of the cells were long spindle-shaped stromal cells, which extended and covered the whole flask surface. Additionally, there were tiny spherical dividing primary cells and stellate-shaped cells with fibroblastic extensions. Around day 8 to 10 the cells were trypsinized and passaged.

Morphological assessment of primary cell lines from all 6 OSCC tissue.

From day 1 to day 21, the cells were observed for morphological changes. The following is a description of the morphological characteristics of the various 6 tissues:

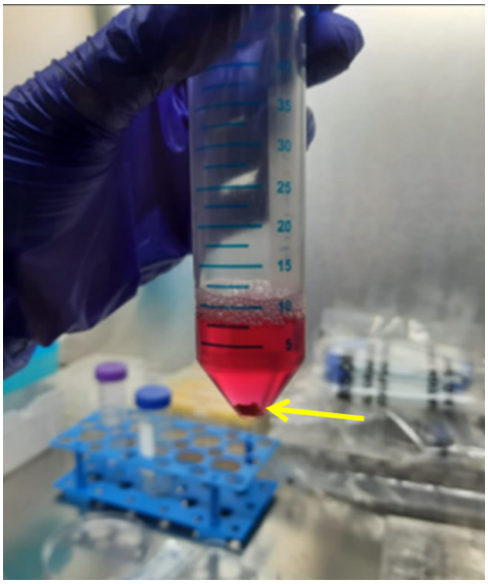

Figure 1. Collection of Tissue Sample [yellow arrow] in 50 ml Tube Containing Complete DMEM Media

Table 1. Reagents and Media Used in Tissue Culture by Explant Method

Name	Reagents/Media	Compony
	Tissue sample collection	
[Transport media] Complete DMEM media	DMEM media	Dulbecco's modified Eagle's medium; DMEM (Gibco; Thermo Fisher Scientific)
	FBS	Fetal Bovine Serum, qualified, Australia, (Gibco; Thermo Fisher Scientific)
	Antibiotic/antimitotic	Antibiotic/antimitotic (100X) (Gibco; Thermo Fisher Scientific, Waltham, MA, USA) 10,000 µg/mL of streptomycin, and 25 µg/mL of amphotericin B
	Tissue Processing	
Providence–iodine solution	10% w/v providence in 70% v/v isopropyl alcohol	Betadine
PBS wash	PBS + 10× antibiotic+ antimycotic + 50 µg/mL gentamicin	Phosphate-buffered saline (PBS) (10×) (Gibco; Thermo Fisher Scientific)
Complete DMEM wash	DMEM with 10% serum + 1% antibiotic+ antimycotic	Dulbecco's modified Eagle's medium; DMEM, Gibco; Thermo Fisher Scientific)
	Mincing of tissue	
Complete DMEM media	Complete DMEM media	Dulbecco's modified Eagle's medium; DMEM (Gibco; Thermo Fisher Scientific +10% FBS + 1% Antibiotic+antimycotic (Gibco; Thermo Fisher Scientific)
Establishing secondary cultures		
Trypsin	0.25% trypsin/EDTA and Complete DMEM media	(Gibco; Thermo Fisher Scientific)
	Cryopreservation of Primary cell lines	
Cryopreservative Media [CPM]	90% FBS + 10% DMSO	DMSO (Sigma-Aldrich)

Tissue 1:

Primary Culture:

On days 1, 2, and 3, there were no cell alterations seen since they kept their spherical to rounded shapes. The cells had a mixed 2 type of cellular shape on days 5 and 6. The first was spindle-shaped, denoting stromal cells, while the second was polygonal, denoting epithelial cells (Figure 6a). On day 7–10 epithelial cells were seen surrounding tissue, and fewer spindle-shaped cells were seen. Wells were confluent at day 16 and tissue bits were immediately surrounded by epithelial cells, with spindle-shaped stromal cells at the periphery (Figure 6b).

Secondary Culture/Passaging

After performing secondary culture in T-25 flask, regular observation was done. On observation after 2 to 3 days of passaging only stromal cells were visible and epithelial cells were absent (Figure 6c).

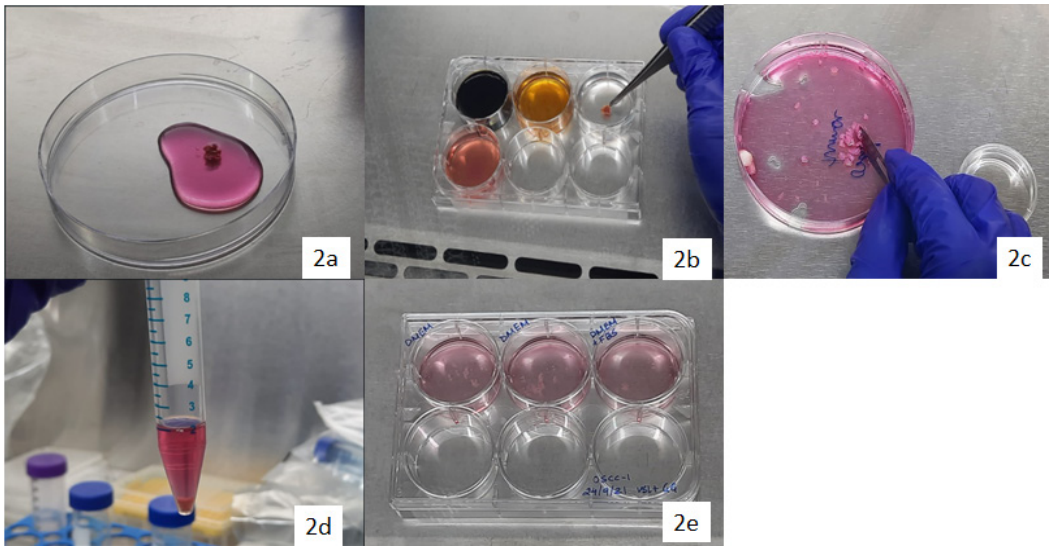

Figure 2. Protocol for Tissue Processing for Tissue Culture. 2a) tissue in petridish with DMEM, 2b) use of different reagents for tissue processing in 6 well culture plate, 2c) mincing of tissue, 2d) pellet formation of minced tissue, 2e) culturing minced tissue in 6 well culture plate with complete DMEM.

Table 2. Summary of all 6 Primary OSCC Tissue Culture

Tissue	Tissue 1	Tissue 2	Tissue 3	Tissue 4	Tissue 5	Tissue 6
Parameters			Primary culture			
Day 1 to 4	Round to spherical cells, RBCs, Desquamated cells	Round to spherical cells, RBCs, Desquamated cells	Round to spherical cells, RBCs, Desquamated cells	Round to spherical cells, RBCs, Desquamated cells	Bacterial Contamination Discarded	Fungal Contamination Discarded
Day 5 to 9	Mixed cellular population	Mixed cellular population	Single cell population	Single cell population		
Shape of Cells	Polygonal: Epithelial cells Spindle: stromal cells	Polygonal: Epithelial cells Spindle: stromal cells	Stromal Spindle shaped cells	Stromal Spindle shaped cells		
			Secondary culture/ Passaging			
10 to 21 days Shape of cells	Spindle: stromal cells	Spindle: stromal cells	Spindle: stromal cells	Spindle: stromal cells, Stellate, Branched cells		
Stain used	H and E stain	H and E stain	H and E stain	H and E stain	Crystal violet	Crystal violet

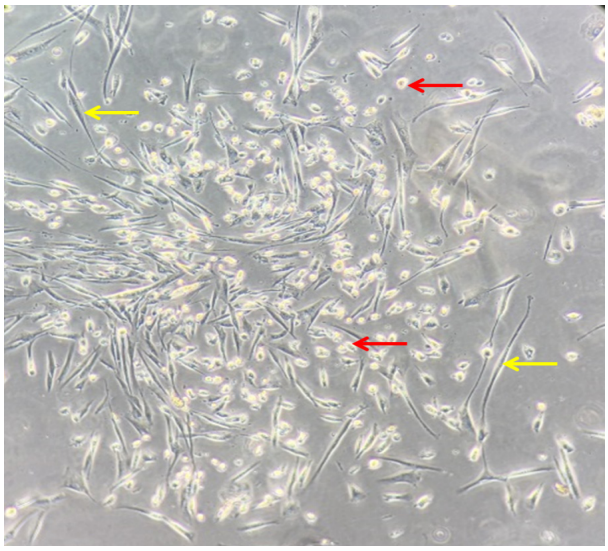

Figure 3. Cells become Round and Float after Using Trypsin; Round Cells [red arrow] that are Trypsinized, Stromal Cells [yellow arrow] that are Still Adherent to Surface [Magnification 100X]

Tissue 2

Primary Culture

Similar primary cell morphological pattern like tissue 1 was also present in tissue 2. Here, the proportion of epitheial cells was lower than that of stromal cells (Figure 7a). At one place, tissue sample showed stromal cells migrating from the primary tissue (Figure 7b).

Secondary culture / Passaging

Only stromal cells were visible and epithelial cells were absent after two to three days of passaging (Figure 7c).

Tissue 3

Primary Culture

Tissue 3 showed only stromal cells with no epithelial cells (Figure 8a). In some areas fibre [collagen] like appearances surrounding tissue bit was observed (Figure 8b).

Secondary culture / Passaging

Only stromal cells were visible and epithelial cells and fibers were absent after two to three days of passaging. (Figure 9a). During this stage few cells were fixed using 4% paraformaldehyde on the cover slip for standard H and E staining for cellular and nuclear morphology. It displayed nuclear and cellular pleomorphism in addition to mitotic figures, enlarged nuclei and stages of mitosis. (Figure 9b).

Tissue 4

Primary and secondary culture exhibited spindle-shaped stromal cells, branched, stellate-shaped cells [Figure 10a]. In comparison to the other 3 tissues, this tissue had lower levels of cellular density, confluency, and proliferation. Here, cells were fixed, and cellular analysis was done using H and E staining. Few areas, the cells were plump with vacuolated cytoplasm and a prominent nucleus (Figure 10b). It also displayed branched cellular

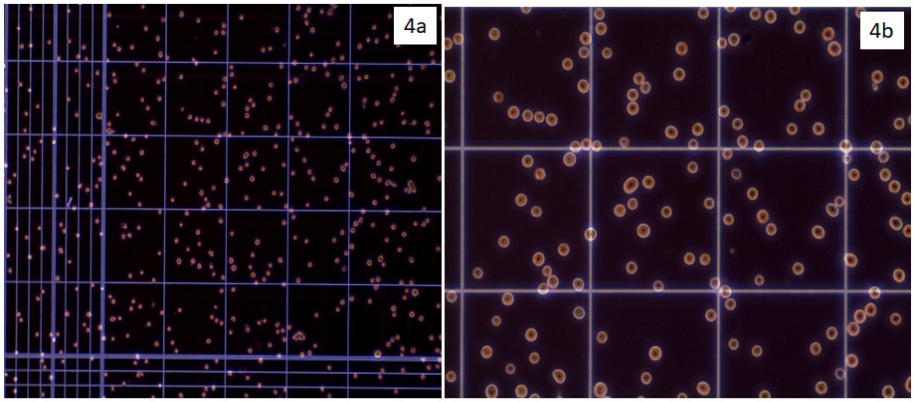

Figure 4. Cell Count Using Hemocytometer and Trypan Blue dye, 4a) Cell Count under 10X; 4b) Cell Count at 20X

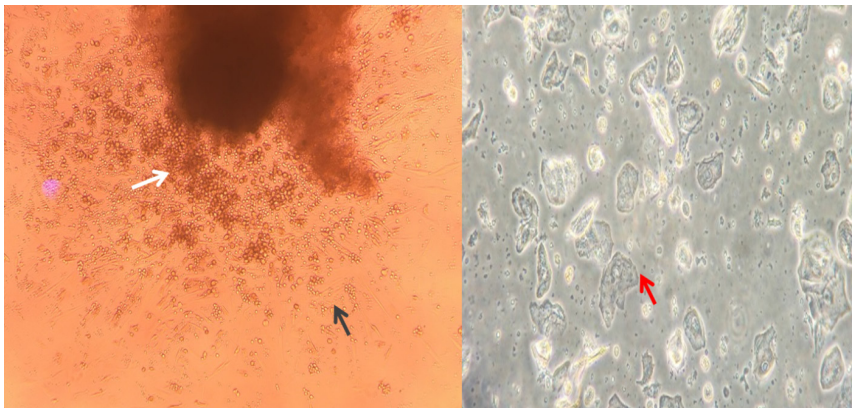

Figure 5. Culture Exhibiting, Round/spherical (Black arrow), Red Blood Cells (white arrow), [Magnification 100X] and Desquamated Polygonal Cells (Red arrow) [Magnification 200X]

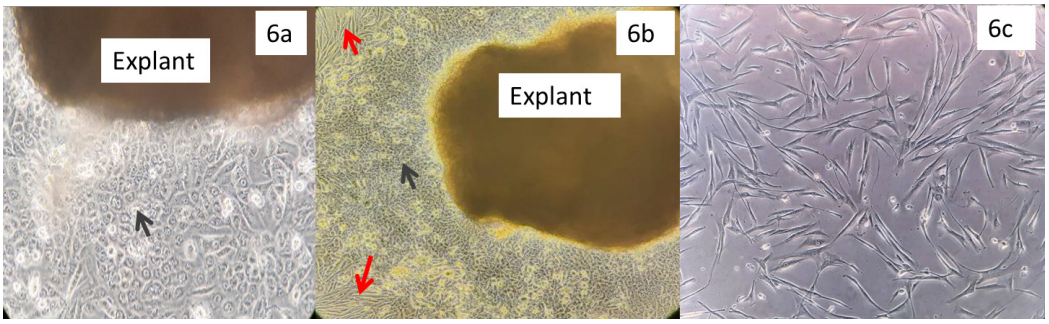

Figure 6. Primary Culture Showing Two Types of Cells; Epithelial Cells [black arrow] and Stromal Cells [red arrow] Seen at Day 7-10 (6a) and Around 16 Days (6b), only Stromal Cells (6c) [Magnification 100X]

appearance, a prominent nucleus, and fibrillar cytoplasmic appearance (Figure 10c).

Tissue 5 and Tissue 6

In primary culture, tissue 5 displayed bacterial contamination as single or multiple, black, rounded forms (Figure 11a). This plate was discarded right away. Tissue 6 exhibited contamination with long, slender extensions between round to oval structures that seemed to be fungal hyphae. On fixed cultures, Crystal violet stain was applied to detail the fungus. In fact, this image might clearly depict the morphology of a fungal hypha (Figure 11b).

Table 2 summarises the morphological analysis of all 6 tissues with respect to time period, primary and secondary culture associated with various parameters and using different routine stains in brief.

Discussion

Cells is a basic building block of life and an excellent resource for biological research. It works as a model system for investigating physiological processes and vetting potentially harmful or beneficial substances for use in medical procedures. Functional enzymes, growth factors, and vaccinations are all produced by cells. The method of cell culture involves maintaining cells alive outside of their natural bodies under controlled circumstances. There has been a substantial advancement in tissue engineering and tissue culture technology. It is becoming more and more common to isolate primary

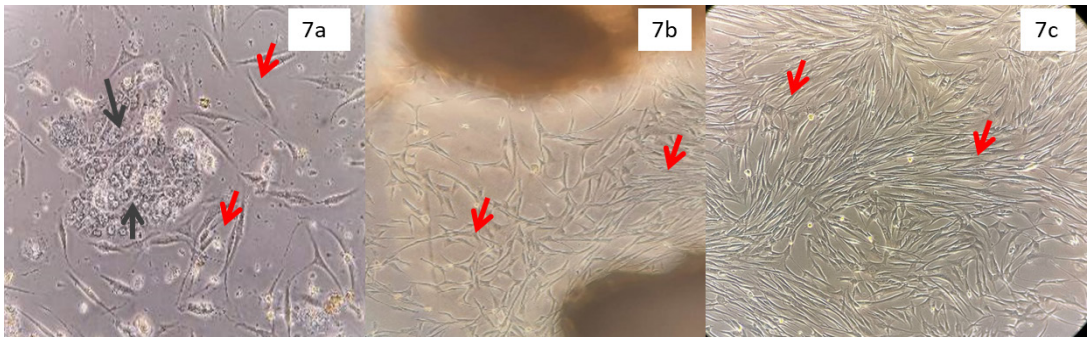

Figure 7. A Primary Culture Demonstrating Two Cell Types, 7a) Less Epithelial Cells [black arrows] and more Stromal Cells [red arrows], and 7b) Predominantly Stromal Cells [red arrows] Migrating from Tissue Explant, 7c) only Stromal Cells Seen [red arrows] [Magnification 100X]

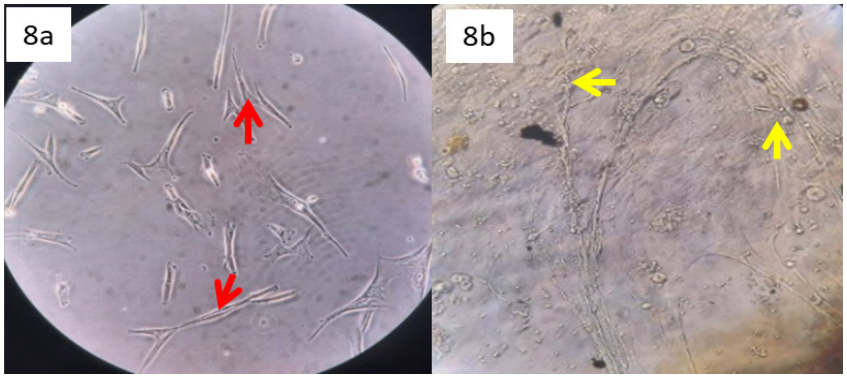

Figure 8. Tissue 3 Shows only Stromal Cells 8a) Spindle Shaped Cells [red arrow] 8b) Collagen Fibre [yellow arrow] Like Structure is Seen Periphery to Cells [Magnification 200X]

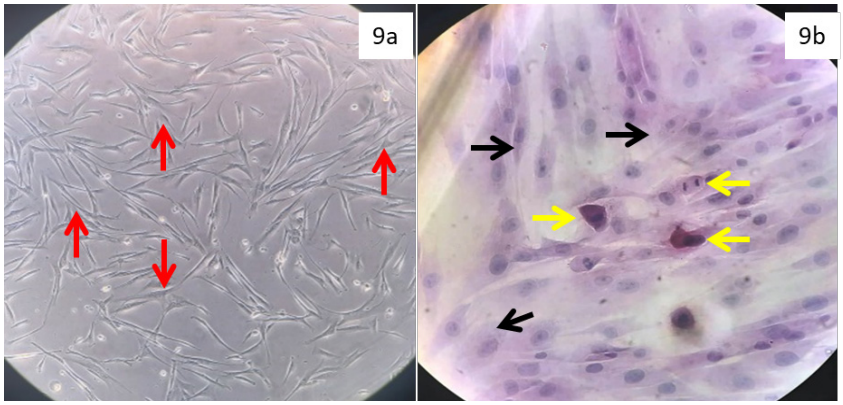

Figure 9. Secondary Culture/Passaging Showing 9a) Spindle Shaped Stromal Cells [red arrows] only; [Magnification100X] 9 b) H and E Stain Showing Cellular [black arrows] and Nuclear [yellow arrows] Pleomorphism [Magnification 200X]

cells from complete organs for scientific investigations in order to better understand the mechanisms underlying the cell cycle, apoptosis, and DNA repair etc. (Southgate et al., 1987; Daniels et al., 1996). Replicative senescence or aneuploidization in primary cells can occur, making isolation and limitless growth challenging. As a result, it's

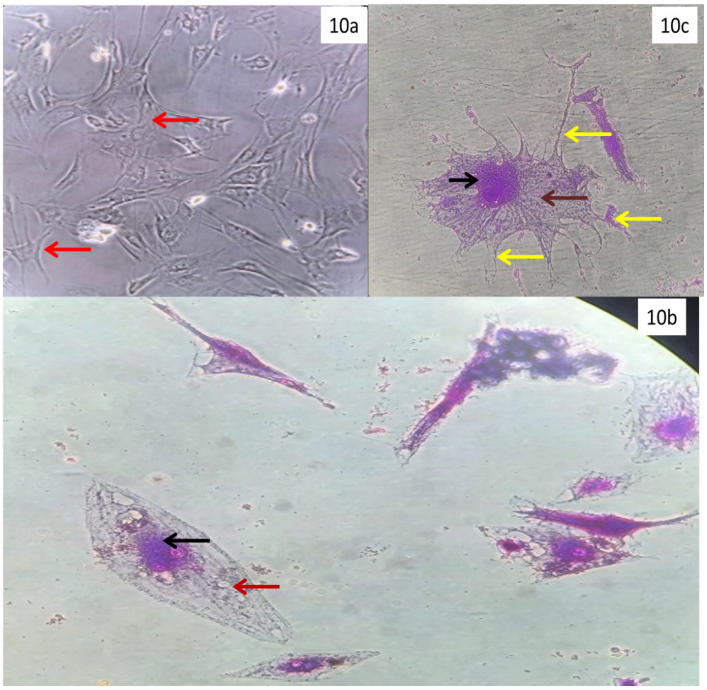

Figure 10. Tissue 4 Exhibited Fibroblast Like Cells, 10a) Stellate-shaped Cells, [Magnification 200X] 10b) Plump Cells with Vacuolated Cytoplasm [red arrow] and a Prominent Nucleus [dark red arrow] 10c) Branched Cellular Appearance [yellow arrows], a Prominent Nucleus [black arrow], and Fibrillar Cytoplasmic Appearance [brown arrow]. [Magnification 400X]

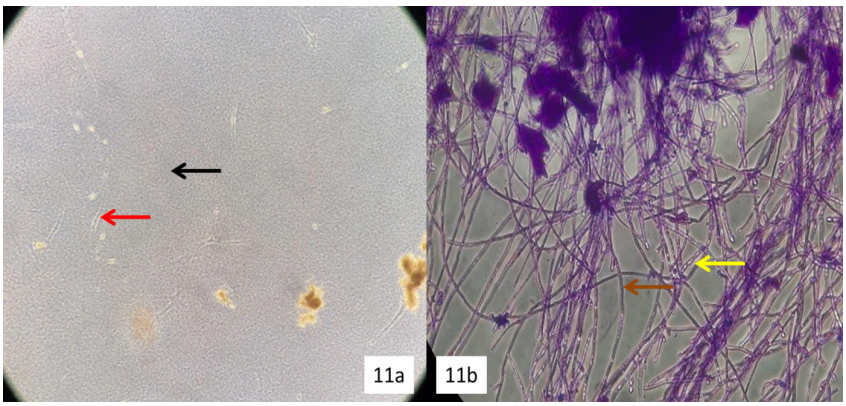

Figure 11. Contamination Seen in the Culture, 11a) Bacterial Contamination. Black Round Bacteria [black arrow], Stromal Cells [red arrow] [Magnification 100X] 11b) Fungal Conatmination [crystal violet stain] Budding [yellow arrow], Hyphae [brown arrow] [Magnification 200X]

important to regularly build new cultures and to maintain them properly. Culturing primary cancer cell cultures by explants has the advantage of retaining the tissue architecture and microenvironment, thus replicating, to some extent, the *in vivo* interactions.

Bolls and Lee presented the enzymatic and explant procedures as the two fundamental approaches for acquiring primary cell culture. Bellingham and Reynolds proposed the enzymatic approach as a technique for separating epithelial cells using an enzyme (trypsin). The tissue dissociation or enzymatic approach uses proteolytic enzymes to produce a single cell suspension or consecutive subcultures (Siengdee et al., 2018; Bayar et al., 2012). For tissue culture in our investigation, we used the explant approach.

Our study's main focus was on protecting and maintaining primary cultures against contamination. The oral cavity tissue samples are consistently contaminated with bacteria and occasionally with fungi, which slows the pace of expansion of primary cell lines. O. W. Merten discussed viral and bacterial contamination and included several precautions to prevent it, such as washing tissue samples in PBS before processing, using sanitised and autoclaved devices, and inspecting the raw materials for contamination. In our research from collecting tissue samples through maintaining and subculturing the cell lines, precautions were taken to avoid any bacterial or fungal contamination. This was modified by adding extra betadine iodine-povidine wash disinfection to the tissue and adding Anti-Anti in PBS wash during tissue processing.

As in the current study, tissue samples from the epithelial malignant tumour OSSC were employed. Because of this, the ideal tissue culture would exhibit both epithelial and stromal cells. However, only 2 tissues from our investigation demonstrated the presence of both epithelial and stromal cells in primary culture and solely stromal cells in subculture. The only tissue that may be obtained during surgery that is solely connective tissue and not epithelial component, allowing only stromal cells to thrive and reproduce, may be the cause of this. Even though the same strict technique was followed for all 6 tissues, there may be another explanation that the culture protocol and environment during tissue culture must have had an impact.

In primary culture, both epithelial and Stromal cells were found in tissue 1 and tissue 2, however after passaging only stromal cells remained. Therefore, it important to remember the following points during subculturing which can have both cellular populations for future research. 1) Epithelial cell growth and multiplication can be favoured over stromal cell growth by the use of selective media. 2) Selective trypsinization can be carried out, in which the early trypsinization time period the cells that are detached and floating be taken, centrifuged, and seeded in new T-25 flasks depending on the need, and the late trypsinization time period the remaining cells be collected, centrifuged, and seeded in T-25 flask. This way you can have both type of cellular population.

With no epithelial cells present in tissues 3 and 4, reason for this can be only connective tissue must have been present at the time of tissue sample selection or in an environment and medium that was conducive to the formation of stromal cells only.

We discovered that the tissues 1, 2, and 3 that predominately exhibit stromal cells were connected with the age group of 40 to 50 years and were all male when these morphological traits were correlated with clinical and pathological criteria. They were all clinically staged between 1 and 3 and had lymph node involvement ranging from level 1 to 3. Well Differentiated Squamous Cell Carcinoma grading was linked to all 4 tissues. Tissue 4 was connected to a 79-year-old patient who had no lymph node involvement, in contrast and clinical stage 1 was present. All four of the patients chewed tobacco regularly. Compared to tissue 4, tissue 2, 3, and 5 displayed higher invasion in various structures. According to this correlation, stromal cells which might be mesenchymal stem cells or CAF can be linked to greater invasion, younger age groups, lymph node involvement, and more affinity for alveoli and the gingiva-buccal location. Due to the extremely small sample size, these results cannot be considered definitive.

In the present study in all 6 tissue cultures we found fibroblast like mesenchymal stromal cells growth and this was in accordance with the study conducted by Oppel et

al., (2019).

In study conducted by Amrutkar et al., (2020) suggested that all six cultures were composed of polygonal-shaped cells with ovoid nuclei and exhibited an epithelial growth pattern and grew as an adherent monolayer. In that 4 tissues were homogenous in size, whereas 2 tissues cells were heterogeneous such that the cultures consisted of both small and large cells with the presence of a few elongated cells. In the present study two tissues exhibited mixed cell type like epithelial and mesenchymal elongated cells whereas in two tissues it was mainly elongated, mesenchymal cells with different size.

Therefore, overall morphological analysis suggests that one should exercise caution when utilising very thorough and flexible, adjusted protocols to obtain the required cellular components during tissue culture, depending on your study's goals and objectives. Use selective medium for epithelial cells when you want them and also have appropriate representative tissue from the lesion. Additionally, when targeting stromal cells, follow the standard procedure as previously indicated without employing selective medium. As in our study, stromal cells of various morphologies and types, including Mesenchymal Stromal/Stem cells, or Cancer Associated Fibroblasts, and others were observed; the characterisation of these cells is required for further studies. These cells are significant components of the tumour microenvironment in OSCC and have pro or anti-tumor activity that affects the progression of the tumour and the prognosis of the patient. Understanding the existence of morphological diversity in solid tumours like OSCC would be made easier by correlating morphological characteristics with clinical, pathological criteria. Therefore, pure cultures of verified primary stromal cell lines were incubated, preserved, and kept for potential use in oral cancer research and therapeutic applications.

In conclusion, the goal of the investigation was to standardise the procedures for obtaining primary stromal cell lines from OSCC tissue in large quantities utilising the explant technique. Despite the fact that contamination was found in 2 tissue cultures, this shows how meticulously cells from the mouth cavity must be isolated and cultured. The technique must also be thoroughly followed and adjusted as necessary. Additionally, the morphological examination of all tissue culture cell lines may be able to provide assistance in a number of cellular and molecular elements of oral cancer and their correlation with clinico-pathological features will be helpful for the understanding of the biology of cancer properly that will help in determining the progress, prognosis and therapeutic options. Using common, inexpensive stains like Hematoxylin & Eosin and crystal violet, which are great for examining the morphology of cells routinely, will be helpful.

Future scope

The additional characterization of cells from each tissue sample, their subsequent passage numbers, and their correlation with patients' clinical and histological data will aid in understanding the cellular and molecular behaviour for the specific tissue and ultimately the patient, which will aid in therapeutic approach. For further cell morphological analysis, simple Hematoxylin & Eosin and crystal violet should be utilised routinely.

Author Contribution Statement

VSL and GMK: conceived, designed, and supervised the study, VSL and GMK: performed laboratory analysis, VSL, KJ, RB and GMK: Analyzed the data and VSL, SK, CR and KJ wrote and edited the final manuscript. All authors read and approved the final manuscript. VSL and GMK have contributed equally and should be considered as a first author.

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Scientific and Ethical consideration

Institutional Ethics Committee (DYPV/EC/404/2019) approval was taken. All patients who took part in our study provided informed consent. The confidentiality of the documentation of the detailed case history, clinical details, and patient personnel information was maintained.

Availability of data

The supplementary data of this study is available on request from the corresponding author, VSL. Because of the privacy of research participants data is not available publicly.

Conflicts of interest

The authors declare that they have no conflict of Interest.

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