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Review Article

Lab-on-a-Chip – Oral Cancer Diagnosis at Your Door Step

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Abstract:

Oral cancer is one of the most common deadliest cancers leading to disfigurement. Despite recent advancement in the treatment modalities, it has less improvement in the prognosis. Early detection plays vital role survival rate of the patients. There is no accurate, cost-effective and reliable method for screening of oral squamous cell carcinoma (OSCC) patients. Hence, many patients are diagnosed at advanced stages. Early detection would, therefore, help to identify patients and modify treatment with close monitoring. There is a need for mass screening with a rapid and reliable oral cancer diagnostic test that can be widely used in a clinical setting. Recent diagnostic techniques for OSCC require modern laboratory facilities, sophisticated equipment with elaborative and lengthy processing techniques by skilled personnel. Lab-on-a-chip (LOC) or micro-total-analysis systems, one of the microfluidics technology that is defined as adaptation, miniaturization, integration, and automation of analytical laboratory procedures into a single device or "chip." This technology assures the replacement of complicated techniques with miniaturized, integrated, programmed and economical diagnostic devices. The detection of oral dysplastic and cancer cells utilizing chip is based on membrane-associated cell proteins. There is unique gene transcription profiles singularly expressed on the cell membranes for its detection. Hence, this system provides a means for rapid, automated, molecular analysis of cancer cells. This review articles emphasis on LOC technology for identification of biomarkers of oral cancer.

Key Words: Diagnostic aids, lab on a chip, microfluidics, oral cancer, pre-malignant lesion

Introduction

The incidence of oral cancer worldwide is estimated around 500,000 per year, accounting for approximately 3% of all malignancies, thus posing a significant health issue[1] and India constitutes 30-40% (80,000 new cases diagnosed annually) cancer load.[2] The oral cancer is the fifth most common cancer in the world, accounting for numerous deaths annually.[1] The 5 years survival rate remains at an approximately 50% for oral squamous cell carcinoma (OSCC) in past several decades.[2] The annual incidence and mortality rates vary among different races, genders and age groups.[1,3] Epidemiologic differences exist in South Asia, where oral cancer ranks first among all types of cancers in male patients and third in female patients.

Oral cancer is associated with chronic irritating factors, such as tobacco, smoking, alcohol, and betel quid use,[1] diet, mouthwash use containing alcohol, infective agents, and socio-economic status.[4] While cigarette smoking and alcohol drinking are the major risk factors in Western countries, betel quid use and smoking are major factors in the causation of oral cancer in South Asia, South East Asia, and Taiwan.[1]

Early detection of cancer is of prime importance. It helps to reduce morbidity and mortality. A major factor lacking in improvement in prognosis over the years is the fact that a significant proportion of OSCCs are not diagnosed or treated until they reach an advanced stage. The diagnostic delay may be caused by patients (who do not report unusual oral features[2] or due to minimal pain during early growth phase leading to delay in seeking professional care[3,5]) or health care professionals (who lack in observing lesions thoroughly, hence additional several weeks or months may elapse before a biopsy is performed[3,5]) and such delays are longer for asymptomatic lesions.[2] Early diagnosis aids in rapid proceeding to treatment thus improve the prognosis. Hence, it is required that patients should undergo an oral and dental examination at an early stage.[1,5] Tumor detection is further complicated by a tendency toward field concretization, thus leading to multicentric lesions.[3]

Aggressive management of OSCC is indecisive as it may lead to severe deformity with increased morbidity rate. As a consequence, many patients with OSCC are either over or under treated with significant personal and socio-economic impact. Most crucial factors that lead to poor prognosis in OSCC patients is the diagnosis of oral cancer at advanced stages and hence treated late. Early detection of lesions can greatly reduce morbidity associated with delayed disease treatment and improve overall patient survival rate. Early detection assists in frequent patient monitoring, dietary

modifications with counseling for cessation of smoking and drinking, preventive drug administration and lesion removal. Early diagnosis and treatment of OSCC may result with a mean survival rate of over 80% with a good quality of life after treatment.[6]

Oral Cancer Diagnostic Aids

This tool helps in detection at an early stage with personalized diagnostic protocols. Thereby considered to be most promising way to reduce mortality from cancer and improve prognosis.[7] Even though with laboratory tools also, definitive diagnosis often remains elusive.[8]

Three roadblocks which have prevented the realization of the potential of clinical diagnostics includes:

1. Lack of definitive disease - Associated protein with genetic markers
2. Absence of easy and inexpensive sampling methods with minimal discomfort
3. Lack of an accurate, portable and easily usable diagnostic platform.[8]

Approaches to early detection of dysplasia and OSCC - Oral cancer screening

Screening and an early detection decreases both the morbidity and mortality associated with OSCC, as unlike many anatomic sites, in oral cavity, pre-malignant lesions are often detected on clinical examination. The precise discrimination between pre-malignant and reactive/inflammatory lesions via conventional visual and tactile examinations alone is challenging. The various criteria for diagnosing and grading dysplasia are known to be controversial, highly subjective with wide range of interpretation, even for qualified pathologist. Hence, conventional histological findings only indicate that whether a lesion may have a malignant potential and cannot be used for prediction of malignant change.[3]

Oral examination, unfortunately, cannot discriminate between potentially malignant lesions from benign lesions. Early diagnosis can minimize morbidity and its treatment associated with severe loss of function, disfigurement, depression with poor quality of life.[2] Oral cancer screening implies searching for oral potentially malignant lesions, typically before symptoms appear. Hence, early detection is necessary to raise awareness in the general public thereby improving access to oral health services. Potentially malignant lesions may also occasionally regress if the health care professional motivates the patient to reduce risk factors (tobacco, alcohol, occupation, pollution, industrial products, medicine and medical products, and geophysical factors) and needs elimination of predominant carcinogens including tobacco and alcohol.[9]

Conventional methods of oral cancer diagnosis

The conventional oral examination is the standard method of revealing pre-malignant lesions and OSCC, confirming the clinical diagnosis by biopsy and histopathological examination.[1,5] Oral cancer as a target for new cancer screening modalities is a good choice because early detection methods are solely lacking.[10]

Conventional methods include

1. Toluidine blue and Lugol's iodine staining
2. Exfoliative cytology
3. Brush biopsy
4. Scalpel biopsy
5. Adjunct based on tissue reflectance (Vizilite, Vizilite plus).[1-5,9]

However, biopsy remains as gold standard and corner stone for early diagnosis of oral cancer; it has following limitations:

1. Invasive procedures, depending on site and usually involves surgical procedures and anesthesia
2. Smaller lesions do not provide sufficient material for proper diagnosis
3. Biopsies from sites with large lesions may not reveal the complete histopathological aspects of lesions
4. Subjective
5. Limited sensitivity and specificity which may lead to flawed diagnosis and inappropriate therapeutic approach[6]
6. It is invasive resulting in both psychological implications for the patient technical difficulties for health practitioner
7. Oral biopsy specimens can be affected by artifacts resulting from either crushing, fulguration or incorrect fixation and freezing
8. Experiments have detected an increased risk of neck metastasis from Stage I to II OSCC after incisional biopsy with presence of tumor cells in peripheral blood 15 min after incisional biopsies with a conventional scalpel.[2,9]

Limitations of conventional method led to development of some modifications in these methods.

Saliva based oral cancer diagnosis

Saliva because of its cellular composition, accessibility, inexpensive and non-invasive methods of collection, can be ideally used as a diagnostic marker for early cancer detection. Estimation of exfoliated cells is considered to be the most direct and reliable method for oral cancer screening. Several variations or improvements in exfoliated cell cytology include staining for presence of micronuclei, detection of microsatellite instability by polymerase chain reaction, fluorescence *in situ* hybridization analysis, promoter hypermethylation, and mitochondrial DNA content.[6]

Blood and saliva are the most widely studied body fluids as they constitute easy quick and non-invasive biomarkers for oral and systemic conditions. It acts as an informative body fluid that contains an array of an analyte (protein, m RNA, and DNA) that can be utilized as biomarkers for translation

and clinical purposes. The biomarkers in saliva are classified into proteomic, genomic and microbiological biomarkers.[2,11]

Salivary genomics and proteomics have led to the discovery of new molecular markers for oral cancer diagnosis, therapeutics, and prognosis.[2] Saliva provides a cost effective and practical approach for screening of large populations. It can be used to measure specific salivary macromolecules and for examination of proteomic or genomic targets such as enzymes, cytokines, growth factors, metalloproteinases, endothelin, telomerase, cytokeratins, mRNA and DNA transcripts. Common epithelial serum circulatory tumor markers in saliva of carcinoma patients are cyfra-21, carcinoembryonic antigen, CA-19-9.[4,9]

To improve the sensitivity and specificity of oral cancer detection, a method that enriches the pre-cancer and cancer cells of saliva are more preferable. Expression of cancer-specific membrane proteins that are used to distinguish cancer cells from normal cells thereby facilitating identification, capture and enrichment of cancer cells. Antibodies against cancer specific membrane proteins that are conjugated with labels including fluorescent, antibodies, and quantum dots or beads (magnetic, metallic) preferentially tag cancerous cells in a sample. Unique proteins expressed by cancer cells must first be identified. Common cancer specific protein biomarkers include 2 membrane glycoprotein (cell membranes of cancer cells not in normal cells), heat shock protein 47 (colligin, endoplasmic reticulum protein with collagen binding properties, overexpressed in oral cancer cells), epithelial cell adhesion molecule (EpCAM) (epithelial transmembrane glycoprotein, strongly expressed in epithelial cancers),[6] CD44 (multi-structural, multifunctional cell surface transmembrane glycoprotein molecule, significantly raised in head and neck cancer patients) (Figure 1).[1]

All these limitations of conventional methods as well as potential use of saliva as diagnostic fluid for oral cancer detection led to advent of following advanced diagnostic aids:

1. VEL scope
2. Positron emission tomography scan
3. Laser and light induced and autofluorescence spectroscopy
4. Elastic scattering spectroscopy
5. Raman spectroscopy
6. Orthogonal polarization spectral imaging
7. Optical coherence tomography
8. Laser capture microdissection
9. Spectral cytopathology
10. Multispectral digital microscope
11. Confocal endomicroscopy
12. Lab on a chip.[1-5,9]

Lab on a Chip Technology

The healthcare sector is nowadays one of the most dynamic fields where the novelty is a strategic and operation imperative.

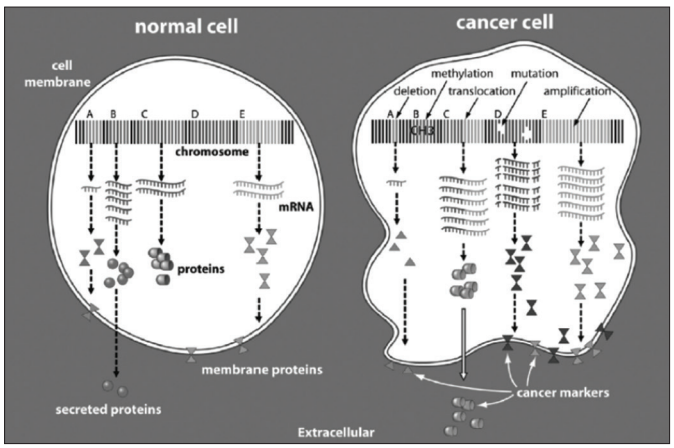

Figure 1: Illustration of differences between normal and cancer cells. Potential cancer biomarkers exemplified by genetic changes in the chromosomal DNA are illustrated in the cancer cell.[6]

The possibility of increasing the quantity and quality of clinical analyses that are performed with instant results and outside the clinical laboratories, contributes to a better quality health care services.[12] The greatest challenges in modern medicine is the ability to provide accurate diagnostic laboratory tests in developing countries and in remote areas where conventional analytical laboratories,[13] sophisticated apparatus and elaborate and lengthy processing by trained and expert personnel[6] are lacking. There is no need for a cumbersome or expensive mass spectrometer, a flow cytometer or even a centrifuge because the application of microfluidics engineering to medical diagnostics has enabled laboratory tests to be performed on a small microchip the size of a credit card that you can carry around in your pocket and may only require the use of a battery as a power source for analysis. As futuristic as this idea seems, the application of microfluidics engineering to the development of medical diagnostic tests is very much a reality.[13]

Microfluidics engineering is a multidisciplinary field of engineering where the intersection of the fields of physics, chemistry, engineering, and biotechnology has come together to develop chips on which the analysis of fluids can occur on a microscale level. A particularly interesting application of microfluidics engineering technology is the introduction of a lab-on-a-chip (LOC); a platform on which one or more laboratory tests is integrated onto a small chip. These chips utilize a wide variety of techniques for analysis. The most successful LOC applications integrate all aspects of sample preparation, sample separation, signal amplification, and signal detection on a single chip. These chips can be easily adapted for use in remote regions since specialized laboratory personnel are not needed for analysis you simply need to apply the sample to the chip and the technology takes care of the rest. A variety of molecular biology, immunology and biochemical techniques have been adapted for use on LOC platforms including protein assays, nucleic acid assays, cell sorting, and

biological analyte detection.[13] Over the past 20 years, there is a speedy development with increasing interest in microfluidic devices known as micro total analysis system (μTAS), LOC or microfluidic paper-based analytical devices. The ability to perform laboratory operations on nano- or pico-scale, with the use of miniaturized equipment has opened new pathways in modern researchers. Application of small volumes of fluids with the use of varied dimension from 10 to 100 μm of dimension is extremely appealing and has been regarded as the most attractive advantage of LOC. Currently, chemists utilize mini-laboratories for synthesis of new molecules. Biologists study complicated cellular processes in the extensive study of new molecule cell biology. Analytical chemists utilize microfluidic devices as convenient tools for detection and determination of many compounds. These devices provide analytical and diagnostic abilities that may revolutionize medicine and pharmaceutical industry. They have been used due to widely for clinical applications in research fields including biomedical science, genomics, forensics, toxicology, immunology, environmental studies, chemistry or biochemistry. In developing countries, miniaturized portable medical diagnostic tools are especially important with basic diagnostic and analytical facilities.[14] Hence, there is great demand for the development of an easy, inexpensive diagnostic biochip with capability of fast and reliable measurements of metabolic parameters from a human body.[15]

History and Development of Microfluidic Devices

The first device was developed in 1975. Manz *et al*. presented a miniaturized open tubular chromatograph using silicon chip technology in 1990.[14] Capillary electrophoresis systems, the first μTAS emerged at the end of 1990s.[16] Concept of paper-based analytical devices was invented and described by Whitesides Group of Harvard University in 2007.[14]

The development of early non-invasive diagnostic protocols was one of the most demanded avenues for decreasing mortality from cancer and better prognosis. The emerging microfluidic analyzing platform promises high through output and high precision to reduced equipment cost and relatively less time. LOC has provided tremendous hope in biomarker measurements with personalized diagnostic strategies.[7]

Microfluidics and Lab on a Chip

Microfluidics is the chemistry or biotechnology equivalent of the integrated silicon chip that has revolutionized electronics equipments. It is suited for handling living cells (with typical diameter is few μm) in a three-dimensional, biologically relevant environment.[1,3,9] It is the miniaturization of biological separation and assay techniques so that multiple experiments can be performed in parallel on a small device. In this technology, minute quantities of media, reagents and even nanoparticles are steered through narrow channels on the device where they are delivered, manipulated and analyzed by fluorescence detection.[17]

LOC is the adaptation, miniaturization, integration and automation of laboratory procedures into a "chip" or single device.[1,3,9] This microfluidic chip accepts saliva sample, can be operated by minimally trained personnel provides a diagnostic answer in a fully automated fashion within limited time. The detection of oral potentially malignant and cancer cells within the chip has membrane-associated cell proteins. The measured profile determines the cancer type and stage.[1,2,5,9] As such, this system provides a means for automated, rapid detection[1] within a time frame of 10-60 min, single use (disposable)[10] and analysis of cancers at molecular level in a miniaturized form.[1] These devices uses 10-1000 μl of different types of clinical specimens including various body fluids as well as samples collected by minimally invasive methods.[10]

One of the most crucial decisions for a TAS platform is the choice of substrate material. Most microfluidic substrate for biochemical analysis systems is fabricated using silicon (Si) or glass. Plastic substrates such as polymethamethylacrylate, polydimethylsiloxane (PDMS), offer a wide range of physical as well as chemical material parameters for the applications of biofluidic chips usually at low cost using replication approaches. Polymers used have numerous advantages for ease of fabrication, and rapid prototyping.[15] Microfluidic devices based on elastomeric materials such as PDMS are rapidly becoming a ubiquitous platform for applications in pharmaceutical industry and biotechnology. Recent growth in the field of PDMS microfluidics has far outpaced that in alternative device technologies based on glass and silicon, due in large part to significantly simpler and less expensive fabrication procedures as well as the possibility of easily incorporating integrated mechanical microvalves at extremely high densities.[17]

Working of LOC

LOC is composed of a microfluidic system, in which mixing and reaction takes place, and has a sensor system, in which detection and quantification takes place. A portable device ensures analysis within consultation time. Colorimetric detection by the optical absorption is the best alternative to fluorescence and electrochemical detectors. This device can be used to perform tests on physiological fluids.[12]

The LOC comprises a microfluidic system and a detection system. There are eight inlets for the reactants and is installed with a unique inlet for the sample to be tested. The sample is mixed with the respective reactants (enzymes catalyzing biological reactions).[12] For many biological and chemical applications, mixers plays an essential role for enhancing mixing efficiency and for rapid homogenization.[14]

A diverse and wide array of microfluidic components and systems have been implied for immunoassays hence the microfluidic technology invented for pathogen detection can be adapted and extended for the more challenging task

of cancer screening and diagnostics. Simply, an automated immunoassay of a single cancer marker or a panel of cancer markers with a cancer-specific gene transcription or expression profile can also be implemented on a credit card - Sized microfluidic cassette used for point-of-care cancer screening. Recently developed bio-barcode assays for multiplex detection provides amplification and detection of proteins and nucleic acids.[10]

LOC in oral cancer diagnosis

Genetic changes in cancer cells leads to altered gene expression patterns that can be identified long before the cancer phenotype has manifested. When compared with normal mucosa, the changes that occur in the cancer cell can be used as biomarkers. Many candidate genes associated with OSCC tumor progression such as p53, cyclin D1, and epidermal growth factor receptor gene have been identified. Microarray analysis of several tumor types has demonstrated that global expression profiling that distinguishes tumor cells from normal cells. The advent of high-density microarrays and advances in bioinformatics, have opened the door for inclusion of these gene signatures into microfluidic LOC devices. Studies have found a set of genes with down or up-regulation in OSCC.[6]

The patient would provide 1 ml of saliva, which is taken up by a sponge-tipped disposable collector. The collector is then inserted into the cassette to inject the collected oral fluid via a sample inlet port (Figure 2).[6] The cancer diagnostic process comprises an initial and first step to removing lymphocytes for isolating cancer cells from the sample. The cancer cells are sorted from the sample using magnetic beads coated with anti-EpCAM antibody, which is aberrantly expressed on the surface of cancerous epithelial cells. The separated cancer cells can be easily detected and counted. The separated cancer cells are then subjected to a thermal and/or chemical lysis step and the mRNA are isolated. Multiplex m RNA amplification by reverse transcription polymerase chain reaction, linear amplification, or a bio-barcode technique.[10] The transcription profile of the isolated sample cancer cells are then compared with cancer signature profiles archived in a database using established statistical rules to identify the type of cancer (Figure 3).[6]

Other applications

1. Drug discovery and development processes,[17] drug effectiveness and resistance.[6]
2. Neurotransmitter detection, cell culture and growth, disease progression and recurrence.
3. Detection and identification of microorganisms[14] (HIV, malaria, tuberculosis, *Escherichia coli*, *Salmonella*, *Shigella*),[13] pathogens, proteins
4. Detect environmental pollutants, explosives, nitroaromatics
5. Urine analysis, sperm counts and motility[14]
6. Detection of acute myocardial infarction.[11]

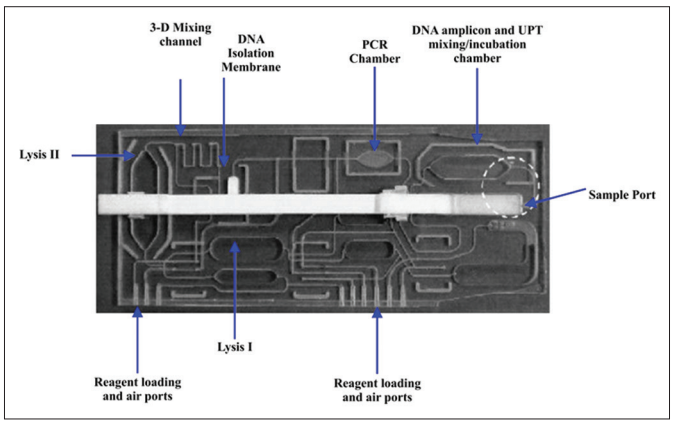

Figure 2: Microfluidic lab-on-a-chip. The saliva sample is introduced into the sample port. The saliva sample is lysed with enzymes, detergent, and chaotropic salts in a two-step, two-chamber lysis process. The nucleic acids are isolated from the lysate by solid-phase extraction using a porous silica membrane as a nucleic acid binding phase. Purified nucleic acids eluted from the silica membrane are amplified by polymerase chain reaction using specific primers.[6]

Simple\microfluidic diagnostic devices such as lateral-flow and consecutive flow assays for home pregnancy testing, drug abuse testing, prostate-specific antigen detection, salivabased HIV testing, etc. are readily available in the market today.[6]

Advantages

1. Small sample size with minimal invasive technique
2. Automated operation
3. Short processing times
4. Reduced reagent consumption, reproducibility, consistency
5. Reduced exposure to hazardous materials or infectious agents
6. Minimal risk of sample contamination
7. Convenient disposal
8. Operability by minimally trained personnel
9. Low cost
10. Small size portability
11. Sophisticated analytical techniques
12. Use battery for power (no need for electrical infrastructure)
13. Elimination of human error.[6,10,13]

Limitations

1. Complicated fabrication process
2. Unsatisfactory interfaces for fluid transfer
3. PMDS used for fabrication have hydrophobicity and propensity for protein absorption, may disturb bioassay results
4. Electrokinetic methods in microchannels suffer from limitations of buffer incompatibility, solvent evaporation and electrophoretic demixing.[7]

Future prospects

LOC technology can serve as an important and vital component to improving both global health and environmental

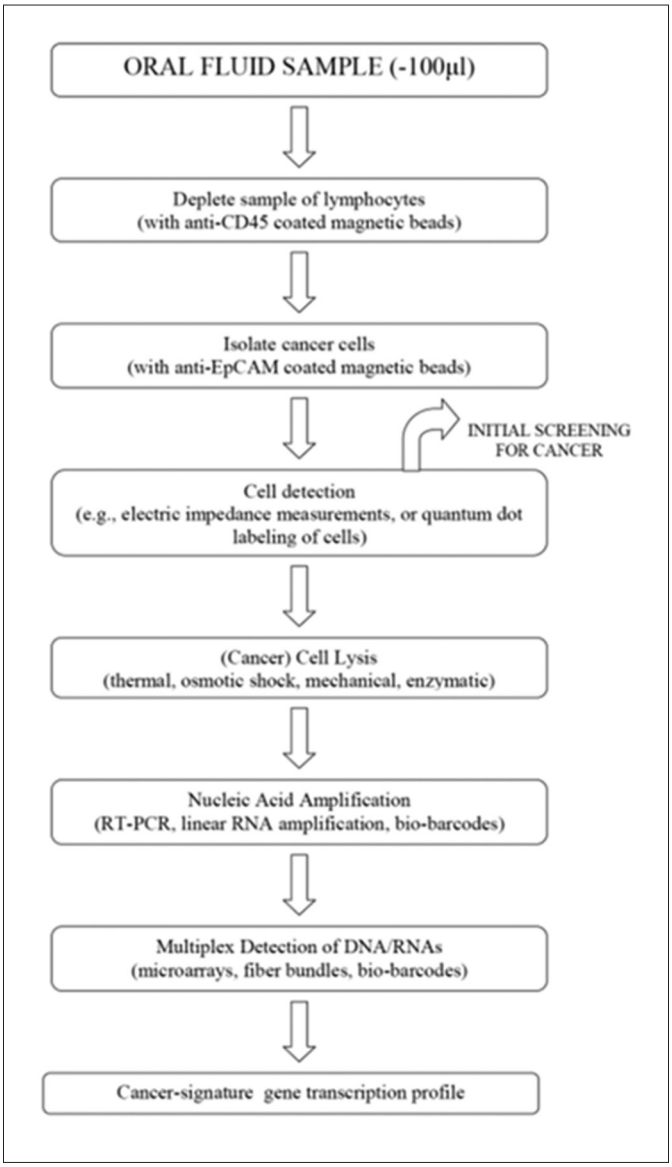

Figure 3: Sample processing steps for isolating cancer cells from saliva and assaying a panel of mRNAs.[11]

protection. LOC area research is still at an infant stage need more matured platform technology in coming future.[14] Recent advances involve applications in neurobiology, neuron culture, manipulation, stem cell differentiation, neuropharmacology, neuroelectrophysiology, neuro biosensors. Nanofluidic devices have the potential to analyze DNA, proteins and preparation of nanoparticles for gene therapy, drug delivery and toxicity analysis.[17] Further to integrate all these processes onto a single device or chip.[6]

Conclusion

LOC devices can be administered at a patient's locale and even by patient himself, which offer not only convenience but significantly more rapid diagnosis. Researchers are transitioning from viewing saliva as a diagnostic outcast in comparison with blood or urine as a valuable biofluid thus closing the gap between saliva and other biofluids for disease diagnostic. Saliva based diagnostics provide incomparable opportunities for research and commercialization for better understanding of genomics, proteomics and transcriptomics. Screening of an entire population for a specific disease type can be made possible in the cuming future by employing saliva diagnostics. Hence, emerging field of microbiology and nanotechnology provides early and rapid diagnosis and improved prognosis of oral cancer. "Think of the test as pathology on a chip."

References

1. Panat SR, Agarwal KK, Gurtu A, Aggarwal A, Mohan S. Current trends in diagnosis of oral cancer and premalignant lesions: An update. JDSOR 2014;5(3):120-4.
2. Tak MM, Dar MD. Avenues to early detection of oral premalignant and malignant lesions: A review of the current technologies. JEMDS 2014;3(58):13165-76.
3. Nigam P, Prasad K, Tak J, Gupta V, Sinha A, Bali R, *et al*. Advanced diagnostic aids in early detection of oral cancer. J Adv Med Dent Sci Res 2014;2(3):39-43.
4. Omar EA. The outline of prognosis and new advances in diagnosis of oral squamous cell carcinoma (OSCC): Review of the literature. J Oral Oncol 2013;2013:1-13.
5. Chaudhary R, Shah A, Shah DM, Singh S, Thakkar P, Goyal S. Advanced diagnostic aids in detection of oral cancer. IJDMR 2014;1(3):139-43.
6. Ziober BL, Mauk MG, Falls EM, Chen Z, Ziober AF, Bau HH. Lab-on-a-chip for oral cancer screening and diagnosis. Head Neck 2008;30(1):111-21.
7. Ying L, Wang Q. Microfluidic chip-based technologies: Emerging platforms for cancer diagnosis. BMC Biotechnol 2013;13:76.
8. Wong DT. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. J Am Dent Assoc 2006;137(3):313-21.
9. Mehrotra R, Gupta DK. Exciting new advances in oral cancer diagnosis: Avenues to early detection. Head Neck Oncol 2011;3:33.
10. Mauk MG, Ziober BL, Chen Z, Thompson JA, Bau HH. Lab-on-a-chip technologies for oral-based cancer screening and diagnostics: Capabilities, issues, and prospects. Ann N Y Acad Sci 2007;1098:467-75.
11. Arunkumar S, Arunkumar JS, Burde KN, Shakunthala GK. Developments in diagnostic applications of saliva in oral and systemic diseases - A comprehensive review. JSIR 2014;3(3):372-87.
12. Miranda JM, Minas G, Vicente AA, Teixeira A. Design of a lab-on-a-chip for clinical tests of human physiological fluids. International Conference on Transport Phenomena in Micro and Nanodevices; 2006.
13. MacPherson MJ, Ravichandran M. Lab-on-a-chip technology: The future of point-of-care diagnostic ability. UWOMJ 2011;80(1):24-6.
14. Lisowski P, Zarzycki PK. Microfluidic paper-based analytical devices (µPADs) and micro total analysis systems (µTAS): Development, applications and future trends. Chromatographia 2013;76(19-20):1201-14.

15. Ahn CH, Choi JW, Beaucage G, Nevin JH, Lee JB, Puntambekar A, *et al*. Disposable smart lab on a chip for point-of-care clinical diagnostics. Proc IEEE 2004;92(1):154-73.
16. Haeberle S, Zengerle R. Microfluidic platforms for lab-on-a-chip applications. Lab Chip 2007;7:1094-110.
17. Sekhon BS, Kamboj S. Microfluidics technology for drug discovery and development – An overview. IJPRIF 2010;2(1):804-9.