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Laser Capture Micro-Dissection – A Premium Tool in Oral and Maxillofacial Pathology

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ABSTRACT --*The dawn of new technologies has enabled deeper insight into processes at subcellular levels, which ultimately improves diagnostic procedures and patient outcomes. Microdissection has greatly contributed to rapid growth in several areas, such as gene expression analysis, proteomics, genomics, and metabolomics. Laser capture microdissection (LCM) as a method of procuring subpopulations of cells under direct visual inspection plays a vital role in these areas. There is ample evidence to substantiate the utility of LCM in understanding the underlying molecular mechanism involving an array of oral physiological and pathological processes. This review provides the indispensable application of LCM in various oral disease from the perspective of an oral pathologist.*

Keywords-- laser ***capture*** micro-dissection – a premium tool in oral and maxillofacial pathology

I. INTRODUCTION

Oral cancer is one of the most consequential public health concerns in today's era. The molecular investigation of pathologically altered cells and tissues at the DNA, RNA, and protein level has revolutionized research and diagnostics in pathology. With the advancement in research technology, various experimental techniques are available for molecular profiling studies such as DNA microarray, differential display, serial analysis of gene expression, massive parallel signature sequencing, and suppression subtractive hybridization. (1) However, the accuracy of these sensitive systems may be compromised if the input DNA, RNA or proteins is not collected from pure population i.e. the accuracy is highly dependent on the sample purity. For example - Imagine a tissue section composed of 80% tumor, 10% stroma and 10% infiltrating lymphocytes in which the lymphocytes may yield more than 10% in overall signal. Thus, the outcome of molecular biological analyses from these samples may be compromised. Hence there is a need for technology that could feasibly distinguish normal, premalignant, malignant, or disease-free cells as distinct populations from the heterogeneous background. (2) Laser capture microdissection (LCM)-based molecular biological analysis is an effective solution for these problems. (3)(4)

The laser capture microdissection (LCM)-based molecular biological analysis allows selective retrieval of specific types of normal and diseased cells from tissue sections that can then be used for DNA, RNA or protein expression analysis. Thus, as per the recent LCM technological/methodological advancement, LCM has been applied to a wide range of oral cancer research that used tissue sections because the results are much more accurate and dependable than conventional methods. (4)

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This article will focus on the principle and technology behind LCM along with its potential application on various fields of molecular biology from the perspective of an oral pathologist.

II. EQUIPMENT OF LCM SYSTEM

LCM was first introduced as a system that can retrieve defined cell populations from human tissue samples and was developed during the mid-1990s by Dr. Emmert-Buck and colleagues at the National Institutes of Health (NIH), Bethesda, ML, USA. (2)(4) Till-date three types of LCM systems are discovered.(2) (5) (6)

TYPE I – Infrared (IR)-laser based LCM system

This type of system damages the tissue very little and is good for small targets. As compared with the ultraviolet (UV)-laser based LCM system, it is not suitable for dissection of thicker samples. Principally it captures a group of or individual cells onto a thermoplastic membrane from histological sections of stained tissue. The IR laser pulse then melts ethylene vinyl acetate (EVA) polymer, which adheres the cells to the melted membrane. Subsequently as the membrane cools it solidifies again and the cells are removed by peeling off the membrane from the cap. (2)(7)

TYPE II – Ultraviolet (UV)-laser based LCM system

This type is suitable for clusters of cells and targets big areas of tissues i.e. to dissect thick sections such as those up to 30 µm. The unwanted tissue is photoablated with the very narrow UV laser beam. The various approaches are use of pulsed UV-A laser to create photonic pressure for sample collection, use of UV laser to cut the tissue by which the dissected cells fall into a collecting tube, use of solid-state UV laser.(8) (9)

TYPE III – Combined IR-UV laser system

This type is the combination of both type I and type II LCM system and carries IR laser capture microdissection and UV laser cutting in one single instrument where the IR preserves the biomolecular integrity and UV microdissects dense tissue structures. Here the solid-state IR laser delivers a capture technique, which preserves biomolecular integrity and is ideal for single cells or a small number of cells while the solid-state UV laser delivers unprecedented speed and precision, which is suitable for micro dissecting dense tissue structures and for rapidly capturing large numbers of cells. (2)(5)

III. PRINCIPLES AND TECHNICAL BASIS OF LCM

LCM is based on the fundamental principle of selective adherence of visually targeted cells and tissue fragments to a thermoplastic membrane which gets activated by a low energy infrared laser pulse. The bedrock conception is (i) visualization of the cells over the microscope; (ii) transfer of laser energy to the area of interest followed by selective removal of the cells of interest from the heterogeneous tissue; and (iii) collection of the cells into a microtube/device. (10)

The components of the LCM system includes (11)(12) –

a) An inverted microscope,
b) A solid-state near-infrared laser diode,
c) A laser control unit,
d) A joystick-controlled microscope stage with a vacuum chuck for slide immobilization,

e) A charged coupled device (CCD) based camera,

f) A color monitor.

The LCM microscope is linked to a personal computer for additional laser control and image archiving. The thermoplastic membrane of approx. diameter 6mm, used for the transfer of selected cells, is mounted on an optically clear cap, which fits on standard 0.5 ml microcentrifuge tubes for further tissue processing. The cap is suspended on a mechanical transport arm and is placed on the desired area of the dehydrated tissue section under standard pressure. The cap is then lowered in a precise juxtaposition to the requisite area and after perceptible selection of the desired cells guided by a positioning beam, the laser gets activated which leads to the focal melting of the ethylene-vinyl acetate (EVA) membrane. The melted polymer expands into the section and loads the extremely small hollow spaces present in the tissue. Soon, within milliseconds the polymer resolidifies and forms a composite with the tissue. The adherence of the tissue with the activated membrane exceeds the adhesion to the glass slide and allows selective removal of the desired cells. **(FIGURE 1)** (2) (13)

Laser impulses, usually ranges 0.5 - 5 ms in duration, can be repeated multiple times across the whole cap surface. This allows the rapid isolation of large numbers of cells, which are harvested by the simple lifting of the cap and transferring the cells into a microcentrifuge tube containing the buffer solutions required for the isolation of the molecules of interest. The minimum diameter of the laser beam of the LCM microscope is currently 7.5 µm, and the maximum being 30 µm. Under standard working conditions, the area of melting polymer is quite exactly the size of the laser spot. Because most of the energy is absorbed by the membrane, the maximum temperatures reached by the tissue upon laser activation are in the range of 90°C for several milliseconds, thus leaving biological macromolecules of interest intact. (2,13) (14)

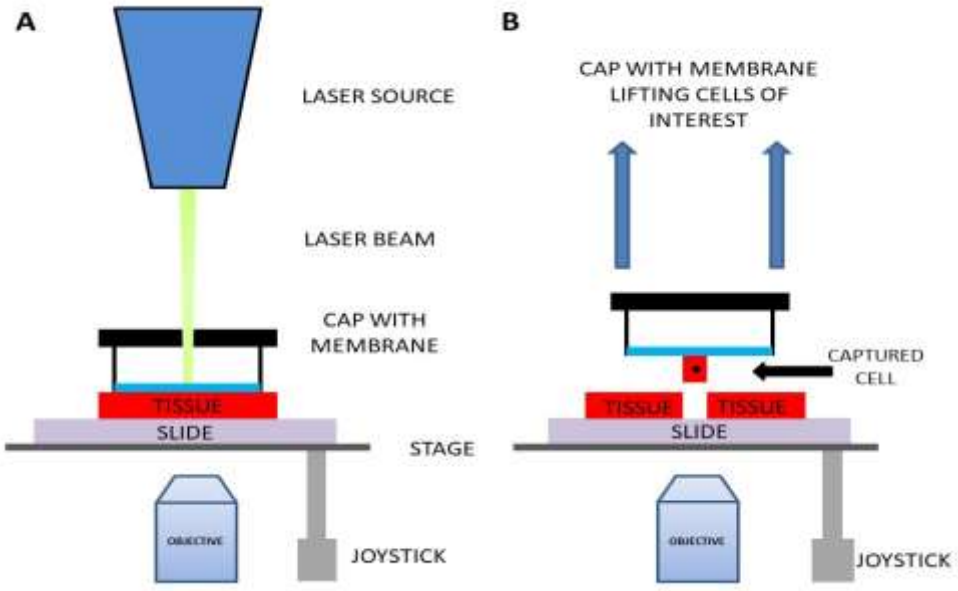

FIGURE 1 : Schematic representation of laser capture microdissection. **(A)** Activation of laser leads to focal melt of the polymer membrane. **(B)** Lifting of the cap selectively to detach the cell adhered to the activated membrane.

IV. TYPES OF SAMPLES

The type of samples typically used for LCM is illustrated in **Figure 2**. LCM is compatible with most cell/tissue staining techniques such as H & E, HICK, toluene blue, fluorescent dyes, and in situ hybridization. The choice of fixative is according to the nature of the investigation. Formaldehyde is an efficient fixative for DNA, but acetone or ethanol fixation yields better quality of RNA. RNAse free reagents should be used whenever RNA based investigations are performed, because the protection of sample from degradation is important. Also, the specimens need to be dehydrated properly, since the presence of water interferes with the bonding of polymer to the captured cells. (2)(4)

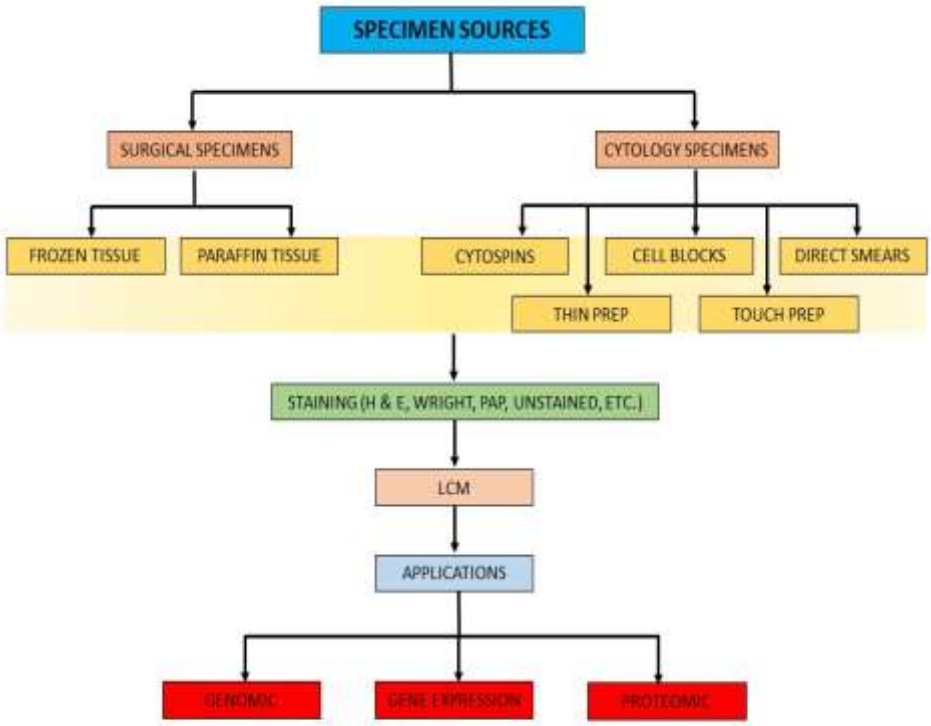

FIGURE 2 : TISSUE SOURCES AND APPLICATION OF LCM

V. APPLICATIONS OF LCM

LCM technology has been used in a wide variety of applications. DNA, RNA, and proteins are the three distinct classes of biomolecules that can be analyzed in LCM specimens. (3) (15) The areas where laser capture microdissection is applied are discussed below:

Genomic Analyses from Microdissected Materials

In cancer tissue, there is a loss of heterozygosity (LOH), which is used for studying the frequency of involvement as well as mapping of tumor suppressor genes in cancer research. And for LOH interpretation pure populations of tumor cells or preneoplastic foci are required which can be achieved through microdissection. Microdissection had revolutionized the path to LOH research in many ways. Besides LOH analysis, other genome

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analyses can also be performed, such as analysis of patterns of X-chromosome inactivation to assess clonality, restriction fragment length polymorphism (RFLP) and single strand conformation polymorphism (SSCP) analysis for assessment of mutation in critical genes. Most recently, analysis of promoter hypermethylation in tumor suppressor genes, which is an early event in carcinogenesis, can also be achieved through LCM. (9)

LCM has been used to study tumour heterogeneity of oral squamous cell carcinoma by picking up cell populations from the tumour invasive front at the advancing edge as well as the central region and the stroma of the same tumor island. It has been reported that there is substantial upregulation of tumor protein 53 (TP53) and ribosomal protein S6 (RPS6) in the tumor invasive front than the center. (16)

Combination of microdissection with primer extension preamplification (PEP) and whole genome amplification (WGA) aids to use smaller and smaller samples of cells, thereby refining the study of preneoplastic lesions, which has opened a whole new frontier in cancer research. Microarrays, in combination with LCM have generated the most excitement of all current expression technologies. (17)

LCM technology is crucial in the study of tumour's that lack solid growth pattern, but rather are categorized by an infiltrative and cystic component that often leads to contamination of tumour tissue by traditional bulk tissue analysis. For example in a study of microgenomics of Ameloblastoma using LCM it was found that most of the overexpressed genes were typical of an Ameloblastoma lineage like wingless-type mouse mammary tumour virus(MMTV) integration site family, member 10A, MyoD family inhibitor (MDFI), protein patched homolog (PTCH), and B-TFIID TATA box-binding protein- associated factor 1 (BTAF1) RNA polymerase II transcription factor. This study shows that LCM of Ameloblastoma tissue provides an accurate way to select a specific tumor cell population, thereby reducing variance introduced by the inclusion of stromal tissue that confounds gene expression analysis. (18) LCM in concomitance with immunohistochemistry (IHC), in situ hybridization (ISH), and polymerase chain reaction (PCR) has been employed to rule out the role of HPV in etiopathogenesis of Ameloblastoma. (19)

Gene Expression Analysis from Microdissected Material

Differential gene expression is a useful parameter to determine how tumors differ from the normal tissues from which they are derived. It can be deliberated by a variety of methods such as differential display, representation difference analysis (RDA), SAGE, ESTs, and differential gene chips. However, a similar issue as in DNA-based studies in the analysis of gene expression is the problem of contamination with inflammatory and stromal cells. Therefore, there has been an increasing need to apply microdissection methods in the expression studies also.

LCM technology has been used for analysis of tumor suppressor gene loci, using DNA, isolated from archived paraffin-embedded OLP tissue specimens. (20) LCM in combination with cDNA microarray analysis provides a powerful new approach to monitor the in vivo gene expression profiles of perineural invasion in salivary Adenoid cystic carcinoma for genes like melanoma cell adhesion molecule (MCAM), amphiregulin (AREG), meningioma expressed antigen 6 (MGEA6), N- terminal caspase recruitment domain 12 (CARD12), peripheral myelin protein 22 (PMP22), taxol resistance-associated gene 3 (TRAG3), MMP-7, and nuclear transport factor 4 (NTF4) gene. (21) Also evaluation of the expression of transcription factors and cytokines in the infiltrating lymphocytes through LCM has led to thought-provoking insights in the progression and development of the Sjogren's syndrome. (22)

Proteomic Analyses from Microdissected Material

Proteomics, a complementary approach to study gene expression, aims to determine the overall set of proteins ("proteome") that are important in normal cellular physiology as well as disease processes such as cancer. LCM has been applied to the study of protein alterations in tumors and their preneoplastic lesions, which is an important step toward formulating treatment and intervention strategies. (23) Several laboratories have studied differential protein expression in microdissected tissue specimens from patients with head-and-neck cancer in efforts to discover novel tumor markers. (24) Proteomic profiling based on the laser capture microdissection of formalin-fixed, paraffin- embedded samples, followed by liquid chromatography-tandem mass spectrometry (LC/MS) analysis was performed for expression of CK10/13/4 or CK14/17. The data obtained from this study indicated that the platform based on LCM-FFPE-proteomic analysis is an effective approach for biomarker identification in OSCC. (25) The expression of cyclooxygenase-2 (COX-2), Bcl2, and Ki67 protein in cells of pleomorphic adenoma has also been analysed. This study implicated upregulation of COX-2 and anti-apoptotic protein Bcl2 in the luminal cells of pleomorphic adenoma aids in its pathogenesis. (26)

LCM and high-throughput surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) have been used in oral leukoplakia and OSCC samples in order to obtain their proteomic profile. This study concluded that LCM usually guarantees approximately 95% purity of the accurate cell population from tissues.(27)

The junctional epithelium is a key feature of innate immunity against periodontitis. LCM can be applied for biomarker discovery for diagnostics in multiple human tissue types and organ systems. LCM studies involving the junctional epithelium have yielded important molecules involved in the pathogenesis of periodontal infections.(28)

Other areas where LCM has also been used include evaluation of tumor microenvironment, forensic analysis of fixed cell samples and hair follicles, studies in developmental biology and embryology.

VI. CONCLUSION

LCM is a high-end research and diagnostic technology that helps in obtaining pure cell populations for the purpose of cell- or lesion-specific genomic and proteomic analysis. As the generation of genomic and proteomic analysis continues to evolve rapidly, the need of the hour is to be highly peculiar with our approach to study the humble cell. LCM technology has significantly advanced since 1990s and has overcome several challenges to address the requirement of large quantities of biomolecules and the need for accuracy. The next few years hold great promise for the use of molecular information in disease management, including design of optimal lower risk, patient tailored treatment. The LCM, being a challenging analytical technique, is sure to become common fixtures of many biomedical research facilities in the field of pathology.

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