10.5005/jp-journals-10024-2286

EDITORIAL


# Laser Capture Microdissection in Oral Cancer

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**How to cite this article:** Muruganandhan J, Sujatha G, Patil S, Raj AT. Laser Capture Microdissection in Oral Cancer. J Contemp Dent Pract 2018;19(5):475-476.

**Source of support:** Nil

**Conflict of interest:** None

Oral cancer is one of the most significant public health concerns in the world. The past decades have witnessed the trend of molecular profiling for diagnostic and prognostic implications. Deoxyribonucleic acid (DNA) microarray analysis, differential display, and serial gene analysis, massively parallel signature sequence, and suppression hybridization are the most commonly employed techniques. The accuracy of these sensitive systems depends on the sample purity. The heterogeneity of cell population and the possibility of contamination by various sources impact the final result. Therefore, laser capture microdissection (LCM)-based molecular biological analysis is an effective solution for these problems. Tissue-based LCM is a method by which specific removal of required cell populations from the sample section can be done.[1] These cells can be then subjected to nucleic acid or protein analysis. The results from LCM are much more accurate and dependable than conventional methods. Thus, LCM has been employed for oral cancer research.

The three types of LCM systems are: the infrared (IR) laser-based, the ultraviolet (UV) laser-based, and the combined IR–UV laser LCM system.[2] The IR-LCM, like Pix-Cell and Auto Pixare, is good for small targets but not for thicker samples. The UV laser-based LCM system, like Leica laser microdissection (LMD), Zeiss PALM, is suitable for clusters of cells and can dissect thick sections up to 30 µm. The Zeiss PALM system uses photonic pressure from pulsed UV laser to collect samples, called laser pressure catapulting. Combined IR–UV laser system, like Arcturus Veritas, Arcturus XT, uses both IR and UV pulses. Here, IR preserves the biomolecular integrity of a smaller number of cells, and UV microdissects dense tissue structures. A combined technique of LCM, ribonucleic acid (RNA) amplification, and gene expression expresses a remarkable molecular characterization of the exact cell and tissue.

The process of LCM involves the following steps.[3] Initially, the tissues are fixed for 8 to 16 hours in neutral buffered formaldehyde at 4°C. Then, it is dehydrated using increasing grades of ethanol followed by immersion at room temperature in xylene for an hour and for 30 minutes at 58°C in paraffin which is subsequently embedded and blocked. These paraffin blocks are stored at 4°C and is cut using a microtome. These paraffin sections are made to float on 43°C nuclease free water. Then, the tissue is mounted on a poly-L-lysine-coated glass foiled polyethylene naphthalate slide for LCM process. The slides are dried in a 35°C incubator for 6 hours. The process of LCM is recommended to be carried out at the earliest and slides are recommended to be used within a week after preparation. Then, the slides are deparaffinized, and washed with DEPC (diethyl pyrocarbonate)-treated water. Nuclear staining is done by Gill no: 3 hematoxylin for 5 to 10 seconds. Avidin-Biotin-Peroxidase complex is employed for immunostaining. The LCM (Leica AS LMD System) dissects only the immunopositive cells which get collected into individual tubes containing TRIZOL reagent and chloroform. Then, the sample is centrifuged at 14,000 rpm for 15 minutes at 4°C. Three layers are formed, namely the lower phenol-chloroform phase,

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an intermediate phase, and the presence of RNA in the upper colorless aqueous phase. This RNA is precipitated by adding isopropyl alcohol. The RNA is precipitated and forms a small dotted pellet at the bottom of the tube; 1 µg of RNA is added to 4 µL of Master mix [high-capacity RNA to complementary DNA (cDNA) converter]. The polymerase chain reaction (PCR) tubes are closed securely and centrifuged. Then, the tubes are loaded in a thermal cycler which synthesizes cDNA. Then, reverse transcription of the cycler is started. The DEPC-treated water is added and stored at –70°C to perform quantitative PCR.

The molecular characterization of specific cells and tissues is made possible with the combination of LCM, RNA amplification, and gene expression. Gene expression analysis from heterogeneous biological samples can obscure changes that occur in particular tissues. In such cases, LCM allows relevant cell types to be purified from complex mixtures of biological samples. Expression of microRNA is also best achieved with LCM in oral oncology. Alteration in microRNA 31 along with p53 mutation is known to cause head and neck squamous cell carcinoma. Additional applications of LCM include differential gene profiling, gene quantification, loss of heterozygosity, and clonal analysis. Cancer Genome Anatomy Project (CGAP) uses LCM to obtain normal, premalignant, and malignant samples from oral tissues.[4] In CGAP, efforts are made to produce cDNA library that catalogs genes that are differentially expressed during tumor progression. Information from CGAP is publicly available through the National Institutes of Health website which provides "*in silico*" answers to various biological questions within a fraction of the time. Research oncologists use these data from LCM for drug discovery and clinical diagnosis.

Thus, the consolidated design, fast functioning, flexible sample preparation, open modular platform, and intuitive operation have proved LCM to be the most powerful and effective in clinical and diagnostic oral oncology. The LCM, being a challenging analytical technique, is sure to become common fixtures of many biomedical research facilities in the field of oral pathology.

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