Research Techniques Made Simple: Laser Capture Microdissection in Cutaneous Research

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In cutaneous research, we aim to study the molecular signature of a diseased tissue. However, such a study is met with obstacles due to the inherent heterogeneous nature of tissues because multiple cell types reside within a tissue. Furthermore, there is cellular communication between the tissue and the neighboring extracellular matrix. Laser capture microdissection is a powerful technique that allows researchers to isolate cells of interest from any tissue using a laser source under microscopic visualization, thereby circumventing the issue of tissue heterogeneity. Target cells from fixed preparations can be extracted and examined without disturbing the tissue structure. In live cultures, a subpopulation of cells can be extracted in real time with minimal disturbance of cellular communication and molecular signatures. Here we describe the basic principles of the technique, the different types of laser capture microdissection, and the subsequent downstream analyses. This article will also discuss how the technique has been employed in cutaneous research, as well as future directions.

Journal of Investigative Dermatology (2016) **136,** e99–e103; doi:10.1016/j.jid.2016.08.005

CME Activity Dates: September 21, 2016
Expiration Date: September 20, 2017
Estimated Time to Complete: 1 hour

Planning Committee/Speaker Disclosure: All speakers, planning committee members, CME committee members and staff involved with this activity as content validation reviewers have no financial relationship(s) with commercial interests to disclose relative to the content of this CME activity.

Commercial Support Acknowledgment: This CME activity is supported by an educational grant from Lilly USA, LLC.

Description: This article, designed for dermatologists, residents, fellows, and related healthcare providers, seeks to reduce the growing divide between dermatology clinical practice and the basic science/current research methodologies on which many diagnostic and therapeutic advances are built.

Objectives: At the conclusion of this activity, learners should be better able to:
- Recognize the newest techniques in biomedical research.
- Describe how these techniques can be utilized and their limitations.
- Describe the potential impact of these techniques.

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PRINCIPLES OF LCM

Emmert-Buck et al. developed laser capture microdissection (LCM) in 1996 at the National Institutes of Health to support the Cancer Genome Anatomy Project (Emmert-Buck et al., 1996). The goal of the Cancer Genome Anatomy Project was to develop a high caliber expression library of human cancers and precancerous lesions. Such an undertaking called for an isolation of specific tumor cells from solid tumors without disturbing the integrity of biomolecules (DNA, RNA, protein) within the collected cells. To accomplish this task, the team developed a microscope-based microdissection platform, now known as LCM.

LCM is a technology used to isolate a single cell or a specific cell population from a heterogeneous tissue section, cytological preparation, or live cell culture by direct visualization of the cells (Emmert-Buck et al., 1996). There are two main classes of laser capture microdissection systems: infrared LCM (IR-LCM) and ultraviolet LCM (UV-LCM). IR-LCM instruments are available as manual or

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Abbreviations: EVA, ethylene-vinyl acetate; FFPE, formalin-fixed paraffin-embedded; IHC, immunohistochemical; IR-LCM, infrared LCM; LCM, laser capture microdissection; UV-LCM, ultraviolet LCM

WHAT LCM DOES

- LCM is a technique that isolates cells of interest or even a single cell from a heterogeneous tissue specimen using a laser source under microscopic visualization.
- Cells isolated by LCM contain intact DNA, RNA, and proteins for downstream molecular analysis.
- LCM is capable of isolating diseased cells from the primary lesion without altering their molecular signatures.
- LCM can be applied to a wide variety of tissue and cellular preparations.

LIMITATIONS

- In the absence of a cover slip, the optical resolution of complex tissues may be limited.
- The reliance on visual identification of target cells creates room for human error.
- Unlike IR-LCM, UV-LCM is limited by the potential to induce UV damage in the circumferential cells, which may be subsequently collected for analysis.

automated systems. Other available platforms include an IR/UV combined system. Regardless of the platform used, the principal steps of LCM are the visualization of cells by microscopy, the transfer of laser energy to isolated cells of interest, and the collection of cells of interest from the tissue section (Espina et al., 2006).

LCM can be applied to a variety of preparations including histological specimens (formalin-fixed paraffin-embedded [FFPE] or fresh-frozen sections) and cytology preparations (direct smears, touch preps, or cell block). Samples can be stained, unstained, or tagged by immunohistochemistry. Frozen tissue effectively preserves RNA, DNA, and proteins, but may distort histologic differentiation. The standard method for preservation of tissue morphology is FFPE. However, it causes unwanted crosslinking between proteins and nucleic acids and proteins are not extractable from FFPE samples (Liu, 2010).

INFRARED LCM

This technique uses a lower energy laser in the IR spectrum of 810 ηm to activate a 100-μm, transparent, and thermosensitive film containing ethylene-vinyl acetate (EVA) saturated with a dye that absorbs IR laser energy. The thermosensitive film is positioned over a stained frozen or FFPE tissue section, which can be visualized with an inverted microscope. The microscope is connected to a computer for laser control and image archiving. A laser beam is directed at the cells of interest, but only the thermosensitive film absorbs the energy of the laser. Consequently, there is no damage to the underlying cells or biomolecules within the cells. The focused pulse from the IR laser produces a conformational change in the EVA polymer, which becomes fixed to the cells of interest underneath. The adhesive force of cells to the film exceeds the adhesive force to the slide, enabling selective removal of cells (Figure 1). Once removed, the cells are transferred to a microcentrifuge tube containing DNA, RNA, or enzyme buffer where the cellular material detaches from the film (Emmert-Buck et al., 1996).

ULTRAVIOLET LCM

The LCM technique using an ultraviolet cutting laser is also known as laser microbeam microdissection. Laser microbeam microdissection uses a high-energy UV laser (355 ηm) capable of cutting tissues. The laser is used to cut around the cells of interest, in contrast to IR capture that focuses the laser on the cells. In UV-LCM, the surrounding unwanted tissue is photoablated whereas the desired cells remain intact (Schutze and Lahr, 1998). Target cells are retrieved through a variety of methods depending on the instrument. The cells can be collected by photonic pressure from a second laser shot that catapults them into a collection cap (PALM/Zeiss system, Oberkochen, Germany), by gravity that deposits them into a collection cap (Leica Microsystems, Wetzlar, Germany), or by a sticky cap to which they are glued after LCM (MMI Instruments, Eching, Germany) (Espina et al., 2006; Liu et al., 2014).

IMMUNO-LCM

Immuno-LCM uses immunohistochemical (IHC) staining to identify and isolate a specific cell population that is challenging to discern visually. For instance, cells that are morphologically similar but immunologically distinct such as B and T lymphocytes can be distinguished using IHC staining for a type-specific antigen before LCM. The common IHC reagents do not adversely affect downstream analysis using assays such as PCR (Fend et al., 1999). RNA degradation from IHC staining can be prevented by prelabeling cells, for example, by injecting animals with a fluorogold label before harvesting the tissue (Yao et al., 2005). However, immuno-LCM is not optimal for studying protein expression, as the protein of interest is bound by antibodies (both primary and secondary) during IHC staining. These bound antibodies can interfere with downstream methods such as polyacylamide gel electrophoresis, western blotting, and mass spectrometry.

DOWNSTREAM ANALYSIS

Once collected, DNA can be subjected to sequencing, DNA methylation assays, and loss of heterozygosity studies. RNA can be used for sequencing and constructing a cDNA library, as well as in gene expression arrays, real-time RT-PCR, and quantitative PCR. Protein can be studied with western blotting, 2D gel electrophoresis, mass spectrometry, and reverse-phase protein microarray. It is important to note that proteomic tests require more material than DNA and RNA analyses (Espina et al., 2006).

ALTERNATIVE METHODS

An alternative approach to isolate and concentrate cells of interest is by cell sorting techniques such as FACS and magnetic-activated cell sorting. These methods require that cells be processed in fluid suspensions, which are suitable for the analysis of hematopoietic and circulating cells but not ideal in the analysis of solid tissue.