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Review

Laser Microdissection: Application to Carcinogenesis

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Abstract. *Although several microscopic techniques are available for separation of small groups of cells from surrounding tissue, the high energy concentrated into a small area, the easy control of beam position and the lack of direct contact with the material to be dissected make lasers the best option for a large scale microdissection. Laser microdissection technology provides samples of homogenous cells, or even a single cell, isolated from whole tissue or cytological materials to ensure that biological molecules such as DNA, RNA or protein are undamaged during sampling in order to define the molecular and cellular biology of diseases, including cancer. This article reviews the various techniques of laser microdissection including capture, catapulting and gravity-assisted, in addition to a cheaper alternative (ultrasound). Methods of sample preparation, including contamination eradication, enrichment, fixation, staining and extraction of biological molecules are also reviewed. Application of genomic and proteomic technology to laser microdissected cells has revolutionized the classification of solid cancer and enhanced its diagnosis, prognosis and drug discovery.*

Abbreviations: CCD, charged coupled device; cDNA, copy deoxyribonucleic acid; DSP, dithio-bis(succinimidyl propionate); EASI, epithelial aggregate separation and isolation; FFT, fresh frozen tissue; FFPET, formalin fixed and paraffin embedded tissue; GAM, gravity-assisted microdissection; H&E, hematoxylin and eosin; IR, infrared; LCM, laser capture microdissection; LMD, laser microdissection; LPC, laser pressure catapulting; mRNA, messenger ribonucleic acid; NA, numerical aperture; PEN, polyethylene naphthalate; PET, polyethylene tetraphthalate; POL, polyester; RT-PCR, reverse transcription polymerase chain reaction; mRNA, messenger ribonucleic acid;UV, ultraviolet light.

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Tissues are a heterogeneous mixture of different cell populations. In the era of genomics/proteomics, the correlation of molecular changes with morphologic pathologies requires procurement of pure populations of cells. Earlier methods employed manual microdissection techniques using scalpels or razor blades (1), fine glass micropipettes (2), needles (3) or touch preparations (4). However, these methods of manual microdissection were time consuming and introduced risk of contamination from surrounding cells (5).

In the 1970s, the principle of laser dissection using pulsed UV lasers was introduced (6). The principle of the cutting method was based on a locally restricted ablative photodecomposition site process without heating the direct environment of the focused high energy beam by using a short pulse duration (in micro seconds) that is shorter than relaxation time of the biological material. A modification of that technique employed a negative selection method termed selective ultraviolet radiation fractionation (SURF) that involved the protection of selected cell population against 337 nm UV nitrogen laser by covering them with dark dyes such as ink, and protected cells were picked up manually (which compounded dexterity issues of contamination) (7). A technique that employed microdissection of membrane-mounted native tissue (MOMeNT), in which tissue sections were mounted onto a thin polyethylene foil and the tissue and the membrane were removed by photolysis using a 337 nm UV laser. This was followed by either transfer into PCR tube by a 30-gauge needle or a small forceps, or by using the laser to catapult the cells directly into the collection tube to alleviate the contamination issues associated with the manual lifting of cells (8).

Because of these contamination issues, newer instruments were developed using various UV or IR lasers to allow researchers avoid touching the specimens by catapulting, dropping the untouched dissected cells into a capture tube, or by lifting the untouched specimens directly into the lid of a microcentrifuge tube. There are several parameters to characterize a laser microdissection system such as type of laser used, resolution, specificity of dissection and integrity of the dissected material, control of stage movement on

which the tissue sits, ease of use, environment in which the instrument is used, and method of sample preparation (9).

Laser Capture Microdissection (LCM)

LCM was initially conceived in 1996 by a team of investigators at the USA National Institutes of Health (NIH) [http://dir.nichd.nih.gov/lcm/lcm.htm] to provide a rapid and direct method for procuring homogeneous cells for molecular analysis (10-12). Arcturus Engineering, Inc. (Mountain View, CA) [http://www.arctur.com] developed the first commercial LCM instrument (PixCell II) in 1997 in conjunction with the NIH team as part of a Collaborative Research and Development Agreement (Figure 1). That system employed a proprietary four-step LCM process to collect tissue. It is a manual LCM only instrument based on an Olympus inverted microscope that used an IR laser (gallium arsenate laser diode, 810 nm λ) focused through a microscope objective to melt a plastic polymer film, containing dyes that absorb the energy, sealed to the bottom of a plastic cap that is placed above the tissue. Because of the mild heat produced, the melted film sticks to selected cells. When the laser beam is turned off, the plastic solidifies and forms bonds with the cells, and the cab can be lifted and placed in a microcentrifuge tube containing solutions for extracting DNA, RNA or protein (Figure 2a). The transfer process is nondestructive and cell morphology is retained. Moreover, there is a record of the original location of cells in the tissue and a visual verification of cell capture (13).

The resolution of the instrument (or the smallest area that be isolated from the rest of the tissue) depends on the numerical aperture (NA) of the objective lens used and on the wavelength of employed light. The NA is defined as the sine of the collection angle multiplied by the refractive index of the immersion medium. Use of dry objectives in LCM instruments limits the NA. A stringent limitation comes from the maximum collection angle used. For limited lens diameter, a larger angle imposes a short working distance. In the PixCell system, light leaving the objective lens passes through the collecting cap, which limits the minimum working distance and consequently the NA of the objective. The size of the beam waist depends on the wavelength of the light used. The IR light used by the PixCell system gives a large beam waist than an UV light, ~7.5 μm or a size of a cell (9). The machine has also a fluorescent capability through the use of various color filters with excitations in the range of 455-630 nm and excitations from >510 to >655 nm. In the PixCell, the bond between the plastic and tissue must be stronger than the bond between the tissue and slide. Uncharged (*i.e.*, without coating) and unsubbed slides are suited when simple stain is used because the tissue adheres more loosely to the slide. However, when the more demanding immunostaining is used, sections may be lost or

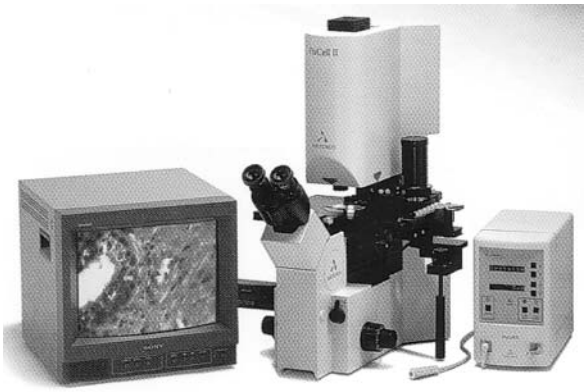

Figure 1. *Photo of the 1996 PixCell II® apparatus. Courtesy of Mike Fazio, Arcturus Engineering, Inc.*

contamination may occur unless sticky prep strips are used to remove contaminants from the cap, as there is no possibility to selectively destroy unwanted cells or tissue in that system (5).

Humidity in the specimen due to incomplete drying or to absorption of air vapor complicates LCM, and samples and instrument should be kept in a dry environment. The PixCell uses a joystick manual control of the stage movement to bring the target into the middle of the field view to fire the laser before moving to the next target. This unautomated process requires skill and concentration as misfire can cause contamination (9). The dynamics of the IR focusing and the melting properties of the thermoplastic transfer film on the cap are optimized with those of cells in 5 to 10 μm thick tissue sections (14). A computer software controls the microdissection process, stores data and allows the pathologist to draw landmarks on the area(s) of interest for LCM.

In 2002, Arcturus launched the first automated LCM system (the AutoPix) that can capture thousands of specific cell types automatically using computer controlled cell recognition and processing technology. In 2004, the company introduced the first system offering both LCM and UV laser cutting capabilities (Veritas Microdissection, see Figure 3) with various models: LCC1701, Veritas microdissection only; LCC1702, Veritas microdissection and fluorescence; LCC1703, Veritas microdissection and laser cutting; and LCC1704, Veritas microdissection, laser cutting and fluorescence. The UV capability allows ablation of nonspecific tissue and contaminants on the capture membrane. The machines use a Nikon microscope with a long working distance 60x objective. Because of its diode-pumped solid-state 349 nm UV cutting laser capability that allows photoablation, Veritas can cut through thick sections (~400 nm), and volatilization of undesired material on slide or cap. An alternate cut and capture method can be used for tissue mounted on membrane such as 2 μm thick

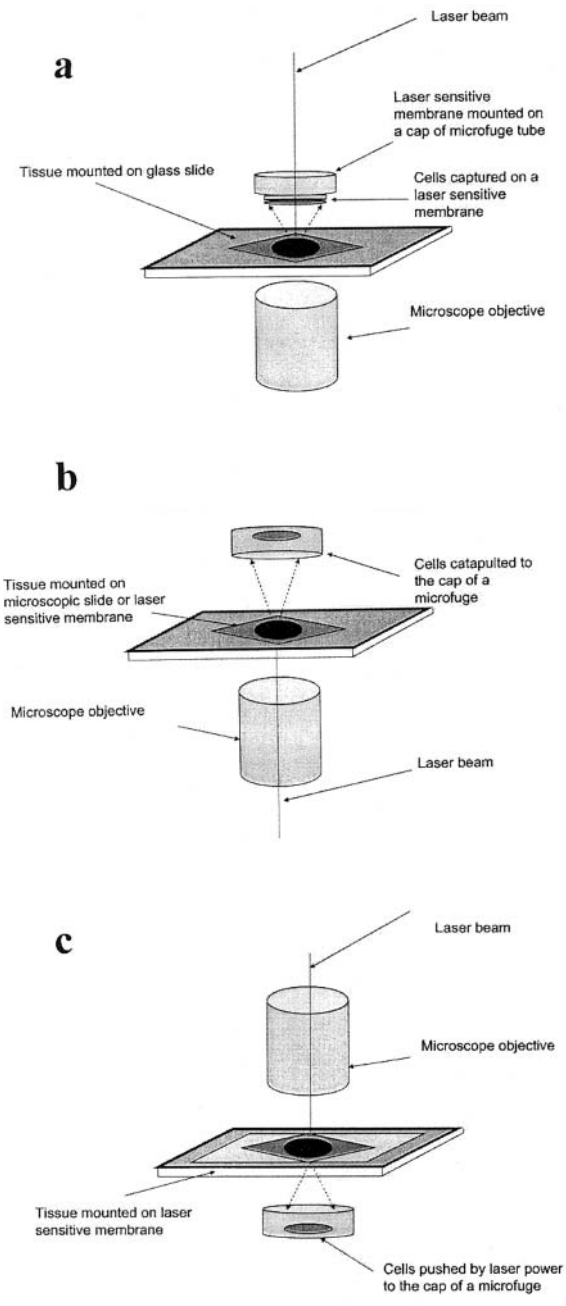

Figure 2. *Principles of (a) LCM by Arcturus, (b) PALM LPC by Zeiss, and (c) GAM by Leica. Modified from Reference 13.*

polyethylene naphthalate (PEN) membrane mounted on either a glass slide or in a metal frame. The use of PEN membrane framed or glass slides enables the use of dehydrated or hydrated samples. Veritas also has barcode reading of slides, which tracks sample chain of custody to ensure integrity of sample identification. Robotic control of caps enables increased flexibility and control of cap

Figure 3. *Veritas® microdissection instrument. Courtesy of Mike Fazio, Arcturus Engineering, Inc.*

processing. Arcturus also has a reagent system for formalin-fixed, paraffin embedded tissue, FFPET (Paradise® Reagent System), in addition to other reagents for DNA extraction, RNA isolation and amplification kits, frozen section staining kit and LCM immunofluorescence staining kit. These LCM are quite expensive. For example, the current list price of the PixCell IIe is US$110,000, and with fluorescence is US$132,000. The list price of Veritas (IR laser LCM only) is US$177,450. One can add fluorescence for an additional US$21,000 and UV laser cutting for another additional US$42,000 (Mike Fazzio, Arcturus, Personal Communication).

Another company, Cell Robotics International, Inc., Albuquerque, NM, employs IR laser tweezers (λ =1064 nm) on an inverted microscope (such as Leica, Nikon, Olympus or Zeiss) to form a powerful single-beam gradient, optical 3-D trapping computerized, motorized workstation to robotically control the stage, which permits easy manipulation of various cellular components with a single beam laser tweezer. The ability to focus the trap beam in the interior of the cells allows manipulating the cell's inside without damage to its membrane or constituents because IR lasers are less detrimental to cell viability than lasers of shorter wavelengths (15). LCM is the most widely used method for dissecting individual cells or groups in cells than other methods described below as judged from the number of articles published in peer-reviewed literature on this subject.

Laser Pressure Catapulting (LPC)

LMD and LPC with a focused pulsed nitrogen UVA laser (λ of 337 nm) allows dissection (cold ablation) and ejection of cells into a cap with no lateral damage to adjacent cellular material, as the wavelength of UVA is outside the range of absorption by biological matter. LPC seems to result from a gas pressure cloud that develops beneath the specimen due to laser-based ablation of material (photonic

cloud) and is sufficient to dislodge selected specimens from the object plane and transport them evenly (beam them up) over long distance along the direction of the incident light into a microcentrifuge tube without fear of contamination (Figure 2b) (16).

The PALM Microbeam modular system (P.A.L.M. Microlaser Technologies GmbH, a company of the Carl Zeiss Group, Bernried, Germany) [http://www.palm-microlaser.com] incorporates a pulsed UV cutting laser (<1 μm) that focuses a power density of >1 megawatt/cm^2, and a continuously emitting IR laser for optical trapping interfaced to a Zeiss Axiovert 200 inverted microscope (17). Add on attachments include: a fluorescence option, advanced image scanning software, and a microtweezer module for micromanipulation of objects in liquids. The system does not require unique disposables such as film, foil or lined caps. Different cutting routines for cryocentrifuged or glass- and membrane-mounted sections, living organisms such as *Amoeba proteus*, phytoplankton or even entire organisms such as the worm *Caenorhabditis elegans*, can be chosen from a sophisticated software to optimize working conditions from the first laser pulsed. The software's selection of drawing tools permits cells or sample areas to be preselected and assigned to special collection vessels (18).

Ready to use LPC membrane slides that use either PEN or POL membrane is recommended for laser ablation prior to cutting in order to avoid contamination with nonselected material (*e.g.*, chromosomes, filaments, cell organelles, *etc.*). Some special preparations, known as sandwich preps, may require covering the specimen with a sheet of LPC membrane to either immobilize a specimen that barely adheres to the membrane, or to conserve humidity so that the specimen does not dry out. On the other hand, humidity between the slide and the membrane may prevent proper membrane cutting (17). This freedom of sample preparation comes with the price of users having to optimize their protocols (9).

The PALM uses computer-controlled stage motors that can move the stage along a predrawn path, a feature that is convenient when large areas of irregular shapes are dissected. A freehand drawing tool allows the operator to outline the target area and then the stage moves as the laser fires and cuts the underlying membrane along the chosen path. The whole area is then catapulted into the collecting cap of a microcentrifuge tube. When several isolated cells are collected into the same cap, they can each be outlined first, and the system can subsequently automatically isolate them one by one (9). The resolution of the system (a higher NA) can be improved if a thin coverslip is used instead of a standard 1 mm thickness microscope slide, as the laser light needs to cross this distance to be focused on the tissue (9); however, this is impractical.

Cell Robotics uses UV laser scissors such as PRO 300 Workstation, with enhanced aluminum mirror for UV reflection, for ablation on an inverted microscope such as Nikon, Olympus, Leica or Zeiss. The workstation is compatible with most fluorescence illumination in its applications. It has motorized stage movement and focus and color video imaging controlled from a custom software interface, with computer controls and user friendly drawing tools for automated cutting along a user-defined path, but can also be adapted to other manufacturers' microscopes. Cell Robotics uses a technique that involves pick-up sticks. Several sticks with film on the ends are placed above the tissue and automatically oriented to the tissue to be collected. Electrostatic forces propel the tissue fragment toward the film, and the film is pushed inside a microcentrifuge tube for collection. Because the fragment is held inside the tube instead of a cap, there is less risk of losing the specimen. The process is motorized for high-throughput collection (19).

Molecular Machines and Industries (MMI) of Glattbrugg, Switzerland [www.molecular-machines.com] provides two UV laser microdissection instruments: SmartCut® and a second generation CellCut® that work with either a Nikon or an Olympus inverted microscope, but can also be adapted to other manufacturers' microscopes. The instruments employ a solid state UV laser, λ 355 nm. The advanced CellCut software allows freehand drawing or use of predefined figures – to encircle single- or groups-of cells mounted on membrane-covered frame, on a glass slide can be mounted – to be ablated by the UV laser. Microdissected samples (*e.g.*, FFPE, FF cryosamples, smears and cell cultures) can then be removed using the MMI isolation Cap® that rests on the membrane and has no direct contact with the specimen in a fast and precise manner, thereby avoiding contamination. The computer controlled motorized microscope stage of the more moderately-priced SmartCut allows for 0.025 μm step width and 2 μm repositioning accuracy. Its software allows for either free-hand or geometric figures drawings. Adjustable laser parameters such as focus, energy and speed allow the user to choose the optimum parameters for particular applications (20).

Gravity-Assisted Microdissection (GAM)

The capture technique used by Leica, Bensheim, Germany [http://www.leica-microsystems.com] relies on gravity to land the specimen in the collecting cap after ablation (21). Tissue is placed on a special membrane (PEN, PET or POL) on a glass slide that can be processed normally and then placed upside down on a microscope stage. Membranes are mounted upside down on regular glass slides (PEN) or steel frames (PET, POL), or on the bottom of special Petri dishes

for cell cultivation. When the powerful diode 355 nm laser passes through the glass it melts the foil as it cuts around the tissue's edge, and the foil and sample fall into a collection tube positioned immediately below the stage (Figure 2c). Single cells or groups of cells can be microdissected from tissue sections, biopsies, smears, cytospins, cell membranes, cell cultures, in addition to a live cell-cutting module. The laser can be also applied for intracellular and cellular ablation. The minimum cutting size for the older model, Leica AS LMD, was reported to be <2.5 μm (9). Multiple collection tubes in automated, programmable cassettes allow for contamination-free cell collection. Lack of humidity contributes to increased electrostatic forces that compete with gravity and affects collection in the Leica systems. Therefore, it is advisable to keep the instrument in a high humidity room free from air drafts (9).

The new Leica LMD600 system is based on the fully automated upright research microscope Leica DM6000B, with motorized transmitted light and fluorescence axes. The images for the tissues are provided by the Hitachi analog 3 CCD camera. Leica SmartCut objective series, in a range of 1.25x to 150x, are optimal for microdissection of individual cells. Leica systems use a computer-controlled mirror to move the laser beam fast, instead of the stage, along a path that can be preselected by a free-hand drawing tool allowing for a fast cutting (9).

Leica systems offer an inspection mode so that when cutting is complete, the operator can inspect both the removed fragment and the area from which it was excised. These systems also offer archiving capabilities so that the image of the captured fragment can be stored (19).

A Cheaper Ultrasonic Oscillator Alternative

For all their power, LMD systems are quite expensive (>US$100,000). Eppendorf, Hamburg, Germany, offers a low-cost alternative, Microdissector [http://www.eppendorf.com]. The Microdissector provides precise dissection of thick sections and tough tissue (*e.g.*, cartilage). The instrument cuts tissue using an ultrasonic MicroChisel. A piezo-powered stepper converts frequency (25-60 kHz) and amplitude (0-1.5 μm) into mechanical force that oscillates the MicroChisel fine metal tip to cut samples as small as a single cell without sample heat up. Because tissue pretreatment is not necessary, both living and archived tissue can be cut. In addition to the MicroChisel, the system comes with the aspiration pipetter and filter micropipette tips. Users can choose between disposable Filtertip® MDS for large samples of tissue (0.2 mm diameter) or TransferTip® MDS glass capillaries for aspirating small samples such as a single cell. To use the system, a micromanipulator such as Eppendorf's TransferMan® NK2 mounted on an inverted microscope is required. For delicate cell-transfer technique, Eppendorf's CellTram® vario is needed. It is a manual microinjector with gears, complete with pressure tube, filling tube, syringe, oil and universal capillary holder with grip head for capillaries with an outer diameter of 1 mm (Figure 4). To cut tissue, the user manually circumscribes the area with the MicroChisel using the foot pedal to determine the amount of force. To provide visual, the system can be combined with a monitor and camera. The microdissector is currently priced at US$14,250 and the NK2 micromanipulator at US$14,800.

Figure 4. *Photograph of the Piezo Power MicroDissector PPMD® System. The MicroChisel® is seen above the stage of a Zeiss inverted microscope on the right, while the NK2® Micromanipulator is on the left. Courtesy of Joel Lopez, Eppendorf USA.*

The characteristics of the main microdissection methods are presented in Table I.

Sample Preparation for Laser Microdissection

The type of sample preparation, fixation, staining and storage impacts laser microdissection, especially when the intent is extraction of a molecule that fragments easily such as RNA. The time following removal of sample tissue and fixation should be kept as short as possible. The fixative used and type of fixation also affect the molecular quality of the tissue. The embedding procedure (*i.e.*, medium employed and temperature used) or cryopreservation affects the results. The use of stains to visualize sample morphology impacts the success of microdissection (22).

There is no single optimal preparation strategy that fits all. If the aim is to isolate RNA from cell and produce full length cDNA, then fresh frozen tissue (FFT) sample will be required. If fixation of samples is desired because of easier storage and handling, then a noncrossing linking protein precipitating fixative such as alcohol- or acetone-based, in combination with low temperature embedding,

Table I. *Characterization of commonly used microdissection techniques.*

	Manual microdissection	Laser capture microdissection	Laser pressure catapulting	Gravity-assisted microdissection	Ultrasonic piezo microdissection
Principle	Acquisition of large tissue are a using a sterile needle or a scalpel	Melting of plastic film due to local heating by an IR laser allows transfer of cells	Cold ablation of unwanted cells using an UV laser, which produces a photonic cloud that exerts pressure to beam cells up	Cold ablation of unwanted cells using an UV laser and allowing cells in a collecting tube placed under microscope stage by gravity	Cutting a group of cells or even a single cells by ultrasound vibrations
Sample size (minimum) & spectrum of use	50-100 μm Large area ($>10^4$ cells)	➢ 7 μm Small cell groups & single cell	≥1 μm Single cell or groups of cells	≥1 μm Single cell or groups of cells	➢7 μm Small cell groups & even a single cell
Specimen specification	FFPET, FFT	FFPET, FFT, fixed and cryocentrifuged cell smear, Hard tissue with newer instruments equipped with a UV laser	FFPET, FFT, fixed and cryocentrifuged cell smear, chromosome and internal cell organelles	FFPET, FFT, fixed and cryocentrifuged cell smear, chromosome and internal cell organelles	FFPET, FFT and even hard tissue like cartilage
Sample procurement	Sterile needle, scalpel	Thermoplastic transfer film	Non-contact laser pressure catapulting directly into a microcentrifuge tube	Samples fall into a collecting tube by force of gravity	Samples cut by a microchisel, or cells aspirated by pipettes
Preparation time	5-10 min	<10s for 1-20 cells	<3 min for 1-20 cells	<3 min for 1-20 cells	15 min for 1-20 cells
Cost	Low	Very high	High	High	Moderate
Advantages	Easy and quick	Raid, fast, versatile, contamination free in new instruments having an additional UV laser; Inspection of removed cells and excised area after capturing; Automation	Isolation of single cells; Low risk of contaminating procured cells; Inspection of removed cells and excised area after catapulting; Automation	Isolation of single cells; Low risk of contaminating procured cells; Inspection of removed cells and excised area after catapulting; Automation	Relatively inexpensive method; Inspection of removed cells and excised area after dissection
Disadvantages	Risk of contamination; Unsuitable for heterogeneous tissue; Unsuitable for lesion < 50 cells	Costly instrument & transfer cap	High cost of equipment; Potential destruction of cells during procurement; Requires special membrane to view intact cell	High cost of equipment; Potential destruction of cells during procurement; Requires special membrane to view intact cell	Slower than laser-assisted capture; Potential for contamination; Requires practice

should be used as these fixatives usually give better immunohistochemical results than aldehyde-based cross linking fixatives (23). Only when an immunohistochemical stain has to be combined with microdissection to enhance phenotype characterization, can a mild neutral buffered-formalin (4.5%) be considered, while accepting a higher degradation rate of nucleic acids. Moreover, fixation time for small tissue specimens should not exceed 2 hours to limit the cross linking of nucleic acids. In this case, digestion with proteinase K (0.5 μg/μl final concentration) is found indispensable in formalin fixed and paraffin embedded tissue to free nucleic acids (22). However, it has been reported that employing frozen sections followed by fixation for a few minutes in succession of cold acetone, methanol, 4% paraformaldehyde, 70& ethanol, ethanol/formalin mixture and ethanol followed by acetone yielded excellent immunostaining of LMD tissue (24). Fixation was the most crucial variable condition under consideration when investigating the effect of fixatives, RNase inhibition, counterstains or protocols on RNA

recovery (23, 24). It should be noted that while nonadhesive slides are adequate for short staining periods, immunostaining requires slides precoated with poly-L-lysine and/or 3-aminopropyltriethoxysilane for proper attachment (25).

The precipitating fixative methacarn consisting of 60% (v/v) absolute methanol, 30% chloroform and 10% glacial acetic acid has been reported to give satisfactory results for RNA and DNA from FFPET (26). Incubation in 30% sucrose at 4oC for 4-12 h, followed by cryosectioning and brief staining in hematoxylin and eosin preserved both morphological features and RNA integrity (27). Use of the reversible cross linker dithio-bis(succimimidyl propionate) (DSP) known as Lomant's reagent as a fixative in immunostained, LMD tissue samples allowed the extraction of unmodified RNA from them (28). Because frozen sections exhibit inferior morphologic quality compared with FFPET, a procedure based on use of methanol-bound fixative UMFIX (methanol/polyethylene glycerol-based fixative) in a fixative-to-tissue ratio of 10:1, coupled with microwave-assisted rapid tissue processing allowed isolation of high molecular weight RNA from LMD paraffin sections stained with H&E (29). A method that employed the use of the RNA preservative, guanidinium-HCl based TRIzol, followed by a fast fixation in 2% paraformaldehyde (4oC for 15 min), then cryostat cutting was shown to improve RNA yield and quality as determined by real-time PCR (30). Although some reports indicated that hematoxylin may have an inhibitory effect on DNA (and therefore methyl green, nuclear fast red or eosin stains should be used as alternatives) (31, 32), others did not support that finding (22, 33).

Examination of cytological preparations after Papanicolaou staining from organs such as uterine cervix for identification of malignancy following LMD and PCR analysis even after storage for several years has been reported (34). Enrichment methods have been devised for concentrating samples with less cellularity and prepared as cytospin preparations (13). Fresh tissues can be readily sampled by scrapping them with a scalpel blade and spreading samples into a glass slide, a procedure termed epithelial aggregate separation and isolation (EASI) that has been used for isolation to enrich epithelial cells from carcinoma samples of breast, lung, colon and prostate, followed by fixation in 95% methanol for about 2 min, followed by H&E staining and LCM. Although visualization of non-coverslipped slides could result in fuzzy images, temporary improvements can be obtained by addition of xylene to provide wetting and refractive-index matching (35). Another alternative to handling samples of low cellularity is to centrifuge them, remove supernatant and make a smear of the sediment. Alcohol was also found to be the preferred fixative to preserving cytological specimens in biological ones (13).

Extraction of the Analyte from Microdissected Samples

The choice of the appropriate lyses buffer for the collected microdissected cells depends on the analyte (DNA, RNA or protein), and on the method of analysis (13). Quantification of extracted analyte is important and there are currently highly sensitive methods to determine the concentration of extracted analyte such as PicoGreen fluorescence (Molecular Probes, Portland, OR) or use of Nanodrop ND-1000 spectrophotometer (Wilmington, DE) that can detect samples at concentrations as low as 2 ng/μl [http://www.nanodrop.com]. Sometimes it may be necessary to extract the analyte from a single cell, as studies have shown that even epithelial cells within a tumor exhibit different expression profiles depending on the location of the cell in the tumor (36). However, large scale amplifications, employing both linear T7 amplification and PCR methods, will be required to generate sufficient quantities of RNA from starting pictogram amounts of this molecule, and to ensure that mRNA populations are faithfully copied (37). A recent method has been reported to perform reverse transcription (RT) of total RNA on detergent-stabilized immunostained single $CD38^+$ plasma cell from the human brain on the cap surface of LCM cell without prior RNA prefixation (38). A lyses buffer (39) has been described for membrane-bound (40) and for cytoplasmic proteins (41).

Applications to Cancer

LMD has provided a dramatic increase in the sensitivity and accuracy of genetic assays by starting with samples of highly homogenous cell populations (or even a single cell) isolated from frozen tumor tissue or paraffin-embedded tumors. Analysis of such homogenous samples of tumor tissue by oligonucleotide microarrays has enabled recognition of distinct genetic subsets of human cancer with distinguishing molecular features, which are in some instances highly associated with clinical outcome in human (42-44) and animal models (45). Proteomic analysis of microdissected hepatocellular carcinoma cells promises to develop new markers for identifying that cancer (40). Differential expression analysis between tumor and nontumor tissue extracted by either LMD or from bulk tissue yielded quite different patterns between the two methods (46).

Adaptation of genomic and proteomic technologies to LMD is likely to revolutionize the classification of most human cancers and it is poised to exert dramatic change on the pace of cancer research, which have potential to open an entire new era in clinical cancer management and drug discovery, all of which would not be feasible without microprecision of LMD (47). Therefore, it is expected that there will be an exponential increase in use of LMD in contemporary cancer research (48-50).

Conclusion

There are a variety of systems on the market from microdissection. The preference of employing a particular system depends on individual requirements such as cell number, procession of single isolation, frequency of use and number of users. Both LCM, LPC and GAM are rapid, reliable and precise techniques. LCM may be adventitious for procuring islets of cells in a short time period. However, the method is not ideal for isolating single cells or long narrow structures like endothelial cells. There are also potential contamination-related problems due to adhesion of loose tissue fragments. Recent instruments combining both LCM and UV ablation overcomes the problems of contamination, allows cutting of hard tissue and isolation of single cells. Strong tissue adhesion to glass after long storage period can result in difficulties in removing the cells. LPC and GAM are noncontact techniques, particularly suited for dissecting a single cell. Problems may, however, arise when there is moisture between membrane and glass slide, or when electrostatic forces divert the captured cell(s).

Laser microdissection provides accurate results in numerous assays such as RT-PCR amplification, loss of heterozygosity, mutation detection, polymorphism, microsatellite instability, differential gene profiling and proteomic analyses; all of which are indispensable to understand and characterize cancer at the molecular level. This expanding technology will have a profound impact on cancer diagnosis, prognosis and on developing more specific targeted therapies (49-51).

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