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Short communication

Manipulation of cells through elastic films

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Abstract

Manipulation of cells in open dishes is often incompatible with preservation of sterility. A dish covered with an elastic or stretchable latex or plastic film allows manipulation of cells through the film with preservation of sterility of the culture and the integrity of the film. The latter forms a 'microglove' for the instrument tip. The idea of manipulation through a thin transparent film is also applicable to general surgery, so that the surgeon's hand operates through a film and without a glove.

Introduction

High resolution microscopy and manipulation of cells are usually impossible with the existing vessels for cell and tissue culture because of the rigidity of glass. Transparent plastic bags, microslide chambers for cell cultivation and film covered dishes (Moroz, 1991, 1992) solve the problem of microscopy, but micromanipulation of the cultivated cells requires aseptic conditions.

Aseptic techniques, including air conditioning, filtration, laminar flow, pipette pluggers and other precautions increasing the sterility of cell culture procedures still do not eliminate the risk of occasional breakdowns while work is done with vessels and dishes which have to be periodically opened (Freshney, 1987), especially for the purposes of cell manipulation. A permanently covered dish would offer a significant advantage for manipulations.

Developments with elastomeric materials resulted in emergence of highly stretchable films with an elongation up to 8–10 times. Among them the rubber still remains one of the most extensible. The rubber film stretched over a Petri dish can provide for a miniature extensible glove to reach out by a microinstrument the cells growing on the bottom of the dish or on other areas of the film. Thus, the cells can be mechanically manipulated on without being removed from the vessel and risking infecting the cells.

The extensibility of latex films, like those used in medical rubber gloves ot birth control devices, can readily be measured by drawing by ink a circle on the film and measuring the diameter of the circle before and after an extreme extension. The difference may approach 10 times.

The silicone rubber and polyurethane are shown to be biocompatible and relatively nontoxic to the cells (Bakker et al., 1988). Elastomeric films, including the latex ones, are transparent in thin layers, which makes them suitable for microscopy especially in a stetched condition. Some leads for microscopy through a thin rubber layer ensue from experiments with elastomer capillaries (Moroz, 1994).

Thus, a thin elastic film of a transparent homogeneous rubber or polyurethane may serve as a sterile 'microglove' for manipulation on cells growing on the film or on the floor of the dish. Some films, such as Seal-View (Norton Performance Plastic Corporation, Akron, OH, USA) or DuraSeal (Diversified Biotech, Boston, MA, USA) combine strength, optical clarity, seal integrity, non-toxicity to cells and high stretchability. These films have low elasticity. Thus, the stretch deformation is irreversible, and in the locus, where the pin is plunged into the film, a deep thin pocket remains. This however cannot be seen as a ser-

ious shortcoming since not so many points of needle manipulation are used in the experiments.

With highly elastic rubber (latex) film its flatness is completely restored. Aladan, Norcross, GA, USA) manufactures gloves with a triple-dipping process, creating three integrated latex plies, which reinforces the whole film. This eliminates preexisting pinholes and increases resistance of the film to needle penetration.

Material and methods

The following preliminary tests have been conducted:

1. Controllable microcompresion and applanation of cells between the film and dish floor by the cap or contact objectives (Moroz, 1991) for microphotometric analysis and high-resolution microscopy.

2. Stretching the film leads to stretching of the cells growing on the film in a way of a 'nonoptical magnification' exposing the thinned and enlarged cell to detailed microscopy. Such stretching may also detach the cells from the film, 'Magnification' through flattening of the cell has also been tested in conditions of centrifugation (Moroz and Mikhailov, 1996).

3. Manipulation by microinstruments deeply protruding into the film but not penetrating it, according to the idea of a 'microglove', to conduct simple manipulations, such as removal of cells, their transfer etc.

4. A sterile microneedle or micropipette lubricated with vaseline oil can penetrate the film cleaned with alcohol to operate on cells at the bottom of the dish or at the film. After the instrument (or a pair of them) is removed from the film, the opening closes itself owing to elasticity of the film, and remains sealed with oil, thus preserving sterility. Light-conducting microtools (Moroz, 1964) may provide for illumination and optical manipulation on the cell in addition to the mechanical ones.

5. Microscopy from the side of the film encounters difficulties with high power objectives: the limited working distance leaves no space for controllable insertion of the microneedles. Therefore, with all films, both transparent, like plastics and opaque, like latex, the microscopical observation is preferable from the bottom of the vessel in an inverted microscope. Only with a stereomicroscope and for rough and preliminary manipulations may observation from the side of the film be advisable.

Conclusion

Elastomeric (or rubber) films allowing typically stretching up to 200% have been incorporated into the design of such popular cell culture vessels as BioFlex culture plates (Flexcell International Corp., McKeesport, PA, USA). They are extensively used to study the changes in various cellular processes such as cell growth rate (Matsumoto et al., 1999), signal transduction (Ingram et al.), gene regulation and cytoskeletal rearrangements (Carson and Booth, 1998) in response to cyclic mechanical stretching modeling physiological conditions such as pressure changes during the cardiac cycle or pathological conditions such as haemodynamic injury.

The idea of manipulation through a sterile transparent film, presented here in a microdimensional form, is applicable to general surgery as well. Surgeon's hand in a glove, impressive as a symbol, is not impeccable as a tool: the area around the organ operated on, remains open and, thus, not protected from microbial contamination from the air, the hand of the surgeon tightly covered by the glove lacks the confort and tactile sensitivity. It is imaginable that a very thin plastic film, transparent and highly elastic, which covers the whole area of operation will provide for higher sterility and will benefit the naked hand of the operator. The hand is plunging, when necessary, into the film slightly stretched on a special frame over the body part operated on. All manipulation goes through the film. Thus, what we call microglove in micromanipulation, in surgery would actually be an antithesis to a real glove.

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References

Bakker DCA, van Blitterswijk WT, Daems & Grote JJ (1988), Biocompatibility of six elastomers in vitro. J Biomed Mater Res 22: 433–439.

Carson J & Booth F (1998) Effect of serum and mechanical stretch on skeletal alpha-actin gene regulation in cultured primary muscle cells. Amer J Physiol 275: 1438–1448.

Freshney RI (1987) Culture of Animal Cells. A Manual of Basic Technique. Alan Liss, Inc., New York.

Ingram A, Ly H, Thai K, Kang M & Scholey J (1999) Activation of mesangial cell signaling cascades in response to mechanical strain. Kidney International 55: 476–485.

Matsumoto T, Kawakami M, Kuribayashi K, Takenaka T & Tamaki T (1999) Cyclic mechanical stretch stress increases the growth rate and collagen synthesis of nucleus pulposus cells in vitro. Spine 24: 315–319.

Moroz PE (1964) Application of light conductors for a light micropuncture of the cell and microelectrodes. Tsitologia (Russ.), 126–129.

Moroz PE (1991) Microscopy of cell cultures throught thin plastic films. Microscopy 164: 169–174.

Moroz PE (1992) Horisontal microscopy in square capillaries. Microgravity Sci Technol 2: 112–114.

Moroz PE (1994) Microscopy and manipulation of cells in elastic capillaries. Trans Am Microsc Soc 113: 412–414.

Moroz PE & Mikhailov AV (1996) Centrifugal flattening of the cell. Cell Vision 3: 57–59.