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Microtools for Microsurgery of a Single Cell in Field of Cellular Engineering

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1. Introduction

Almost all main problems of natural science lead to necessity of studying the processes occurring in single cell. At present intensive work is going on in order to improve the technology of reconstruction of cells, and after that of the whole organism. At the level of an individual cell it is possible to change its biochemical processes, its physiology and morphology, its genetic status. Microsurgery of a single cell, replacing its elements, the introduction of foreign genetic material makes this area of medicine, agriculture, basic and experimental biology especially relevant. Appearance in the hands of microsurgeon of a single cell of the new microtools that have the ability of active interference in functioning of the cell without causing a significant damage to it have changed the experimental biology. The cellular engineering solves a wide range of problems, extensively using microsurgical methods and approaches, which include microinjection into the cell and its organelles - for nuclear transfer and transfer of the individual chromosomes, transgenesis, dividing of early preimplantation embryos for twinning, etc.

2. Microtools

Microtools for microsurgery of single cells are microscopic things comparable in their dimensions with a cell or its organelles; microtools are made with a microforge on the tip of a round forged out glass micropipette or of a glass blank of some other profiles (Fig. 2). The figure shows a diagram of the micropipette, which can serve as a blank (foundation) for many microtools.

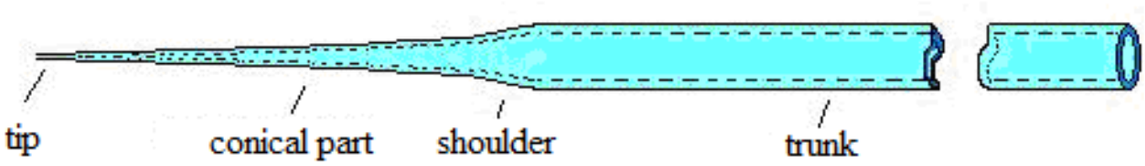

Fig. 1. Scheme of a micropipette – a blank for manufacture of many microtools

Round capillary, from which microtools are manufactured, can be presented in form of a blank of various profiles. Drawn tip of a capillary or other profile is, in fact, a blank for microtools manufacture.

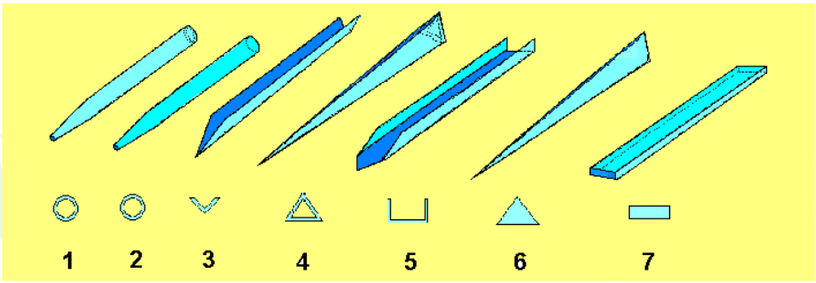

Fig. 2. Types of glass profiles for microtools manufacture

Using specialized microtools has many advantages. For example, a triangular profile of a micropipette allows to produce micropipettes, which do not leave long-lived perforations on a cell; two-channel pipettes enable to transplant organelles and to enucleate cells with single puncture, etc.

Further microtools will be shown according to the chart:

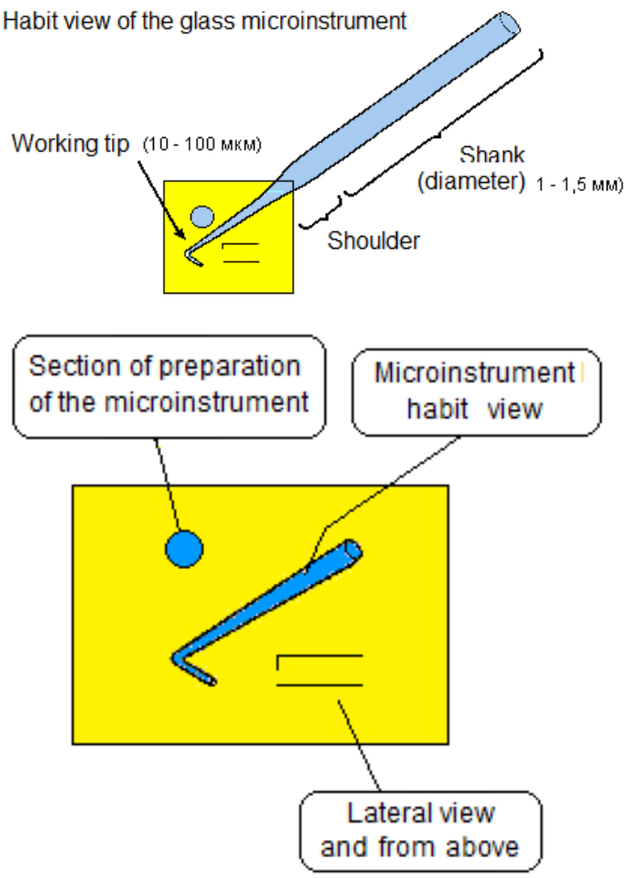

Fig. 3. Scheme of a microtool tip

Microtools for micromanipulation on cells can be distributed into several groups according to their functions: fixing and keeping; actually operating; auxiliary and special purpose microtools. Such a distribution is convenient from a practical point of view, since it allows making the right choice from a variety of microtools. This greatly reduces time of an operation and enables to make it with minimal damage to cells. For example, a seemingly simple task of cells fixing appears not a simple problem. For protoplasts, spheroplasts or individual organelles in cases when they should not be exposed to negative pressure, inevitable at aspiration or at microsucker fixation, microtools like microspatula-holder or capsule-holder are recommended (Fig. 4).

Use of such tools allows to fix an object without negative pressure, merely by a shape of tool itself.

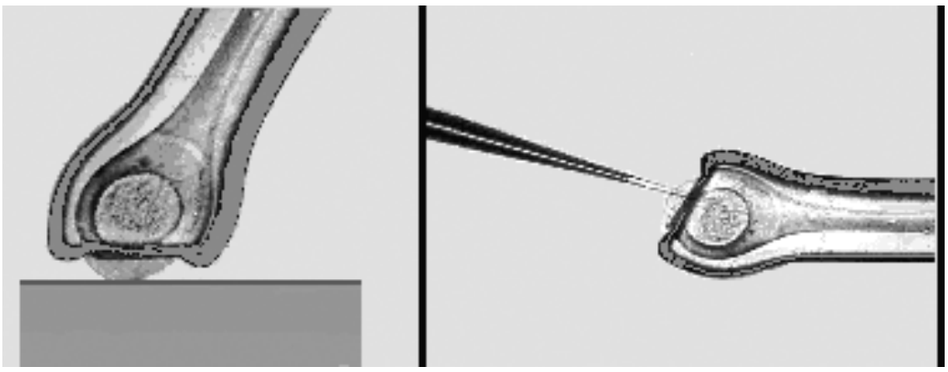

Fig. 4. Capsule-holder

Microtools must be separated by the nature of their use in active and passive. By active, we usually mean those able to damage a cell to a greater or lesser degree (operating and some special purpose instruments, such as microexpander, microprobe, microloop, etc.). Passive microtools usually only contact cells and have little possibility to damage it (fixing and auxiliary tools, such as microhook, capsule-holder, spatula-holder, etc.).

3. Microtools for single cell microsurgery

3.1 Microneedles, microhooks, microloops

Microneedle is an oldest microtool that emerged from the practical work with cells. The most important mission of microneedles was first manipulations with cells. However, using it one could perform various simple and complex operations: cell cutting; its destruction; perforation of a cell wall or membrane. Nowadays role of microneedles in microsurgery became much more important because of development of technology for production of monozygotic genetically identical twins of laboratory and farm animals. Microneedles are actively used for preparation of a given size incisions in zona pellucida of early mammalian embryos during retrieval of blastomeres from them and for preservation of integrity of transparent envelope for its further use.

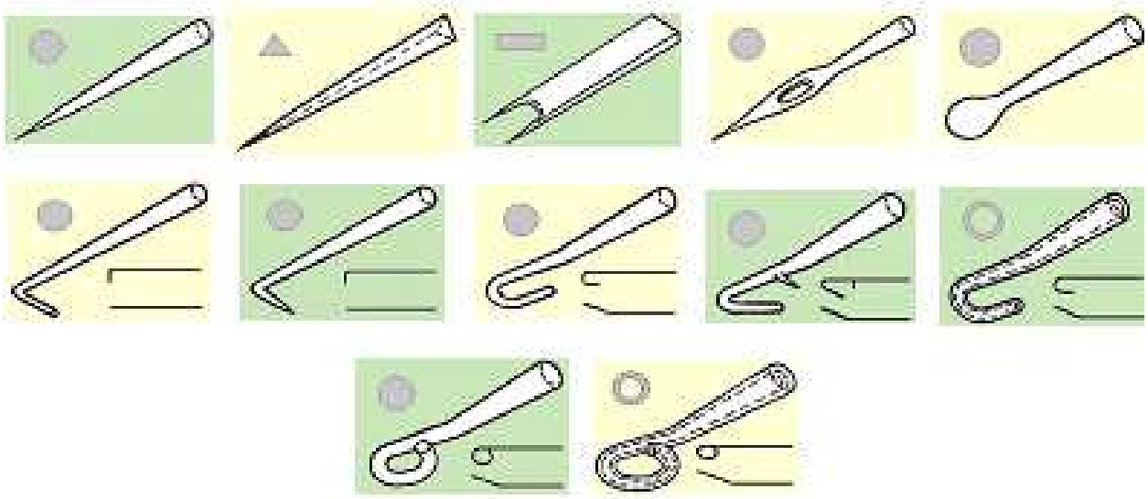

Fig. 5. Microneedles, microhooks, microloops

According to our findings, change of section profile of microneedles, for example, for triangular one, can greatly expand field of its activity at atraumatical separation of blastomeres and their further separation. In addition, perforations made with triangular microneedle (the same as with a micropipette) are repaired much faster than those made with rounded microtools.

Using of flat section profiles allows making special forks for retention of large cells, tissues, capillaries.

Microneedles with a hole enable to apply microligatures relatively easy on small capillaries, spermatic cord, etc., surrounded by tissues. These are made from carefully soldered microloop on microforge it until piercing tip is formed. A hole shape and size depend on ratio of internal to external holes of a microloop and its dimension in whole.

Microspheres at the end of microneedles are made on microforge at very high temperature of the filament and used to keep cells and manipulate with them and to hold tissues or organs; use of them minimizes mechanical damages.

Different types of microhooks are used primarily to hold cells; to isolate them from cell mass; to isolate individual cells from tissue. Hollow semicircular microhook, holding cells, allows to change solutions.

3.2 Microtools: Microloop with variable diameter

Application of microligaturing method for small size early embryos of mice, for instance, appeared far simple. The method was firstly used by Spemann on embryos of Triton with a newborn baby hair. We improved this method and made it much easier, having designed a special micromanipulator for microligaturing. Even simpler became a method of microligaturing with variable diameter microloops, at that we used Nylon thread with of 5-7 microns diameter as a loop. With the help of such microloopscan one can not only separate blastomeres, but can cut early embryo into two halves as well. These loops may be used

repeatedly. For this purpose it is necessary to insert a Nylon thread into a loop before the beginning of the work: this thread will repeatedly return a loop into its start position.

Fig. 6. Micro-loops with variable diameter

To reuse a microloop, a thread was inserted into them, as shown for a microloop; free ends of the thread enabled returning of the loop at its start position. We used some of microtools designed for single cell microsurgery/: microneedle and microspatula of profile glass and a special polyvinyl microsucker for unilateral removal of a tadpole eyes with the so-called "waist" method (the same as production of monozygotic twins "halves" from blactocyst) in series of morphogenesis studies on amphibian Mauthner cells; it showed possibility of using these tools for microoperations at organ level as well. This technology has dramatically increased the viability of the tadpoles.

3.3 Microsupports for cells holding

When working with isolated, single cells it may be necessary to fix them for the microsurgical operations. Capsule-holder (Figure) allowed holding a cell without application of negative pressure (suction), which made it possible to practically avoid damage to the cell at its fixation.

Fig. 7. Clamping stops of different profiles

Cells holding during operation is always an important issue and can somehow affect a result of the operation and integrity of operated cells.

First, you need to avoid excessive deformation of cells during holding. If you do not use microsuckers aspirating cell contents and serving as "hidden factor" of cell damage, you can use end and clamping stops as shown in the figure. The stops must be bent in a way allowing it only to contact a cell but not deform it in general. End stop is used for holding cell near a Petri dish wall or some piece of objective or cover glass. Profile of such a stop can be round or rectangular. These stops are convenient for holding large cells like amphibian or fish eggs.

Profile microhook and profile microloop can be good stops when it is necessary to use a cover glass or microspatula. It is practically impossible to damage a cell placed under such microtools.

These unique microtools allow to place a cover glass on them and hold the cell during microscopy. In addition, profile microloop allows to work in a flow microchamber.

3.4 Microspatulas for cells

Microtools - microspatulas have various profiles and serve for pressing, dissection, separation and holding cells in microchamber. They are necessary for work with tissues or with cells clusters. Microspatulas are applied to press cells to the bottom of a microchamber and to hold it. In addition microspatula-hemisphere is used to crush cells, scraping and cell preparation. They have various configurations and help to solve specific problems of cells fixing at special experimental situations. Various spatulas that can successfully hold and manipulate single cells are presented further.

Fig. 8. Microspatulas of different profiles

Cell microsurgery often meets situations when a cell must be held with a microtool to immobilize it, or, conversely, move it along the surface of a microchamber object-slide, or, finally, to reduce its thickness uniformly, thus facilitating detailed study of its internal structure. For this purpose, flat or convex or concave micropress-spatulas may be useful.

The Figure 8 shows the spatulas scheme (left to right):

Microspatula from fragments of plane cover glass for pressing cells to the bottom of the microchamber, holding of one or several cells.

Profile microspatula for preparation, pressing, separation and holding of cells in microchamber.

Microspatula from fragments of plane cover glass with stops.

Microspatula from glass melted in platinum microloop.

Microspatula-semisphere to separate cells, scrapings, preparation of cells and tissues, pressure of cells or tissue to the bottom of a microchamber.

Microspatula-holder for holding cells, fixing it during microsurgery, quick change of solution around the cell (part of the sphere is polished). The universality of this

microspatula-holder is obvious, since the cell can be transferred and held without use of negative pressure, making a variety of saw cut (abrasion), and fixing it additionally with negative pressure, sticking a cell rigidly to a holder. There are some operations that do need such a fixation.

3.5 Microspatulas-microscrapers for cell culture and flattened cells

Single cells can be isolated from the culture by scraping them from a substrate on which they are cultivated. This eliminates the effect of lytic enzymes or substances weakening cell adhesion to the substrate. Scraping cells with sharpened microtools can damage cells themselves. A cell must be not cut but very carefully "peel off" from substrate, and only after that it becomes "spall", isolated and may be used for work.

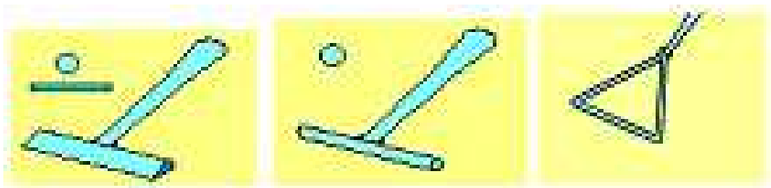

Fig. 9. Microscrapers of different profiles (left to right): Triangular profile microscraper; Round profile microscraper; Microscraper of platinum wire

3.6 Microscalpels

Microscalpels are the most common and frequently used microtool for division of early embryos; for removal of zona pellucida; for dissection of the chromosomes, etc. Microtools end are made from profiled glass blanks. This somewhat extends and simplifies manufacturing of microscalpel with simple cleavage or sharpening with an abrasive disk.

Since a cell reconstruction is in one way or another related with microsurgical operations of varying degrees of complexity, we have paid great attention to various kinds of scalpels.

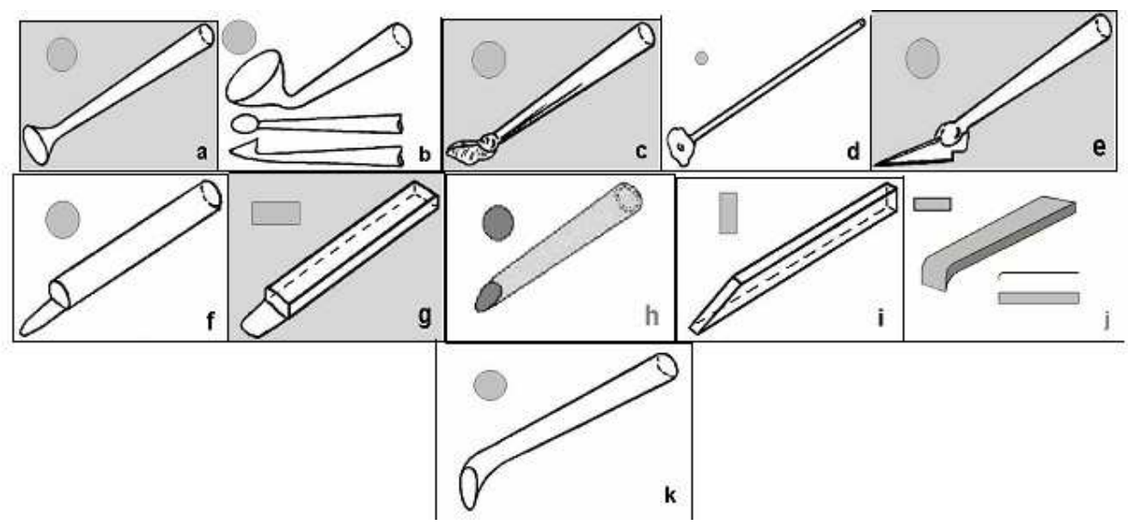

Fig. 10. Microscalpels of different kinds

Later on, we began to use them for microsurgical operations less frequently as cutting tools cause the most severe damages. Sometimes, however, these tools cannot be avoided, for example, at work with plant cells and various tissues.

A scalpel is the most common tool in surgery. However, in the case of a single cell it should be reasonably applied only in exceptional circumstances. A scalpel is a cutting tool and it can severely damage integrity of cells during operation. Nevertheless, a scalpel is indispensable at preparative manipulation with a study object or at preparation for surgery.

Let us review various types of microscalpels for work with single cells and cell tissues.

a. Circular microscalpel for cutting cells of early embryos or tissues. This microscalpel is made with a microforge, producing a separation of the weld microbead technology, perfectly described in the monograph.
b. Microscalpel-hemisphere for cell cutting, scrapings, cell preparation and tissue surgery. By making technique this knife is similar to the previous one with the only difference being that position of a hemisphere can be changed by bending of microforge neck at any angle. To use such a scalpel is much more convenient because you can cut with a top section or with lateral parts of the hemisphere.
c. Microscalpel from a crystal to cut cells or tissues of early embryos (crystal is glued). Reliable and easy microscalpel can be made by using of finest pieces of artificial crystals of ruby, sapphire or quartz. Only water-resistant glue should be used for this purpose.
d. Circular microscalpel for cutting cells or tissues (at a platinum wire). If a thin platinum wire is inserted into molten bead, and then torn on cooling of glass, you can get round microscalpel. It requires besides a scalpel holder in form of a glass capillary in which a platinum wire is welded to install it on a micromanipulator. It should be noted that according to, Shouten showed excellent qualities of this instrument, cutting with it such small objects, as bacteria, into three or four parts.
e. Microscalpel preparative to cut a cell or tissue (from a piece of the blade). Preparation works with biological samples are easy to carry with a simple knife blade made of pieces of blade glued with water resistant glue to a holder.
f. End microscalpel to cut cells or tissues. Tempered glass (glass after heating and cooling) with a transverse fracture sometimes gives a sharp cutting edge, which can act as an end microscalpel.
g. End microscalpel from profile blank to cut cells or tissues. Glass, depending on their composition on the Mohs scale, can be scaled 5 to 7 (diamond is 10). The hardest are quartz glass and "Pyrex" type glass. Profile blanks from such glasses can be used for the manufacture of end microscalpel by cleavage.
h. End microscalpel (micro-engraver, oval) from a large rod for cutting cells or tissues (sharpened and polished).
i. End microscalpel profile (narrow) to cut cells or tissues (sharpened and polished).
j. Microscalpel bent from profile blank (with given different sharpening angles) to cut a cell or tissue (can be sharpened at a different angle, as shown below).
k. Microscalpel bent from profile blank (can be sharpened at a different angle, as shown below).

The last microscalpel in this table (bottom right) is offered by Fonbrun. We have found a way to make cutting edges of it with different bevel angles (Fig.11).

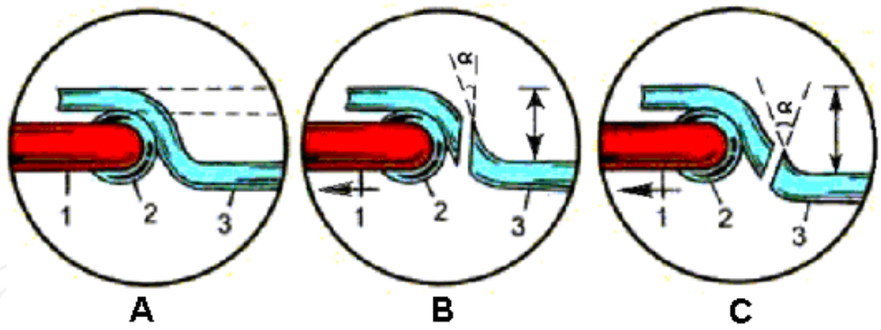

Fig. 11. Technology of manufacture of microscalpels with different angles of grinding with the help of microforge 1 - microforge filament, 2 - a drop of molten glass, 3 - a tip of drawn glass rod,α - angle of grinding. Position B and C - /cooling of the filament

3.7 Micropipettes

The micropipetes proposed in the paper and used in experiments, have different construction. These are:pipettes with inserts and triangular ones, that are used for microinjection to puncture a cell; a micropipette with a limiter allowed to microinject into tissue at a certain depth, for example, at embryo transplantation into horn of an animal uterus. Using microsurgical pipette we performed nuclear transfer in mice. It is four-sided, sharpened by special technology, with the aid of an original device for microtools sharpening, and has the shape of a pupil pen. Many micropipettes were used for the selection of eggs and other isolated cells and organelles, including nuclear transfer. Various profile inserts, limiting cell advance into a micropipette pot, were made inside the micropipettes.

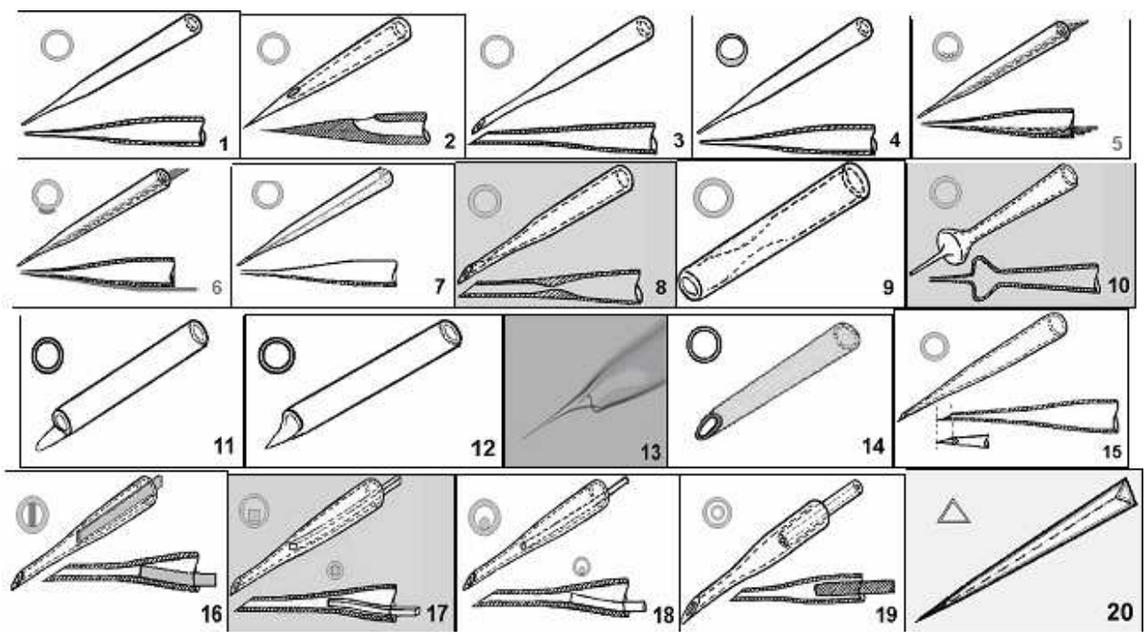

Fig. 12. Micropipettes of different kinds

The most part of microsurgical operations and all kinds of micromanipulation are made with the help of a well - known micropipette.

1. Micropipette for microinjections of solutions and organelles into cells, microelectrophoresis, may be used as microelectrode.
2. Micropipette-needle for perfusion, isolation of organelles from cell
3. Micropipette with a bevel for nuclear and other organelles transfer
4. Micropipette with non-co-axial profile for microinjections of solutions into cell, microelectrodes, microelectrophoresis, iontophoresis
5. Micropipette with inserts (filaments)
6. Micropipette with an external insert
7. Micropipette with abrasion
8. Micropipette with a "waist" (sharpened)
9. Micropipette with a "waist" (melted) and slightly melted tip
10. Micropipette with a limiter for microinjections into the tissue and vessels
11. Micropipette with a cutting edge
12. Micropipette with a piercing tip
13. Micropipette with a polished tip
14. Microsurgical micropipette (four-sided sharpened) for nuclear transfer and intracellular organelles
15. Micropipette with a flat rectangular insert – stop
16. Micropipette with an inner profile
17. Micropipette with an inner square stop
18. Micropipette with an inner circular stop
19. Micropipette with a calibrated capillary – stop
20. Micropipette profile (triangular) for injection, organelle transplantation, cutting, preparation of cells or tissues

3.8 Microtweezers

Rarely used for work with the single cell microtweezers are presented to give an idea that they are easy in manufacturing but hard in use, as they require special micromanipulators or various devices with micrometer displacement of tweezers tips.

Fig. 13. Microtweezers

3.9 Microsyringes

Microsyringes are widely used in cell microsurgery, even a microinjector by itself is a complex Microsyringe, consisting of microneedles, conductive wire for compressed air or hydraulics, and pressure source.

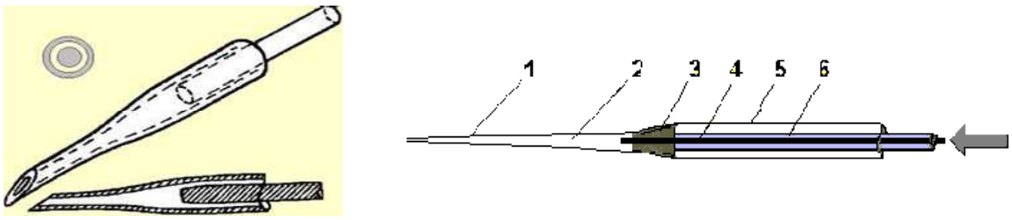

Fig. 14. Micropipette with a plunger inserted into its cavity. 1 - tip of the micropipette, 2 - injectable solution, 3 - seal (silicone tube), 4 - metal plunger, 5 – micropipette body, 6 - capillary sealing silicone tube and plunger guide.

The figures show two nearly identical microsyringes with the only difference being that the first

Movement of a plunger in an injectable solution enables to make multi-dose injections or, vice versa, to produce suction micropipette and then a micropipette itself serves for transfer of organelles.

3.10 Profile microtools

For solving various problems in the field of microsurgery of single cell, depending on the complexity of operations, one should always seek to expand microtools species. Round capillaries and glass solid rods practically exhausted their potential. Therefore, a further step can be choice of different types of profile available for making them both as over the burner flame and at special pull devices as well.

Different profile of a blank allows to produce cutters of different shapes. Their distinctive difference from other penetrating microtools is that perforations on a cell are closed immediately after microtools leaves a cell.

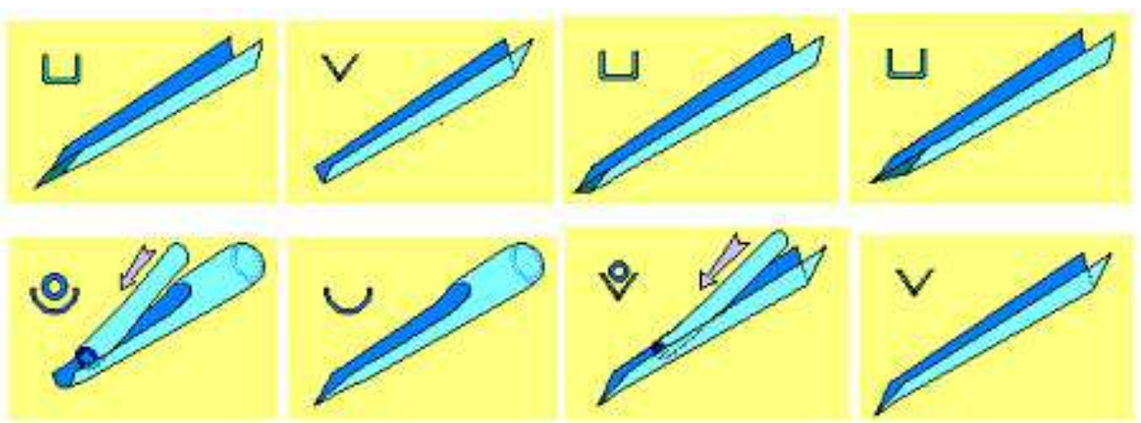

Fig. 15. Microcutters of different configurations

4. Microtools from paired capillaries

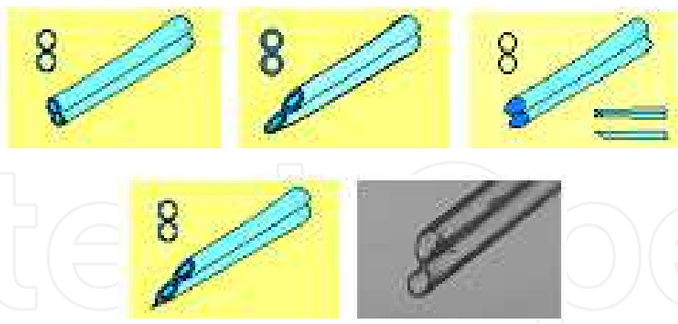

Fig. 16. Paired microcapillaries

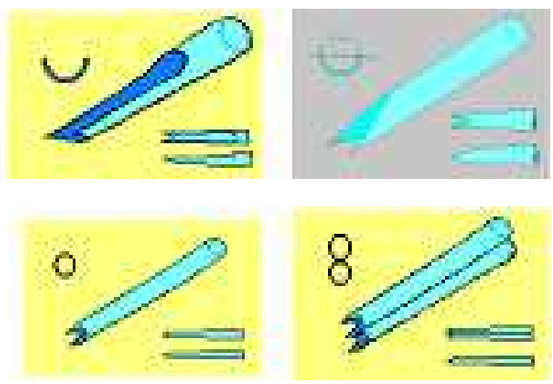

Fig. 17. Microtools for work with chromosomes and tissue microcapillaries

5. Microprobes

The demonstrated flexible microprobes are universal. Microaspirator-irrigator, made from polyethylene tubing (high pressure) over a spirit lamp flame or on a microforge, was used for washing of eggs out from oviducts or uterine horns of animals. They were also used for washing of organs cavities, for perfusion, for change of solutions in the flow microchamber. Microprobes with side and end holes were used for transplantation of eggs into uterine horns of laboratory animals.

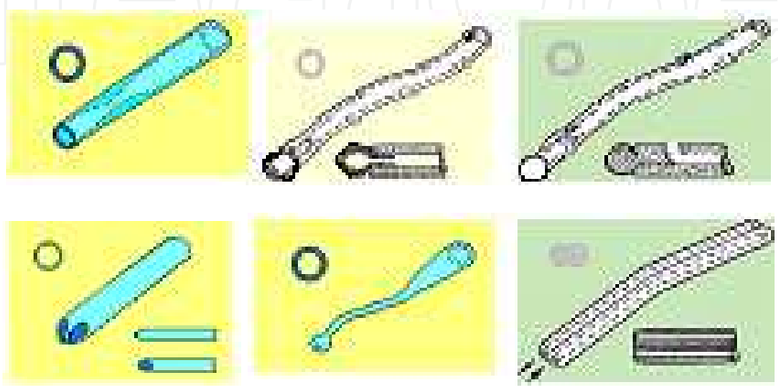

Fig. 18. Microprobes of different material and different configuration

6. Microsuckers and holders

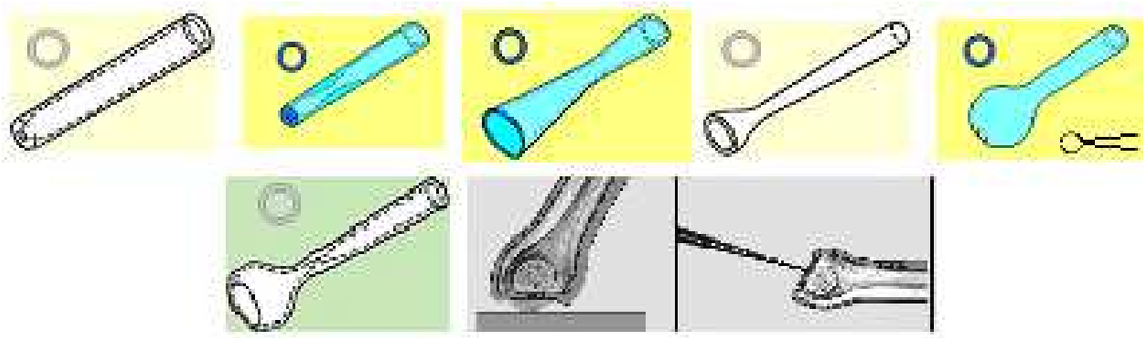

Fig. 19. Microsuckers and Holders

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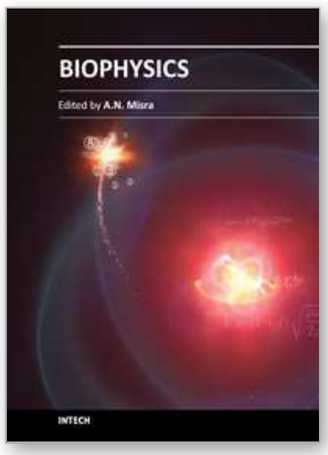

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