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Micro-Nano Technologies for Cell Manipulation and Subcellular Monitoring

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1. Introduction

Currently, mechanistic and biological phenomena within the cellular level are not well understood (Bao & Suresh, 2003) and the evolution of those during disease or treatment is also unclear. Cells are complex structures with nuclei and organelles, whose dimensions require the development of micro and nanotechnologies for effective manipulation and monitoring. Indeed, sizes of nuclei vary from 3 to 7 μm for differentiated cells. In embryos, nuclei are not surrounded by a membrane although the genetic material is mostly compounded in a 9μm diameter region. Other organelles, with specific functions in eukaryotic cells like mithocondria, can have smaller sizes (1 μm). In addition, histologists know that important parameters such as pH or Ca/Na/K concentration can greatly vary locally within the cytoplasmic region. Recently, Weibel (Weibel et al., 2007) argued the need for microbiology to evolve into a quantitative field. The argument predicted the development of microsystem tools to enable individual cell manipulation, growth, and the study of **subcellular organization**, while mechanisms of differentiation and communication between cells would be unveiled. Indeed, current research efforts aim at investigating the genetic, biochemical, and behavioural differences among cells. On-going development of microstructures to quantitatively study parameters within single cells will lead to a thorough understanding of subcellular activity, including pathogenesis with an unprecedented level of detail. In addition, these efforts will pave the way for highly-effective, cell-tailored, drug delivery. The problems are of course how to effectively manipulate cells, how to position a sensor at specific locations within the cell, and even how to penetrate the different organelles, as depicted in Figure 1. This chapter will review current concerns on cell manipulation and recent developments in micro and nanofabrication technologies aiming at the increased functionality of cellular tools.

2. Microfabrication technologies for cellular handling

A first step in the study of cells inevitably starts by designing tools and protocols for cell handling, as the manipulation of the uttermost external membranes in life cells are likely to

introduce perturbations in the system that could ultimately impact either the sub-cellular or intercellular processes to be elucidated. Pipettes are typically pulled and thinned by pullers, cut to the appropriate inner diameter by microforging techniques, and polished. Human fertility scientists identified earlier (Huang et al., 1996) the difficulties around tool preparation, and the importance of appropriate cell handling during in vitro fertilization (IVF) procedures. Following this line of thought, and in view of the traditional manufacturing approach currently active in the trade, technologists have pondered whether cell handling is hindered by rudimentary-manufactured manipulators (Campo et al., 2009 a and b).
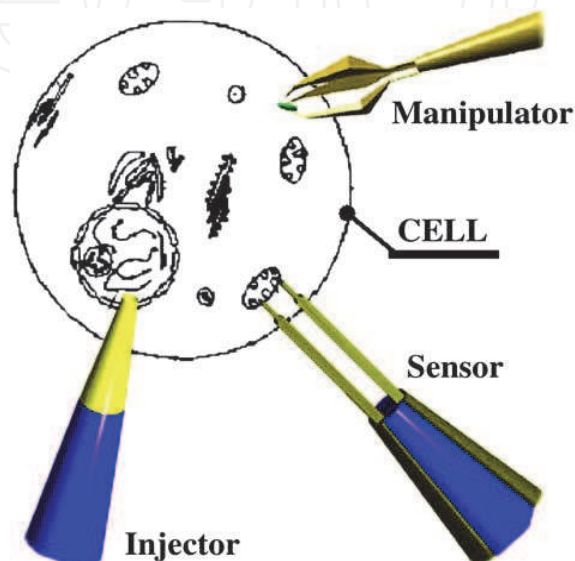

Fig. 1. Schematic describing functionalities of intracellular manipulator.
After Kometani et al., 2005b[1].

Conventional pipettes consist of borosilicate glass capillaries that are subsequently thinned, cut, and polished to achieve appropriate tip geometries with adequate dimensions. Figure 2 shows commercially available glass capillaries whose difference in geometry has been controlled by a puller with a combination of axial tension and thermal treatment (Sutter Instruments, 2008). In recent years, cell microinjection has become a crucial procedure in cell and reproductive biology. Microinjection of cells is routinely used in various biotechnological and biomedical applications such as reproductive cloning of animals by nuclear transfer (Kishigami et al., 2006), production of transgenic animals by DNA injection into embryos (Ittner and Götz 2007) or in vitro fertilization of oocytes by intracytoplasmic sperm injection (ICSI) (Palermo et al. 1992; Yoshida and Perry 2007). Higher cell survival rates have been reported to result from minimized microinjection damage when high quality pipettes are used. With common-practice microinjection methods, 5-25% of mouse oocytes lyse during ICSI (Lacham-Kaplan & Trounson, 1995) and 20-30% of mouse embryos lyse after pronuclear microinjection of DNA (Nagy et al. 2002). With all, experimental evidence suggests that crucial conventional pipette manufacturing procedures involve tedious artisanal methods that are prone to failure (Yaul et al., 2008; Ostadi et al., 2009).

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[1] Reprinted from *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, Vol. 232, No. 1-4, Kometani, R.,Hoshino, T., Kanda, K., Haruyama, Y., Kaito, T., Fujita, J., Ishida, M., Ochiai, Y., & Matsui, S. , Three-dimensional high-performance nano-tools fabricated using focused-ion-beam chemical-vapor-deposition, pp. (362-366), 2005, with permission from Elsevier

Despite the absence of a thorough bio-mechanistic explanation to pipette-cell interaction, trial and error-based initiatives have accumulated a wealth of specifications applicable to tools and piercing techniques in different scenarios. Figure 2 shows electron microscopy images of pre-processed pipettes (top) and optical microscopy images of conventionally processed pipettes to different geometries. In particular, for most applications involving oocytes and embryos, injection pipettes must be bevelled and often spiked at the tip (Yaul et al. 2008; Nagy et al. 2002). Bevelled spiked tips (as those shown in Figure 3) favour penetration through a thick zona pellucida and an elastic plasma membrane.

Fig. 2. Electron and optical microscopy images of pre-processed (top) and conventionally processed capillaries.

The different geometries achieved by forging and polishing techniques have some versatility to accommodate for different piercing scenarios. Microinjection protocols for sperm injection, particularly those used for injecting mouse oocytes, occasionally need flat-end micropipettes to first "core" through the zona pellucida and then through the oolemma using minute vibrations from a piezo device. Pipettes used with a piezo drill require a clean 90-degree break at the tip (amenable to be produced by a microforge) and a bevel or spike at the tip is not needed to assist perforation. The inner diameter of these pipettes must be carefully controlled for the pipette to be most effective. The pulled pipette is usually cut on a microforge to the appropriate diameter (Sutter Instruments, 2008). Figure 3 shows SEM images of tips from glass pipettes, revealing rough edges in pre-processed pipettes and polished edge in a conventionally-used pipette in human ICSI; where smooth edges and a bevelled tip with a spike assist during perforation.

Indeed, edge finish is an important factor in pipette quality. Abrasion, acid and fire polishing are common techniques to remove morphological irregularities from pipette edges. In a recent study, Yaul and coworkers (Yaul et al., 2008) investigated the impact of different gas chemistries during fire-polishing of IVF pipettes. Typically in IVF industry, glass micro tools are drawn from hollow glass capillaries of 1 mm diameter (Yaul et al., 2008). These thinned capillaries are cut manually to a length of 100 mm from hollow glass rods resulting in sharp and chipped edges, similar to those shown in Figure 3 (left). Resulting sharp and uneven edges are known to easily pick up debris, rendering them ineffective for IVF treatments. Yaul's experiments involved analysis of fire polishing process

using candle, butane, propane, 2350 butane propane, and oxyacetylene gas flames to find the appropriate gas chemistry, the optimum distance of the capillary relative to the flame, and the optimum heating and cooling times as the tip morphology is modified by the glass phase transition. The results show that the temperature range in 2350 butane propane gas (between 925–1,015°C) is optimum for fire polishing of borosilicate glass capillaries, as the softening point of borosilicate glass is 820°C and the working temperature lies between 1,000 to 1,252°C.
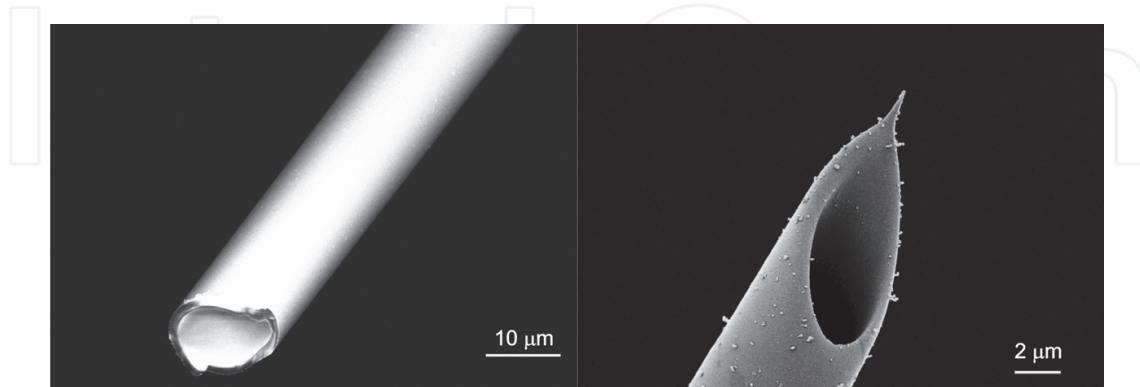

Fig. 3. Electron microscopy images of unpolished glass pipette (left) and commercially-polished (Humagen, Virginia) pipette (right).

The uneven pattern in heat radiance from a non-punctual heating source was also tentatively addressed by exposing capillaries to the top, middle, and bottom section of the flame, as shown in Figure 4. Inspection of edge roughness was conducted by optical microscopy and discussion was only provided in a qualitative manner.
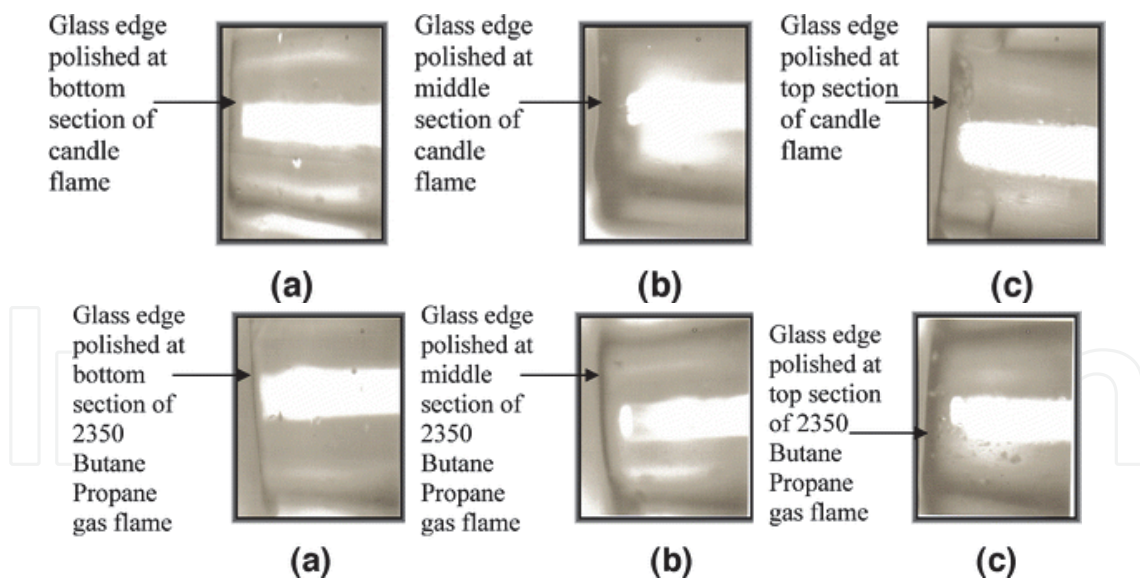

Fig. 4. Optical microscopy images showing the effects of pipette exposure to candle flame (above) and to 2350 Butane Propane (below) at different sections in the candle flame. (after Yaul et al., 2008)[2]

[2] With kind permission from Springer Science+Business Media: *Biomedical Microdevices*, Evaluating the process of polishing borosilicate glass capillaries used for fabrication of in - vitro fertilization (iVF) micro-pipettes, Vol. 10, No. 1, (2008), pp. (123-128), Yaul, M., Bhatti, R., & Lawrence, S. Figures 8 and 9.

Reportedly, fire-polished capillaries were tested in IVF clinics in the UK, with great acceptance over pipettes with non-fired polished edges. Although fire polishing seems to have an impact on pipette performance, there is still a lack of quantitative measure of both thermal parameters (for automation) and effects of this treatment on edge roughness.

At this time, it is unclear what levels of roughness are acceptable for adequate cell handling. A number of questions arise as the importance of the edge surface to be atomically flat for cell handling or even for organelle perforation. Incidentally, Malboubi and coworkers (Malboubi et al., 2009) unequivocally correlated pipette surface roughness (in the order of nanometers) with giga-seal formation in patch clamping, providing semi-quantitative evidence of improved roughness based on the resolution provided by electron microscopy images. Commonly used characterization techniques to measure roughness in microelectronics, such as atomic force microscopy (AFM) are amenable to be deployed in the context of tools for the biological sciences. This is possibly the most sensible step looking forward in the process to understand edge roughness and its consequences on cellular manipulation.

To this effect, a number of efforts to introduce current microtechnologies into the context of pipette processing (Kometani et al., 2005-2008; Malboubi et al., 2009; Campo et al., 2010a) and piercing techniques (Ergenc & Olgac, 2007) have appeared in the literature. In particular, the use of focus ion beams and electron microscopy may have opened a new avenue to the generation of improved or even, altogether new, tools in the biomedical sciences. In the next sub-sections we will review the functionalities of focus ion beams and the incipient efforts to apply those to the life sciences.

2.1 Micro-nano fabrication using focus ion beam technologies

In the last few decades, technological advancements in computing capacity, vacuum pumps, and differential vaccumm columns among others have had a large impact on electron and ion microscopy. Scanning electron microscopes (SEMs) can now operate in dry vacuum or wet mode, possibilitating imaging of hydrated specimens in their native environment. To the myriad of developments, it is worth emphasizing the revolutionary contribution of dual focus ion beam systems (FIBs), where the usual single electron gun in SEM is now complemented with a gallium ion gun. The complementary gun keeps a coincidental point with the electron gun, and their intersecting angle depends on the manufacturer. In addition, the systems are usually complemented with in situ gas sources. The action of a focus beam of gallium ions is schematically depicted in Figures 5 and 6.

Gallium ions are larger and heavier than electrons. When accelarated, they (brown spheres in Figure 5) impinge on a surface and their interaction with the outmost atoms on the substrate will result in atomic ionization and breaking of chemical bonds. As a result, outer atoms in the substrate are sputtered away, as seen in the grey spheres in Figure 5. The availiability of gas injectors in the FIB chamber can actually assist the atom ejection process by first adsorbing, and then chemically etching the targeted surface (blue spheres in Figure 5). The use of gases can acelarate milling in large regions and facilitate etching through deep trenches. In addition, gallium ions are likely to be unintentionally implanted during etching. Unintentional gallium ion implantation could have deleterious effects over the applicability of biomedical tools, as will be discussed later.

The other-less known- functionality adscribed to FIB is the gas-assisted deposition of metals and insulators, as seen in Figure 6. In this scheme, the combined action of gallium ions (brown spheres) and gas molecules (blue and green sphere-coplexes) emerging from the in

situ sources, results in the adsorption of gas molecules to the substrate, which leads to a thermally-driven gas molecule disocciation. Finally, the unwanted dissociated species are sputtered. Similarly to milling, implantation of both gallium ions and dissociated species are likely to occur during deposition.

The reader is directed to the monograph on Focus Ion Beams by Lucille Giannuzzi and Fred Stevie (Giannuzzi & Stevie, 2005) for a thorough and rigorous description of the technique.
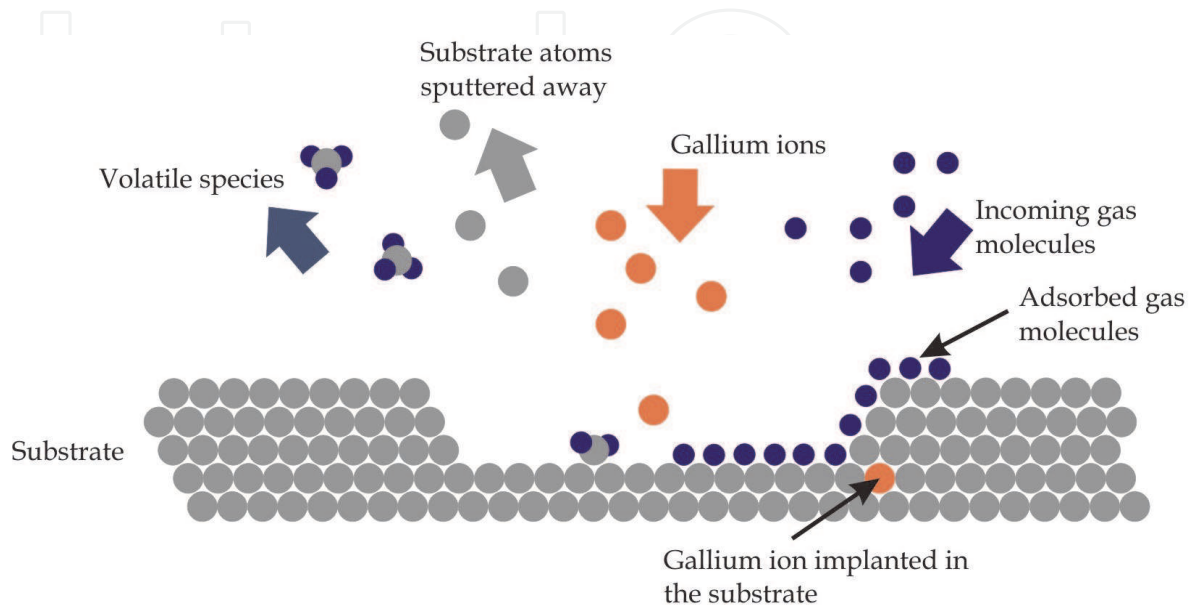
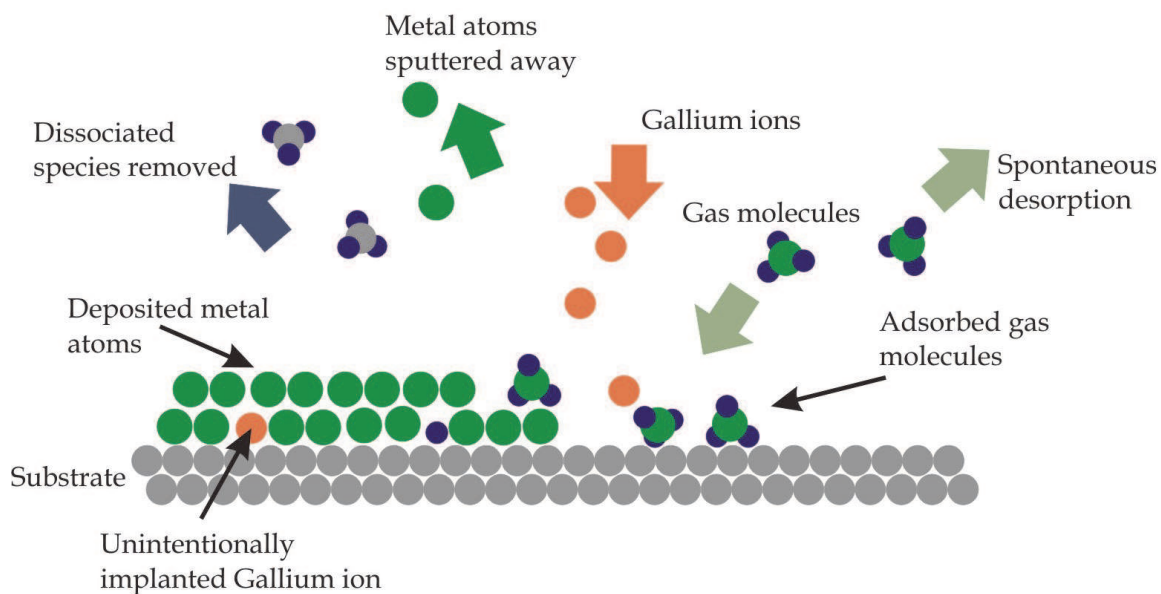Fig. 5. Schematic describing atomic processes during ion beam-promoted sputtering.

Fig. 6. Schematic describing atomic porcesses during ion-beam assistend gass deposition.

2.2 FIB micro-nano fabrication for customized pipettes
Fabrication of microinjection pipettes and micromanipulators with finely-controlled piercing angles, nanometer polishing, and versatile tip geometries is the next step to

improve cell manipulation efficiency. Microsystem-based technologies hold the promise to mass-manufacture such highly functional tools with highly customized tips. This high customization is likely to have an impact on both current and future cell handling and cell probing. In particular, FIB micromachining has been receiving some attention lately in this application context. Indeed, FIB is rapidly becoming useful in diverse environments, serving multiple applications. Some of which differ greatly from the initial aims of FIB towards, for example, identification of failure mode analysis in the semiconductor industry or the thinning of lamellas (Gianuzzi and Stevie, 2005) for transmission electron microscopy inspection in materials science.

The pervasive nature of FIB capabilities, combined with the increasing cross-talk between the physical and the life sciences have been conducive to the pursuit of FIB as means of pipette customization. Some of the first evidence in this effort came from Kometani and coworkers, who retrofitted conventional glass pipettes (glass capillaries) by building nanostructured nozzles (Kometani et al., 2005a). The process is depicted in Figure 7 (Kometani et al., 2005b), where a conventional pipette is subjected to pulling (for thinning purposes), gold coating (to minimize electrical charing during electron and ion beam bomdardment), and FIB customization by chemical vapor deposition (CVD) and further milling.
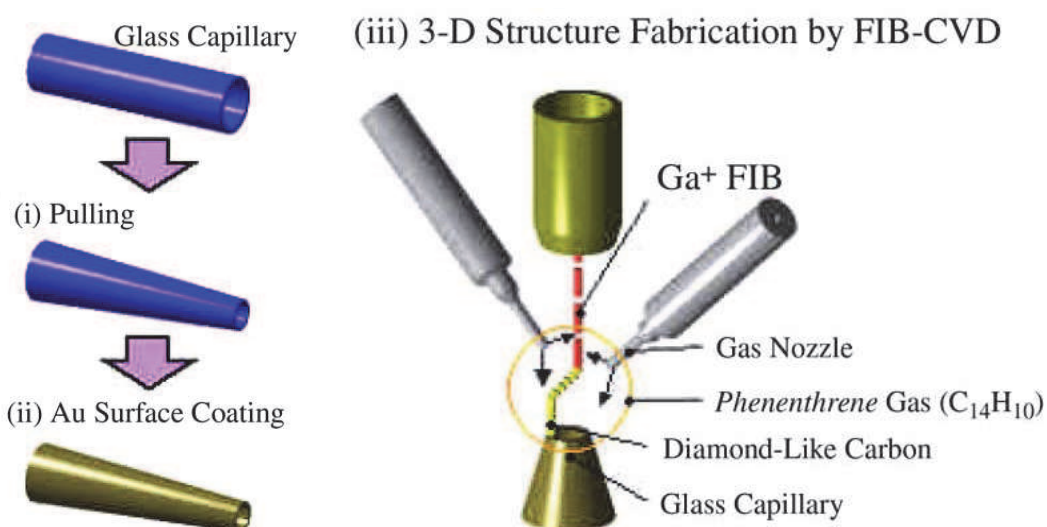

Fig. 7. Pipette microfabrication sequence in FIB. After Kometani et al., 2005b[3].

CVD deposition refers to the gas deposition process described in the previous section. *Phenanthrene* gas ($C_{14}H_{10}$) is well known for yielding three-dimensional diamond-like carbon (DLC) when used as a precursor in FIB-assisted CVD. Reportedly, this precursor is a good choice for either filling predetermined voids or for growing a fill tube directly above a fill hole (Biener et al. 2005). Other precursor gases are readily available on conventional commercial FIBs, such as tungstene (W) and silicon oxide (SiO_2), opening up the materials space to applications with conductivity and structural requirements (Kometani et al., 2007).

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[3] Reprinted from *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, Vol. 232, No. 1-4, Kometani, R.,Hoshino, T., Kanda, K., Haruyama, Y., Kaito, T., Fujita, J., Ishida, M., Ochiai, Y., & Matsui, S. , Three-dimensional high-performance nano-tools fabricated using focused-ion-beam chemical-vapor-deposition, pp. (362-366), 2005, with permission from Elsevier

In this scheme, nozzle structures were first fabricated on silicon substrates (Kometani et al., 2003). Irradiation of selected areas in the surface under a constant *phenanthrene* gas flow was performed following a scanning sequence dictated by a function generator. This function generator specified the pathway of the ion beam which determines the local DLC deposition, as depicted in Figure 6, with nanometric presicion. The pathway is not only being dictated on the x-y plane perpendicular to the tip by the usual lateral scanning action of beams. It also suffers modification on the vertical z axis, parallel to the pippette axis, presumably by varying the focal depth of the ion beam. The convolution of both lateral scanning and vertical height variation, would produce a helix trayectory, as shown in the schematic of Figure 8 top, which summarizes the synthesis of nozzle structures by DLC deposition. Nozzle fabrication is followed by spot milling of the inner channel, and tip customization by sectioning at specific angles.

Figure 8 (middle row) shows the progression of nozzle building with final inner and outer channels of 150 nm and 1580 nm at tip and base, respectively. The post-fabrication cross-section images (as seen in the last images in middle and bottom row) suggests even deposition and absence of voids, although the DLC surface has probably been unintentionally polished during FIB cross-sectioning. Further discussion would be needed to clarify the structural quality of as-grown DLC in these architectures.
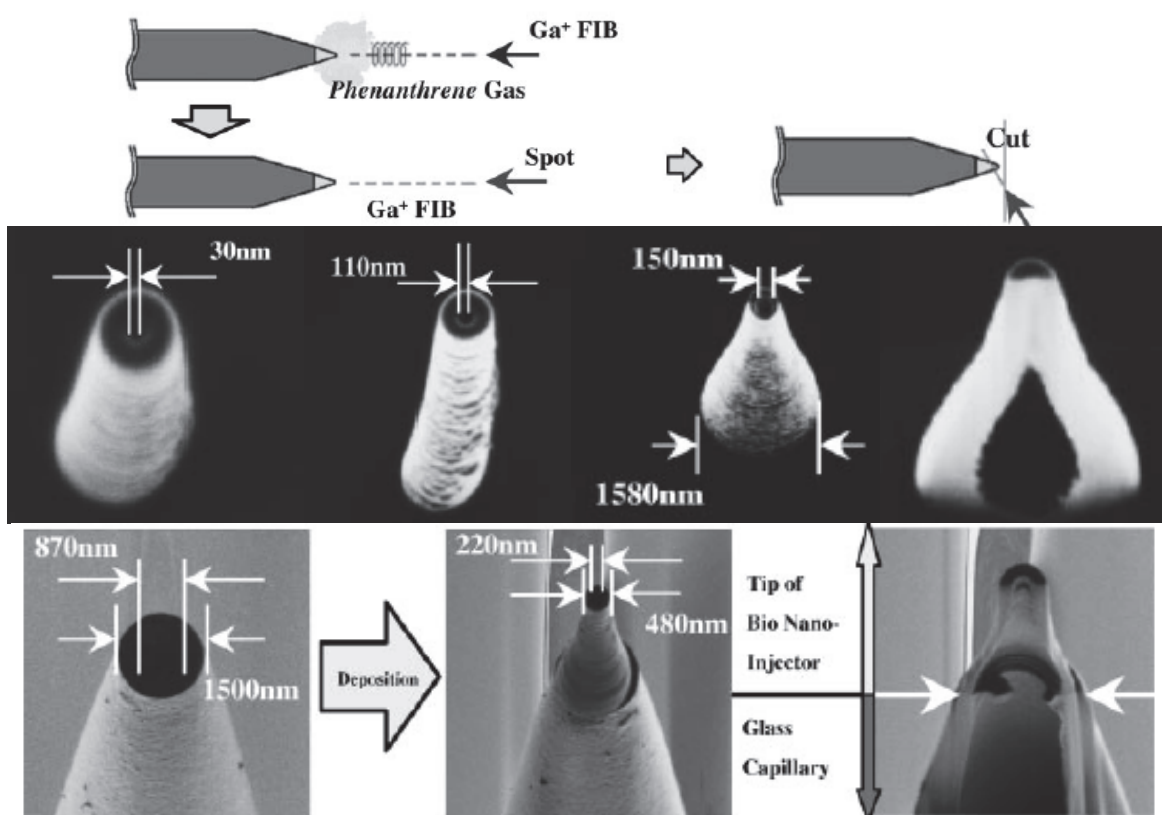

Fig. 8. FIB sequence for pipette micromanufacturing through vapor deposited DLC. After Kometani et al., 2003[4].

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[4] This material is the reproduction from . *Japanese Journal of Applied Physics*, Vol. 42, No. 6B, (February 2003), pp. 4107-4110, Nozzle-Nanostructure Fabrication on Glass Capillary by Focused-Ion-Beam Chemical Vapor Deposition and Etching, 2003, Kometani, R., Morita T., Watanabe, K., Kanda K., Haruyama, Y., Kaito, T., Fujita, J., Ishida, M., Ochiai, Y., & Matsui, S.

Similarly, nozzle architectures can be implemented on pipette surfaces (Kometani et al., 2003). Again, by combining etching and deposition FIB capabilities, a final nozzle of 220 nm tip inner diameter can be tightly sculpted on the edges of a commercial glass pipette, as seen in Figure 8 (bottom row). In this process, an initial polishing of the edges takes place to subsequently facilitate a smooth deposition. The function generator designed for sculpting this nozzle tapered a cone-like shell, from 1500 nm to 480 nm outer diameters and from 870 nm to 220 nm inner diameters. A cross-section view of the structure shows the sharp interface between glass and DLC. Although values of interface strength were not reported along with fabrication methodology, this approach could be sound for cellular handling.

We have mentioned earlier how the versatility of FIB milling and deposition is not only due to the high position ability of the ion beam itself, but also to the high programmability of the scanning lenses (magnetic fields). Coupled with design software, sophisticated 3-dimensional patterns can be sculpted at pipette edges, as seen in Figure 9 (Kometani et al., 2005a, and 2006). Nanonets could be sculptured at the edges of pipettes (Kometani, 2005b) (not shown), offering a singular approach to collecting 2μm polystyrene spheres submerged in an aqueous environment.
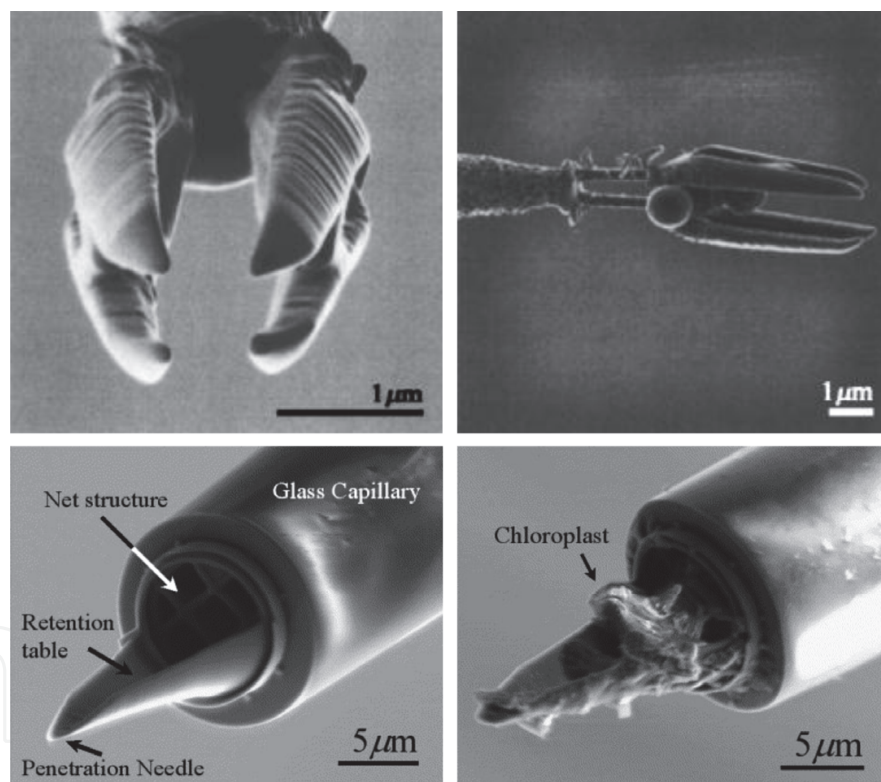

Fig. 9. SEM images of nozzle architectures, after Kometani et al., 2005a[5], and 2006[6].

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[5] Reprinted with permission from *Journal of Vacuum Science & Technology B: Microelectronics and Nanometer Structures*, Vol. 23, No. 1, pp. (298-301), Performance of nanomanipulator fabricated on glass capillary by focused-ion-beam chemical vapor deposition, Kometani, R., Hoshino, T., Kondo, K., Kanda, K., Haruyama, Y., Kaito, T., Fujita, J., Ishida, M., Ochiai Y., & Matsui, S. (2005) American Vacuum Society.

[6] Reprinted from *Microelectronic Engineering*, Vol. 83, No. 4-9, , Kometani, R., Funabiki, R., Hoshino, T., Kanda, K., Haruyama, Y., Kaito, T., Fujita, J., Ochiai, Y., & Matsui, S., Cell wall cutting tool and nano-net fabrication by FIB-CVD for subcellular operations and analysis, pp. (1642-1645), (2006), with permission from Elsevier

The nanonet design serves as a proof of concept conducive to manipulation and analysis of subcellular organelles. Design possibilities go beyond passive components and an electrostatically-active manipulator has also been demonstrated, as shown in Figure 9 (top) by a 4-finger nanomanipulator, clampling 1μm-diameter latex microspheres. The scenario to selectively capture subcellular organelles of a specific size in a cell of choice was explored by constructing a multi-component tool. The multicomponent tool consisted of a cell wall-cutting needle and a nano-net of a pre-defined mesh size (Figure 9 bottom). Chloroplasts in an Egeria densa leaf were selectively captured by size-filtration in a nanonet. Filtration was activated by the naturally-occurring capillary suction at the tip of the pipette (Kometani 2006). Indeed, the filtering tool was manipulated to approach a specific cell (Kometani 2006), and the needle was inserted into the cell wall with the assistance of an external micromanipulator. Filtering of smaller organelles took place, leaving chloroplasts in the cell cutting component, also acting as a surgical scoop. Reportedly, this process was completed in 7 s, and the filtered chloroplasts from the cell of interest are shown in Figure 9 (bottom right).

The wealth of examples provided by Kometani and co-workers has paved the way for a radical approach to pipette design and manufacturing, opening a whole new perspective in procedures for biological handling at the cellular and subcellular level. Following the initial stages of proof of concept, a few questions arise regarding reliability; such as the mechanical properties of the ensemble. As-deposited FIB DLC shows a large Young's modulus (600 GPa-Kometani 2003 and references therein), yielding a stiffness comparable to other ceramics such as SiC and WC. In addition, the nanomanipulators did survive tests involving microsphere manipulation and chloroplast filtration. However, a more detailed analysis of mechanical durability of these structures in the relevant context applications is necesary.

Albeit, questions regarding toxicity and biocompatibility need urgent attention (Campo et al., 2011). It is worth emphasizing that gallium ions are likely to be implanted during both milling and deposision in FIB. Further studies are needed to address the impact of ion implantated tools on cellular activity. The next section will describe the work by Campo and co-workers who milled conventional glass pipettes at specific angles (Campo et al., 2010a) by FIB, yielding extremely polished edges with high precision. Tests during mouse embryo piercing, correlated increased penetration rates with decreased pipette angle. In addition, optimum embryo development after manipulation revealed little impact of residual implanted gallium ions, suggesting biocompatibility. It was also observed that milled pipettes maintain structural integrity after repeated piercing. Micromachining of glass pipettes by FIB could be amenable to mass production, presenting a promising avenue for future, increasingly demanding, cell handling.

2.3 FIB microfabrication for cell handling: Piercing and biocompatibility

Recapitulating, prior sections have described how manufacturing embryo injection pipettes is a delicate task. Typically conducted manually, trial-and-error pulling and heating control the inner and outer diameters as well as the length of the taper (Nagy et al., 2002). In FIB, areas can be milled selectively by gallium ions, as discussed earlier. In this section, we will describe how careful choice of time and current in the ion beam allows for high-quality polishing, providing qualitatively smooth, regular tapers. The high spatial selectivity in FIB has been effectively used to design sharp pipette tips, creating highly customized capillaries (Campo et al., 2010a). In this context, customization involved controlled bevelled angles as well as regular taper finishes. In an attempt to address the usability of FIB-customized

pipettes in an impactful application context and also to partially address biocompatibility, mouse oocytes and embryos were used in piercing tests. Mouse embryos pose an ideal test bench for initial assessment of biocompatibility since small perturbations at the initial developmental stages are likely to impact embryonic development.

Important technological advances have taken place on oocyte and embryo microinjection recently. Oocyte and embryo microinjection can be performed with flat-ended pipettes which are easier to fabricate but often require piezoelectric-driven drilling to mechanically advance the pipette tip through the zona pellucida and plasma membrane (Yoshida & Perry, 2007). Piezo-driven drilling has been key to the success of experimental procedures involving microinjection of mouse oocytes in processes such as ICSI. The high elasticity of mouse oocyte plasma membrane complicates manual perforation without rupture using conventional bevelled and spiked pipettes, systematically requiring piezo-drilling. Although not strictly necessary, piezo-driven drilling greatly simplifies mouse embryo piercing. In this scheme, a small amount of mercury is typically loaded in the micropipette to minimize lateral vibrations, preventing cell damage (Ediz & Olgac, 2005). However, the additional cost of piezo-drills and, more importantly, toxicity issues arising from the use of mercury, have limited the use of piezo-assisted microinjection to just a few species (Yoshida & Perry, 2007; Ergenc et al., 2008).

Following this line of research and given the increasing need to improve current cell piercing (Ergenc & Olgac, 2007) and pipette quality (Yaul et al. 2008; Ostadi et al. 2009), Campo and coworkers (Campo et al., 2010) have sharpened conventional glass capillaries by FIB and tested them in mouse embryos. For the first time, the effect of sub-nanometer pipette polishing on zona pellucida and plasma membrane piercing is investigated. In addition, in vitro development of injected embryos was monitored, providing an early assessment on damage and toxicity from FIB-processed pipettes. A total of three capillaries were sharpened at 30, 20, and 15° (capillaries number 1,2, and 3, respectively), with profiles shown in Figure 10. The resulting edges were smooth after successive polishing. The insert in Figure 10a (previously shown in Figure 3 right) shows a commercial glass pipette used in human ICSI (Humagen, Virginia) featuring a sharp spike that, according to commercial specifications, has an angle between 25 and 30°. This angle is similar to those of pipettes number 1 and 2. Table 1 lists the different capillaries describing the final angular features.
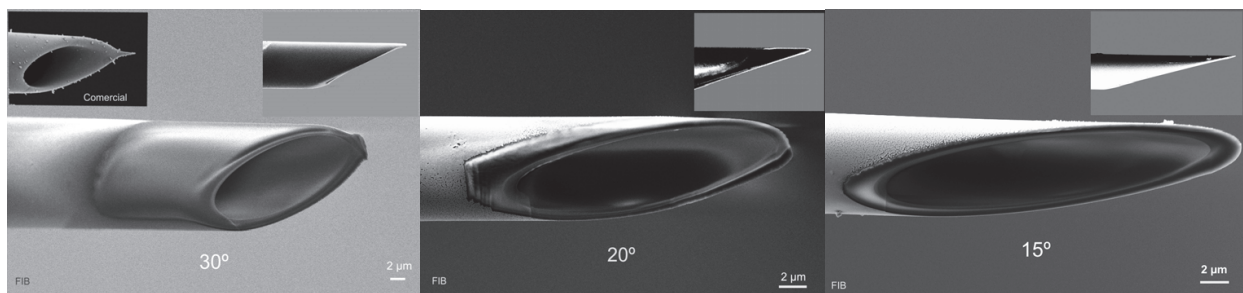

Fig. 10. SEM images of pipettes (left) 1, (centre) 2 , and (right) 3, sharpened at 30˚, 20˚, and 15˚, respectively and showing beveled cut. Insert in (a) top left shows a commercial ICSI pipette. Inserts in (a), (b), and (c) top right show side-views of the milled pipettes.[7]

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[7] With kind permission from Springer Science+Business Media: *Biomedical Microdevices*, Focus Ion Beam Micromachined Glass Pipettes for Cell Microinjection., Vol. 12, No.2, 2010, pp. (311–316), Campo, E.M., Lopez-Martinez, M. J., Fernández, E., Barrios, L., Ibáñez, E., Nogués, C., Esteve, J., & Plaza, J.A.

Capillaries number 1, 2, and 3 were used to pierce zona pellucida and plasma membrane of mouse embryos at the one cell stage (Figure 11). When successful, once inside the perivitelline space, the sharp tip was positioned to deform the plasma membrane and pierce it, penetrating within the cytoplasm. This process was conducted with a micromanipulator; applying pressure manually, in the absence of piezo-drilling.

A total of 20 embryos were used to test the injection efficiency of each pipette and the results are summarized in Table 1. Successful penetration of both the zona pellucida and the plasma membrane was achieved with all pipettes tested, with varying efficiencies and rates of embryo lysis. Pipette number 1 went through the zona pellucida in 60% of the tested embryos, leaving 40% unperforated. Further plasma membrane penetration was achieved with a 45% success rate, applying high pressures that resulted in extensive embryo deformation, possibly promoting lysis. All of the tested embryos were perforated with pipette number 2, totaling 75% penetration rate of both the zona pellucida and the plasma membrane. Pipette number 2 failed to perforate the plasma membrane in only 25% of the tested embryos. Finally, zona pellucida and plasma membrane penetration was achieved in 100% of the embryos punctured with pipette number 3.

Pipette number	Beveled angle [°] of micropipette	Number of embryos used	Penetration of ZP and PM (%)[*]	Penetration of ZP only (%)[**]	Lysis (%)[b]
1	30	20	9 (45)[a]	3 (15)[a]	3 (33.3)[a]
2	20	20	15 (75)[a]	5 (25)[a]	7 (46.7)[a]
3	15	20	20 (100)[b]	-	6 (30)[a]

[*] ZP: zona pellucida; PM: plasma membrane
[**] Number (percentage) of embryos that lysed after successful penetration of both ZP and PM
[a–b] Values with different superscripts within the same column differ significantly ($p < 0.05$).

Table 1. Injection efficiency and rates of lysis in mouse embryos penetrated with capillaries beveled at different angles.[8]

Lysis was observed only in embryos in which both the zona pellucida and the plasma membrane penetration was successful. Rates of embryo lysis were 33%, 46.7%, and 30% for capillaries number 1, 2, and 3, respectively. Pipettes were structurally sound, as no chips or fractures developed after piercing multiple embryos. Embryos that survived micromanipulation with pipette number 3 (n=14) were kept in culture for 96 h, and their development was assessed every 24 h, as seen in Table 2. These will be referred to as micromanipulated embryos throughout this discussion. An additional collection of embryos that were cultured in parallel with the micromanipulated embryos but without going through micromanipulation was used for comparison. We will refer to these as control embryos (n=23). In vitro embryo development results are summarized in Table 2. Control and micromanipulated embryos cleaved at similar rates (86.9% and 92.8%, respectively) and developed to the blastocyst stage (see Figure 12) also at similar rates (82.6% and 78.6%, respectively).
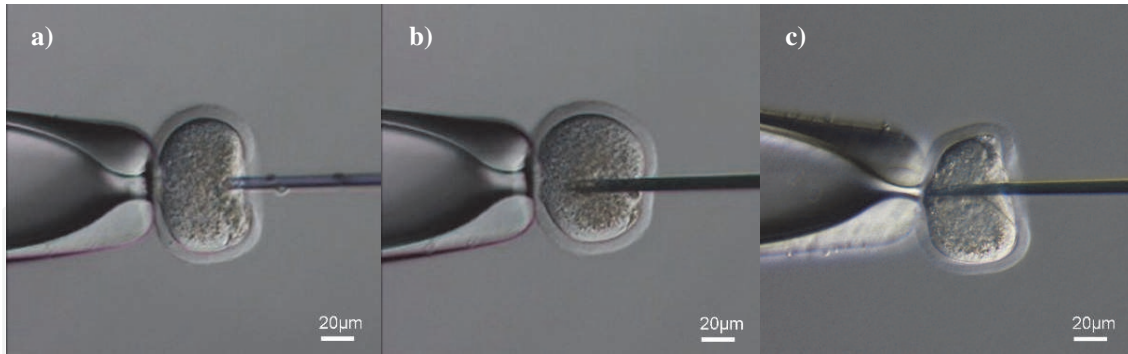
Fig. 11. Optical images of injection with pipettes number (a) 1 (b) 2, and (c) 3. The images were acquired at different stages in the piercing process. The observed embryo deformation is not indicative of the penetration depth prior to piercing.[9]

Group of embryos	Number of embryos	Embryos (%) developed to			
		2-cell	4-cell	Monula	Blastocyst
Micromanipulated	14	13 (92.8)	13 (92.8)	13 (92.8)	11(78.6)
Control	23	20 (86.9)	20 (86.9)	20 (86.9)	19 (82.6)

Table 2. In vitro development of mouse embryos successfully penetrated with pipette number 3.[10]

Summarizing, only pipette number 3 could penetrate the zona pellucida and the plasma membrane with 100% success and avoid lysis with 70% success. Piercing with pipettes 1 and 2 resulted in a more difficult plasma membrane penetration, as summarized in Table 1. Penetration rates increase with a decreased pipette angle. However, some lysis was unavoidable for all three pipettes. The correlation between sharpness and lysis is unclear, as sharper tips do not appear to minimize damage during piercing or decrease lysis. The finish in the pipette surely has an impact both in the piercing effect and in embryo development. Indeed, roughness and chipped edges in pipette tips are thought to be responsible for introducing contaminants into the oocyte, therefore inhibiting fertilization (Yaul et al., 2008). Some additional work has also been done assessing the effect of FIB- taper finish on penetration and damage (Campo et al., 2009b), although only in a qualitative fashion. These results show that capillary number 3 with 15°-sharp, smooth, finished tips by FIB could penetrate the zona pellucida and the plasma membrane efficiently, in good agreement with classic piezo-assisted drilling (Nagy et al., 2002).

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[9] With kind permission from Springer Science+Business Media: *Biomedical Microdevices*, Focus Ion Beam Micromachined Glass Pipettes for Cell Microinjection., Vol. 12, No.2, 2010, pp. (311–316), Campo, E.M., Lopez-Martinez, M. J., Fernández, E., Barrios, L., Ibáñez, E., Nogués, C., Esteve, J., & Plaza, J.A.
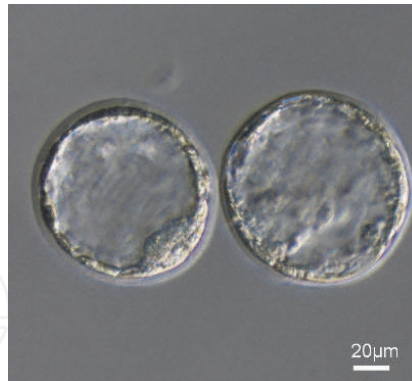
[10] With kind permission from Springer Science+Business Media: *Biomedical Microdevices*, Focus Ion Beam Micromachined Glass Pipettes for Cell Microinjection., Vol. 12, No.2, 2010, pp. (311–316), Campo, E.M., Lopez-Martinez, M. J., Fernández, E., Barrios, L., Ibáñez, E., Nogués, C., Esteve, J., & Plaza, J.A.

Fig. 12. Optical images of previously injected embryos showing development to the blastocyst stage.[11]

Survival rates during the in-vitro development of embryos previously manipulated with pipette number 3 were similar to survival rates of control, non-manipulated embryos (Table 2). All 14 embryos pierced with pipette number 3 were monitored for in-vitro development, noting the survival rates at the two-cell, four-cell, morula, and blastocyst stages. Most micromanipulated embryos successfully developed to the two- and four-cell stages as well as at morula. The survival rate at all these stages was constant at 93%. Similarly, control embryos maintained a constant 87% survival rate at the same intervals. Only one of the 14 micromanipulated embryos remained arrested at the one-cell stage. This phenomenon was also observed in control embryos, suggesting that causes inhibiting division might be unrelated to micromanipulation factors.

Gallium ions (unintentionally implanted during pipette milling) did not appear to affect development of mouse embryos to the blastocyst stage. While more detailed analyses are needed to fully address biocompatibility, these preliminary results are encouraging and could ultimately lead to improved piercing and survival rates during cell handling.

3. Sub-cellular monitoring, drug-delivery and manipulation

The prior section reviewed recent developments in technologies aiming at improving the funtionalities of celllular tools (mostly manipulators and injectors) and addressing the quality of finishes in injection pipettes. In the spirit of completeness, in this section we will describe in a cursory manner, some of the current landscape for subcellular technology.

3.1 Monitoring and drug-delivery by sub-cellular smart particles

The idea of targeting specific areas within the cell for detailed study is not new. Dyed biocompatible polymeric nanoparticles have been used for apoptosis imaging (Kim et al., 2006), and morphology of intracellular polymeric systems have been studied to address bio-compatibility along with their suitability as imaging markers and as drug delivery carriers (Nishiyama, 2007). Carrier morphology studies suggest that particles of specific sizes are

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[11] With kind permission from Springer Science+Business Media: *Biomedical Microdevices*, Focus Ion Beam Micromachined Glass Pipettes for Cell Microinjection., Vol. 12, No.2, 2010, pp. (311–316), Campo, E.M., Lopez-Martinez, M. J., Fernández, E., Barrios, L., Ibáñez, E., Nogués, C., Esteve, J., & Plaza, J.A.

required to prevent deleterious interaction with cell organelles. On those lines, vehicle morphology studies concluded that phagocitic cells responded differently to micelles (assemblies of hydrophobic/hydrophilic block-copolymers) of different sizes (Geng et al., 2007). Walter et al. examined polymeric spheres that were phagocited for drug delivery (Walter et al., 2001) and Akin et al. (Akin et al., 2007) used microbots (nanoparticles attached to bacteria) to deliver therapeutic cargo to specific sites within a cell. Microbots delivered nanoparticles of polystyrene carrying therapeutic cargo and DNA into cells by taking advantage of invasive properties of bacteria. Recently, Kataoka's group (Mirakami et al., 2011) has sucessfully delivered chemotherapeutic drugs to the nuclear area of cancerous cells using micelles carriers. The specific delivery to the nuclear region is believed to have played a role in inhibiting the development of drug-resistance tumors.

Within the subcellular domain, different approaches have aimed at manufacturing devices to interact with organelles. Some groups have contemplated the possibility of constructing micro total analysis stystems (µTAS) suitable for biological applications (Voldman et al., 1999), where the mechanisms to extract information out of the cellular entity are challenging. However, few attempts have been made to address viability and functionality of standard microtechnology processed systems. Recently, our group has reported silicon microparticles embedded in live cells, suggesting an outstanding compatibility between conventional microtechnology devices and live systems down to the cellular level (Fernandez-Rosas et al., 2009; Gomez-Martinez et al., 2010). In terms of sensing, initial functionality mechanisms have identified apoptosis. These revolutionary findings constitute a paramount paradigm shift on cellular metrology, histology, and drug delivery; which are likely to have a profound impact in future research lines.

3.2 Manipulation by biomimetics

Another approach to sub-cellular exploration is inspired by nature. Indeed, understanding, mimicking, and adapting cellular and molecular mechanisms of biological motors in vitro has been forecast to produce a revolution in molecular manufacturing (Dinu et al., 2007, and Iyer et al., 2004). Biomolecular motors are biological machines that convert several forms of energy into mechanical energy. During a special session at Nanotech 2004 in Boston, MA, DARPA commissioned-overview by Iyer argued that functions carried out within a cell by biomolecular motors could be similar to man-made motors (i.e. load carrying or rotational movement). Researchers have already pondered about ways to transport designated cargo, such as vesicles, RNA or viruses to predetermined locations within the cell (Hess et al., 2008). Professor Hess during his keynote lecture at SPIE Photonics West (January 2008) also proposed biomolecular motors as imaging and sensing devices. Biomolecular motors such as the motor protein kinesin have been suggested as efficient tractor trailers within the cell. Efficiency of these systems could generate useful tools (conveyor belts and forklifts) as nanoscale bio-manufacturing tools. Kinesin moves along a track and is responsible for transporting cellular cargo such as organelles and signaling molecules. However, a detailed explanation of this walking mechanism is still missing (Iyer et al., 2004), currently inhibiting spatial and temporal control of kinesin molecular motors.

3.3 Monitoring and manipulation by FIB and microfluidics

Trends to intracellular manipulation also revolve around scaling down conventional pipettes. This trend is facilitated by microfluidics. Microsystem technologies have produced in the last decade an array of microfluidic devices (Verpoorte & De Rooig, 2003) that could

potentially probe the subcellular domain. By combining our prior experience learned in FIB glass pipettes (Campo et al. 2010a) with microfluidics (Lopez-Martinez et al. 2008 and 2009), micropipettes have been milled and tested in live embrios (Campo et al., 2009a and b). In this approach, micropipettes dimensions are comparable to some organelles and the sharp tips are likely to induce less damage on external cell walls. Details on the bottom-up microfabrication squeme can be found elsewhere (Lopez-Martinez et al., 2009). Similar experiments to those with glass pipettes (described in Section 2.3) revealed that silicon oxide (SiO$_2$) FIB-sharp nozzles successfully pierced mouse oocytes and embryos, without prejudice to the embryo and without producing structural damage to the nozzle.

Lack of structural damage is an important concern in FIB-modified structures as puncture devices reside on mechanical strength. Ideally, micronozzles will be sturdy enough to perforate zona pellucida and membrane without curving the tip of the micropipette or causing any other structural damage such as cracking or fragmentation. The tested micropipettes mantained their structural alumina layer, which provided sturdier structures. Figure 13 shows the structural layer (darker filler) surrounded by the silicon oxide channel. The tips did not show signs of mecanical failure during puncturing, as seen in Figure 14, or after repeated puncturing. Success from this initial assessment on mechanical strength and sucessful piercing has led to further work on hollow, fully microfluidic-functional micropipettes (*Lopez-Martinez & Campo-under preparation*). In addition, a study to assess viability and the adequate angular range for embryo piercing is underway. A better understanding of this procedure could eventually lead to commercial production and set pattern in cell handling.
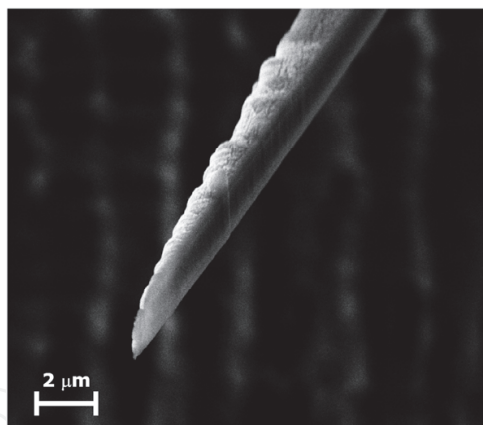

Fig. 13. SEM image of a 2 µm-wide silicon oxide nozzles FIB-sharpened at 5º (after Lopez-Martinez et al. 2009).[12]

Scaling down further to nanofluidics has also been achieved by ingenious building of carbon nanopipettes on conventional glass pipettes (Schrlau et al., 2008). Compared to conventional glass pipettes, these structures have suggested enhanced performance for intracellular delivery and cell physiology due to their smaller size, breakage and clogging resistance. Carbon nanopippettes have been reportedly used for concurrent injection and electrophysiology.

3.4 Smart materials in the sub-cellular domain

Materials science also has an important role in the development of cellular tools. Indeed, development of biocompatible smart materials with novel functionalities could provide the needed non-incremental advancement for sub-cellular monitoring and manipulation. Historically, there is a large presence of polymers in biomedicine. In fact, liquid crystal elastomers have been proposed as artificial muscles under the heating action of infrared lasers (Shenoy et al., 2002 and Ikeda et al., 2007), and an early proof-of-concept observed liquid crystal elastomers "swimming away" from the actuating light (Camacho-Lopez et al., 2004). This rudimentary motor was submerged in water and the source was an Ar+ ion laser (514 nm). Despite their potentially large application space, photoactuating materials have not been used in the broader context of biological systems (Campo et al., 2010b), posing an unique research opportunity for innovative functionalities.
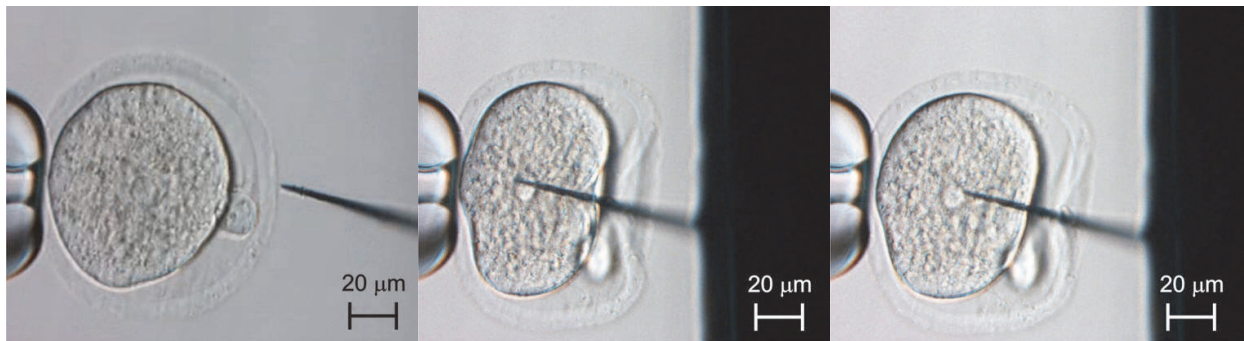

Fig. 14. Optical images of piercing test progress, (left) microdispenser nozzle outside a embryo, (centre) nozzle trying to penetrate embryo and (right) nozzle inside the embryo. (After Lopez-Martinez et al., 2009)[13]

4. Conclusions and future directions

An engineering analysis of the currently restrictive designs, finishes, and probing methods of glass pipettes and micromanipulators, suggests that those suffer from limited functionality and often damage cells; ultimately resulting in lysis. With all, the physical parameters that identify a high-quality pipette for a specific application need of a more quantitative description. In particular, the finishes of a pipette seem to be lacking a quantitative measure that could be provided by commonly-used characterization techniques in microsystem technologies, such as atomic force microscopy.

There seems to be plenty of leeway in advancing the state of the art in pipette design, manufacturing and piercing techniques. The great flexibility posed by microsystem technologies in the context of microfluidic devices and micromanufacturing with ion beams, present an unique opportunity in the biomedical sciences. In this scheme, tools for cell handling and monitoring can be tailored to specific tasks with unprecedented level of detail. Indeed, the possibility of affordable custom-made tools opens the door to improved sucess rates in common cellular procedures such as cell piercing. Highly-customized tools can also be designed to accomplish subcellular manipulation that would be, otherwise, unattainable

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[13] Reproduced with permission from IOP: *Journal of Micromechanics and Microengineering*, Versatile micropipette technology based on Deep Reactive ion Etching and anodic bonding for biological applications, . (2009), Vol. 19, No. 10, pp. 105013, Lopez-Martinez, M.J. , Campo, E. M., Caballero, D., Fernandez, E., Errachid, A., Esteve, J., & Plaza, J.A

with the limited functionalities of conventional pipettes. The use of ion beams for surface finishes can possibly alivieate some of the tedious work often involved in finishing capilaries. Ion beam polishing could also contribute to the characterization of roughness and finishes in a quantitative manner. In fact, ion beam milling is a useful tool to reverse engineer the morphology of pipettes altogether by sequential polishing and further image reconstruction (Ostadi et al., 2009). These tomographic capabilities could prove useful in quality control assessment of current and upcomming cellular tools.

Kometani et al. have provided a wealth of examples in highly customized micromanipulators, pending application in relevant cellular and subcellular scenarios. Future experiments should aim at inseminating mouse oocytes with FIB-polished glass pipettes, as initial tests by Campo et al. merely addressed piercing feasibility, i.e. mechanical sturdiness, sharpness, and early indication of biocompatibility. However, the real application scenario has not yet been demonstrated since no injection tests have been performed to show functionality. Similarly, FIB-sharpened microfluidic-pipettes are pending injection testing. In addition, microfulidic-pipettes manufacturing is ameanable to exploring materials other than silicon oxide, that could be of interest to complementary applications such as electrophysiology. Similarly to glass pipettes, microfluidic pipettes could be fitted with additional components, either by bottom-up or top-down microtechnologies. Resulting structures from the addition of sensors and actuators with different functionalities need to be tested in adequate scnearios and further assess biocompatibility.

We have discussed in detail how FIB with the assistance of gallium ions and carbon deposition, has gone well beyond proof of concept in terms of innovative design and micromanufacturing. Future directions in the microtechnology applications to the life sciences are likely to build upon FIB capabilities and also explore upcomming ion-bem microscopies. Looking forward, building upon FIB capabilities could be explored in the materials space, as well as in the functionality space of ion beam-produced tools. On the materials front, most FIB manufacturing for cellular tools has exploited the structural robustness of DLC. However, a number of chemistries are available in commercial FIB, with increasingly purified sources (Botman et al., 2009). Deposition of gold (Au), paladium (Pd), and platinum (Pt) could be specially interesting for devices requiring electrical conduction, such as those used in electrophysiology. Tipically, higher purity nanostructures are deposited by ion beam than by electron beam-assited deposition (Utke, 2008). However, further work will need to assess the effects of source purity on chemistry and mechanical characteristics of ion beam-deposited structures.

Amongst emergent novel micromachining and micromanufacturing technologies ameanable to contributing to cellular tools, Helium Ion Microscopy (HIM) is possibly the most relevant. Seminal papers describe this novel microscopy that serves both as a characterization (Scipioni et al., 2009) and a manufacturing tool (Postek et al., 2007, Maas et al., 2010) in micro-nano systems. With the use of hellium (He) ions and, smilarly to FIB, highly customizable milling capabilities, HIM could have a possitive impact on the pending biocompatibility assessment. Adequate biocompatibility studies are needed to assess ion dose implantation on tools and devices and the effects at the subcellular and cellular levels, as well as in vivo. These will be critical parameters that could hinder the implementation of ion-beam technologies in the life sciences. In all likelihood, these strategies will need to be developed by multidisciplinary teams. In fact, assembly of highly multidisciplinary teams, encompassing bio-medical scientists and microsytem technologists, are surely needed to fully explore the possibilities of impactful task–specific tools in the context of subcellular manipulation.

It is also crucial to develop a mechanistic understanding of how design, manufacturing, and piercing techniques affect cellular structures. Indeed, the impact of pipette parameters on handling is unclear, as mechanisms responsible for different failure modes during conventional piezo-assisted piercing only recently have been subject of investigation (Ediz et al., 2005). Mechanistic studies would establish a much-needed correlation between the (quantifiable) physical parameters of pipettes and piercing techniques with cellular response in the context of elasticity theory and biology. In terms of operator training and quantification of the exerted force, the advent of haptics in the context of robotics could provide quantification of cell injection force and also to improve success statistics in piercing and other operational procedures. There is already enough evidence suggesting that the combination of haptic and visual feedback improves handling (Pillaresetti et al., 2007). Further development of these technologies will, most likely, make them available to the bio-medical community at large. Novel piercing technologies have also appeared in the recent literature, such as Ross-Drill, promoting a rotational approach to cell piercing, rather than tangential (tipical of piezo-assited drilling) and claiming decreased training effort for operators. The possibility of combining Ross-Drill with FIB-polished pipettes has already been sugested (Campo et al., 2010a).

SPECIALTY	APPLICATION	CITATION
CELL INJECTION	ROTATIONAL OSCILLATION-DRILL	ERGENC, EDIZ & OLGAC
CELL INJECTION	MICROMANUFACTURING OF CUSTOMIZED TIPS IN GLASS CAPILARIES AND MICROFLUIDIC PLATFORMS	CAMPO & PLAZA
CELL INJECTION	3-D STUDY OF GLASS PIPETTE GEOMETRY BY MICROMACHINING TECHNIQUES	OSTADI & OLGAC
CELL MICROINJECTION	USE OF CARBON NANOTUBES FOR ELECTROPHYISIOLOGY AND NANOFLUIDIC INJECTION	SCHRLAU&BAU
CELLULAR/ SUBCELLULAR HANDLING	MICROMANUFACTURING OF CUSTOMIZED MANIPULATORS IN GLASS CAPILARIES	KOMETANI& MATSUI
SUBCELLULAR MONITORING	MICROMANUFACTURIG OF CUSTOMIZED SENSORS AND ACTUATORS IN GLASS CAPILARIES	KOMETANI& MATSUI
SUBCELLULAR DRUG DELIVERY	POLYMERIC MICELLE CARRIERS	GENG & DISCHER
SUBCELLULAR DRUG DELIVERY	BACTERIA-MEDIATED DRUG DELIVERY	AKIN&BASHIR
SUBCELLULAR DNA DELIVERY	POLYMER MICROSPHERES	WALTER & MERKLE
SUBCELLULAR MONITORING AND DELIVERY*	PROOF OF CONCEPT: BIOCOM-PATIBLE INSERTION OF MICROCHIPS ON CELLS	FERNANDEZ-ROSAS, GOMEZ-MARTINEZ & PLAZA
SMART MATERIALS**	PROOF OF CONCEPT: LCE PHOTO-PROPELLED IN AN AQUOUS ENVIRONMENT	CAMACHO-LOPEZ, PALFFY-MUHORAY & SHELLEY
MECHANICAL ACTUATORS*	PROOF OF CONCEPT: BIMORPH THERMAL NANO- ACTUATORS BY FIB	CHANG & LIN
HAPTIC TECHNOLOGY IN CELLULAR HANDLING	HAPTIC FEED-BACK IN COMBINATION WIH VISUAL INSPECTION DURING CELL PIERCING	PILLARISETTI & DESAI

*This is a promising approach in subcellular monitoring and delivery.
* *This approach has not been applied to cellular or subcellular environments.

Table 3. List of highlighted technologies according to specialty, detailing specific application and citation included in the references in Section 6.

Future directions in micro-nanotechnologies applied to the life sciences are likely to build upon the approaches described in this chapter, which have been summarized in Table 3. Beyond piercing, technological developments such as cell-embedded silicon microparticles are likely to develop into micro-chips in the near future; posing a new paradigm shift in sub-cellular probing. In addition, novel actuation capabilities have been temptatively explored by Kometani's group producing an electrostatic-operated micromanipulator. Further, Chang et al., (Chang, 2009) have recently discussed a bimorph thermal actuator that combined thermal conductivity of FIB-depostied tungsten (W) with structural rigidity of DLC. This work is innovative as it introduces smart materials in microtechnology manufacturing in the production of cellular tools. On-going efforts to incorporate electro and photoactuators in the biomedical arena as artificial muscles are likely to expand to the subcellular domain and potential application contexts will be suggested, further paving the way for the incorporation of nano-opto-mechanical-systems (NOMS) in main stream research (www.noms-project.eu).

5. Acknowledgments

6. References

Akin, D., Sturgis, J., Ragheb, K., Sherman, D., Burkholder, K., Robinson, J.P., Bhunia, A.K., Mohammed, S., & Bashir, R. (2007). Bacteria-mediated delivery of nanoparticles and cargo into cells nature. Nature Nanotechnology, Vol. 2 (April 2007), pp. (441 – 449), ISSN: 1748-3387

Bao, G., & Suresh, S. (2003). Cell and molecular mechanics of biological materials. Nature materials, Vol. 2 (November 2003), No. 11, pp. (715-725), ISSN : 1476-1122

Biener, J., Mirkarimi, P.B., Tringe, J.W., Baker, S.L., Wang, Y.M., Kucheyev, S.O., Teslich, N.E., Wu, K.J., Hamza, A.V., Wild, C., Woerner, E., Koidl, P., Bruehne, K., & Fecht, H.-J. (2006). Diamond Ablators for Inertial Confinement Fusion. *Fusion Science & Technology*, Vol. 49, No. 4, pp. (737-742), ISSN: 1536-1055

Biggers, J.D., McGinnis, L.K., & Raffin, M. (2000). Amino Acids and Preimplantation Development of the Mouse in Protein-Free Potassium Simplex Optimized Medium. *Biology of Reproduction*, Vol. 63, No. 1, (July 2000), pp. (281-293), ISSN: 0006-3363

Botman, A., Mulders, J. J. L., & Hagen C.W. (2009) Creating pure nanostructures from electron-beam-induced deposition using purification techniques: a technology perspective *Nanotechnology*, Vol. 20, pp(372001 (17pp), ISSN: 1748-3387

Camacho-Lopez, M., Finkelman, H., Palffy-Muhorray, P., & Shelley, M (2004) Fast liquid-crystal elastomer swims into the dark. *Nature Materials*, Vol. 3 (May 2004), pp. (307-310), ISSN: 1476-4660

Campo, E.M., Lopez-Martinez, M.J., Fernandez, E., Ibanez, E., Barros, L., Nogues, C., Esteve, J., & Plaza, J.A. (2009). Sharpened transparent micronozzles fabrication for cell membrane piercing. *Proceedings of SPIE, the International Society for Optical Engineering*. ISBN 978-0-8194-7450-6, San Jose, California, United States, January 2009

Campo, E.M., Lopez-Martinez, M. J., Fernández, E., Esteve, J., & Plaza, J.A. (2009). Implementation of Ion-Beam techniques in microsystems manufacturing: Opportunities in cell biology. *Proceedings of SPIE, the International Society for Optical Engineering, Scanning Microscopy*. ISSN 0277-786X , Monterey, May 2009

Campo, E.M., Lopez-Martinez, M. J., Fernández, E., Barrios, L., Ibáñez, E., Nogués, C., Esteve, J., & Plaza, J.A. (2010). Focus Ion Beam Micromachined Glass Pipettes for Cell Microinjection. *Biomedical Microdevices*, Vol. 12, No.2, (April 2010), pp. (311–316), ISSN 1572-8781 .

Campo, E.M., Campanella, H., Huang, Y.Y., Zinoviev, K., Torras, N., Tamargo, C., Yates, D., Rotkina, L., Eteve, J., & Terentjev, E.M. (2010). Electron Microscopy of Polymer-Carbon Nanotube Composites. *Proceedings of SPIE, the International Society for Optical Engineering, Scanning Microscopy*. ISBN: 978-0-8194-8217-4 , Monterey, May 2010

E.M. Campo, B. Mamojka, D. Wenn, I. Ramos, J. Esteve, and E.M. Terentjev, "*Education and dissemination strategies in photoactuation,*", in preparation, SPIE NanoScience + Engineering August 2011

Chang, J., Min, B.K., Kim, J. & Lin, L. (2009) Bimorph nano actuators synthesized by focused ion beam chemical vapor deposition, *Microelectronic Engineering* 86, pp.(2364–2368), ISSN: 0167-9317

Dinu, C.Z., Chrisey, D.B., Diez, S., & Howard, J. (2007). Cellular Motors for Molecular Manufacturing. *The Anatomical Record*, Vol. 290, No. 10, (Setember 2007), pp. (1203-1212), ISSN: 1932-8494

Ediz, K., & Olgac, N. (2005). Effect of Mercury Column on the Microdynamics of the Piezo-Driven Pipettes. *Journal of Biomechanical Engineering-Transactions of the ASME*, Vol. 127, No. 3, (June 2005), pp. (531-535), ISSN: 0148-0731

Ergenc, A., & Olgac, N. (2007). New technology for cellular piercing: rotationally oscillating µ-injector, description and validation tests. *Biomedical Microdevices*, Vol. 9, No. 6, (December 2007), pp. (885-891), ISSN: 1387-2176

Fernandez-Rosas, E., Gomez, R., Ibañez, E., Barrios, L., Duch, M., Esteve, J., Nogues, C., & Plaza, J.A. (2009) Intracellular Polysilicon Barcodes for Cell Tracking. *Small*, Vol. 5, No. 21, (November 2), pp. (2433–2439), ISSN: 1613-6829

Geng, Y., Dalhaimer, P., Cai, S., Tsai, R., Tewari, M., Minko, T., & Discher, D.E. (2007). Shape effects of filaments versus spherical particles in flow and drug delivery. *Nature Nanotechnology*, Vol. 2, (April 2007), pp. (249-255), ISSN: 1748-3387

Giannuzzi, A., & Stevie, F. A. (2005). *Introduction to Focused Ion Beams: Instrumentation, Theory, Techniques and Practice* (1st edition), NY: Springer, ISBN 978-0-387-23116-7 North Carolina State University (Eds.)

Gomez-Martinez, R., Vázquez P., Duch, M., Muriano, A., Pinacho, D., Sanvicens, N., Sánchez-Baeza, F., Boya, P., de la Rosa, E., J, Esteve, J., Suárez, T., & Plaza, J.A. (2010). Intracellular silicon chips in living cells. *Small*, Vol. 6, No. 4, (February 2010) pp. (499-502), ISSN: 1613-6829

Hess, H., Fischer, T., Agarwal, A., Katira, P., Finger, I., Mobley, E., Tucker, R., Kerssemakers, J., & Diez, S. (2008). Biomolecular motors challenge imaging and enable sensing. *Progress in biomedical optics and imaging, Proceedings of SPIE, Nanoscale imaging, sensing, and actuation for biomedical applications V*, ISSN: 1605-7422, San Jose, January 2008

Hoshino, T., Kawamori, M., Suzuki, T., Matsui, S., & Mabuchi, K. (2004). Three dimensional and multimaterial microfabrication using focused-ion-beam chemical-vapor deposition and its application to processing nerve electrodes. *Journal of Vacuum Science & Technology B*, Vol. 22, No. 6, (November 2004) pp. (3158-3162), ISSN: 1071-1023

Huang, T.,Kimura, Y., &Yanagimachi, R. (1996). The use of piezo micromanipulation for intracytoplasmic sperm injection of human oocytes. *Journal of Assisted Reproduction and genetics*, Vol. 13, No. 4, pp.(320-328), ISSN: *1573-7330*

Ikeda, T., Mamiya, J., & Yu, Y. (2007). Photomechanics of Liquid-Crystalline Elastomers and Other Polymers. *Angewandte Chemie International Edition*, Vol. 46, No.4 (January 2007), pp. (506 – 528), ISSN: 1521-3773

Ittner, L., & Götz, J. (2007). Pronuclear injection for the production of transgenic mice. *Nature protocols*, Vol 2, No. 5, (April 2007), pp. (1206-1215), ISSN: 1754-2189

Iyer, S., Romanowicz, B., & Laudon, M. (2004). Biomoleculars motors. *Proceedings Nanotech, the Nanotechnology Conference and Trade Show*, Boston, March 2004

Kim, K., Lee, M., Park, H., Kim, J., Kim, S., Chung, H., Choi, K., Kim, I., Seong, B.L., & Kwon, I.C. (2006). Cell-Permeable and Biocompatible Polymeric Nanoparticles for Apoptosis Imaging. *Journal of the American Chemical Society*, Vol. 128, No. 11, (March 2006), pp. (3490-3491), ISSN: 0002-7863

Kishigami, S., Hikichi, T., Van Thuan, N., Ohta, H., Wakayama, S., Bui, H.T., Mizutani, E., & Wakayama, T. (2006). Normal specification of the extraembryonic lineage after somatic nuclear transfer. *FEBS Letters*, Vol. 580, No. 7, (March 2006), pp. (1801-1806), ISSN 0014-5793

Kometani, R., Morita T., Watanabe, K., Kanda K., Haruyama, Y., Kaito, T., Fujita, J., Ishida, M., Ochiai, Y., & Matsui, S. (2003). Nozzle-Nanostructure Fabrication on Glass Capillary by Focused-Ion-Beam Chemical Vapor Deposition and Etching. *Japanese Journal of Applied Physics*, Vol. 42, No. 6B, (February 2003), pp. 4107-4110, ISSN: 0021-4922

Kometani, R., Hoshino, T., Kondo, K., Kanda, K., Haruyama, Y., Kaito, T., Fujita, J., Ishida, M., Ochiai Y., & Matsui, S. (2005). Performance of nanomanipulator fabricated on glass capillary by focused-ion-beam chemical vapor deposition. *Journal of Vacuum Science & Technology B: Microelectronics and Nanometer Structures*, Vol. 23, No. 1, (January/February 2005), pp. (298-301), ISSN 0734-211X

Kometani, R.,Hoshino, T., Kanda, K., Haruyama, Y., Kaito, T., Fujita, J., Ishida, M., Ochiai, Y., & Matsui, S. (2005). Three-dimensional high-performance nano-tools fabricated using focused-ion-beam chemical-vapor-deposition. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, Vol. 232, No. 1-4, (May 2005), pp. (362-366), ISSN 0168-583X

Kometani, R., Hoshino, T., Kondo, K., Kanda, K., Haruyama, Y., Kaito, T., Fujita, J., Ishida, M., Ochiai, Y., & Matsui, S. (2005). Fabrication of nanomanipulator with SiO_2/DLC heterostructure by focused-ion-beam chemical vapor deposition. *Japanese journal of applied physics*, Vol. 44, No. 7B, (July 2005), pp. 5727–5731, ISSN 0021-4922

Kometani, R., Funabiki, R., Hoshino, T., Kanda, K., Haruyama, Y., Kaito, T., Fujita, J., Ochiai, Y., & Matsui, S. (2006). Cell wall cutting tool and nano-net fabrication by FIB-CVD for subcellular operations and analysis, *Microelectronic Engineering*, Vol. 83, No. 4-9, pp. (1642-1645), ISSN: 0167-9317

Kometani, R., Koike, H., Kanda, K., Haruyama, Y., Kaito, T., & Matsui, S. (2007). Evaluation of a Bio Nano-Sensing Probe Fabricated by Focused-Ion-Beam Chemical Vapor Deposition for Single Organelle Analyses. Japanese Journal of Applied Physics, Vol. 46, No. , (December 2007), pp. (7963-7965), ISSN 0021-4922

Lacham-Kaplan, O., & Trounson, A. (1995). Intracytoplasmic sperm injection in mice: increased fertilization and development to term after induction of the acrosome reaction. *Human Reproduction*, Vol. 10, No. 10, (October 1995), pp. (2642-2649), ISSN 0268-1161

Lopez-Martinez, M. J., Caballero, D., Campo, E. M., Perez-Castillejos, R., Errachid, A., Esteve, J., & Plaza, J. A. (2008). FIB assisted technology in sub-picolitre micro-dispenser fabrication. *Journal of Micromechanics and Microengineering*, Vol. 18, No. 7, (July 2008), 075021, ISSN 1361-6439

Lopez-Martinez, M.J. , Campo, E. M., Caballero, D., Fernandez, E., Errachid, A., Esteve, J., & Plaza, J.A. (2009). Versatile micropipette technology based on Deep Reactive ion Etching and anodic bonding for biological applications. *Journal of Micromechanics and Microengineering*, Vol. 19, No. 10 (October 2009), 105013, ISSN 1361-6439

Maas, D., van Veldhoven, E., Chen, P., Sidorkin, V., Salemink, H., van der Grift, E, & Alkemade, P. (2010). Nanofabrication with a Helium Ion Microscope. *Proceedings of SPIE, the International Society for Optical Engineering, Scanning Microscopy*. Vol. 7638, ISSN 0277-786X , Monterey, May 2009

Malboubi, M., Ostadi, H., Wang, S., Gu, Y., & Jiang, K. (2009). The Effect of Pipette Tip Roughness on Giga-seal Formation. *Proceedings of the World Congress on Engineering 2009 Vol II WCE 2009*, ISBN: 978-988-18210-1-0 London, U.K. July, 2009

Morita, T., Kometani, R., Watanabe, K., Kanda, K., Haruyama, Y., Hoshino, T., Kondo, K., Kaito, T., Ichihashi, T., Fujita, J., Ishida, M., Ochiai, Y., Tajima, T., & Matsui, S. (2003). Free-space-wiring fabrication in nano-space by focused-ion-beam chemical vapor deposition. *Journal of Vacuum Science & Technology B*, Vol. 21, No. 6, (November 2003), pp. (2737-2741), ISSN: 1071-1023

Mirakami, M., Cabral, H., Matsumoto, Y., Wu, S., 3 Kano, M.R., Yamori, T. , Nishiyama, N., Kataoka, K. (2011) Improving Drug Potency and Efficacy by Nanocarrier-Mediated Subcellular Targeting. Science of Translational Medicine 3, 64ra2 , ISSN: 1946-6242

Nagy, A. , Gertsenstein, M., Vintersten, K., & Behringer, R. (2002) *Manipulating the Mouse Embryo: A laboratory Manual*, 3rd ed, Cold Spring Harbor Laboratoy Press, ISBN 978-087969591-0, New York

Nishiyama, N. (2007). Nanocarriers shape up for long life. *Nature Nanotechnology*, Vol. 2 (April 2007), pp. 203-204, ISSN: 1748-3387

Ostadi, H., Malboubi, M., Prewett, P.D., & Jiang, K. (2009). *Microelectronic Engineering*. 3D reconstruction of a micro pipette tip, Vol. 86, No. 4-6, (April-June 2009), pp. (868-870), ISSN: 0167-9317

Palermo, G. , Joris, H., Camus, M., Devroey, P., & Van Steirteghem, A. C. (1992). Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, Vol. 4, No. 340, (July 1992), pp. 8810-8817, ISSN 0140-6736

Pillarisetti, A., Pekarev, M., Brooks, A.D., & Desai, J.P. (2007) "Evaluating the Effect of Force Feedback in Cell Injection," *IEEE Transactions on Automation Science and Engineering*, vol. 4(3), pp. (322-331), ISSN: 1545-5955

Postek, M., Vladár, A.E., Kramar,J., Stern, L.A., Notte, J., & McVey, S. (2007) "The helium ion microscope: a new tool for nanomanufacturing", . *Proceedings of SPIE, the International Society for Optical Engineering, Instrumentation Metrology and Standards for nanomanufacturing*, Vol. 6648 (2007)

Schrlau, M., Brailoiu, E., Patel, S., Gogotsi, Y., Dun. J., & Bau, H.H. (2008). Carbon nanopipettes characterize calcium release pathways in breast cancer cells. *Nanotechnology*, Vol. 19, No. 32, (July 2008), 325102, ISSN 1361-6528

Scipioni, L., Sanford, C., Notte, J., Thompson, B.,& McVey, S. (2009). Understanding imaging modes in the helium ion microscope. *Journal of Vacuum Science and Technology B*, Vol. 76, No. 6 (November-December 2009), pp. (3250-3255), ISSN 1520-8567

Shenoy, D.K., Thomsen III, D.L., Srinivasanb, A., Kellerc, P., & Ratna, B.R. (2002). Carbon coated liquid crystal elastomer film for artificial muscle applications. *Sensors and Actuators A: Physical*, Vol. 96, No. 2-3, (February 2002), pp. (184-188), ISSN: 0924-424

Sutter Instruments. (2008). P-97 Pipette cookbook (Rev. D). Retrieved from www.sutter.com

Utke, I., Hoffmann, P., Melngailis, J. (2008). Gas-assisted focused electron beam and ion beam processing and fabrication. *J Vac. Sci. Technol. B* 26, pp.(1197-1272), ISSN ISSN 0734-211X

Verpoorte, E., & De Rooig, N.F. (2003). Microfluidics meets MEMS. *Proceedings of the IEEE*, Vol. 91, No. 6 (June 2003), pp. (930 - 953), ISSN: 0018-9219

Voldman, J., Gray, M.L.,& Schmidt, M.A. (1999) Microfabrication in Biology and Medicine. *Annual Review of Biomedical Engineering*, Vol. 01, (August 1999), pp. (401–425), *ISSN*:1523-9829

Walter, E., Dreher, D., Kok, M., Thiele, L., Kiama, S.G., Gehr, P., & Merkle, H.P. (2001). Hydrophilic poly(DL-lactide-co-glycolide) microspheres for the delivery of DNA to human-derived macrophages and dendritic cells. *Journal of Controlled Release*, Vol. 76, No.1-2, (Setember 2001), pp. (149–168), ISSN: 0168-3659

Weibel, D.B., DiLuzio, W.R., & Whitesides, G.M. (2007). Microfabrication meets microbiology. *Nature Reviews Microbiology*, Vol. 5, No. 3, (March 2007), pp. (209-218), ISSN: 1740-1526

Yaul, M., Bhatti, R., & Lawrence, S. (2008) Evaluating the process of polishing borosilicate glass capillaries used for fabrication of in - vitro fertilization (iVF) micro-pipettes. *Biomedical Microdevices*, Vol. 10, No. 1, (February 2008), pp. (123-128), ISSN: 1387-2176

Yoshida, N., & Perry, A. C. F. (2007). Piezo-actuated mouse intracytoplasmic sperm injection (ICSI). *Nature Protocols*, Vol. 2, No. 2, (March 2007) pp. (296-304), ISSN 1754-2189
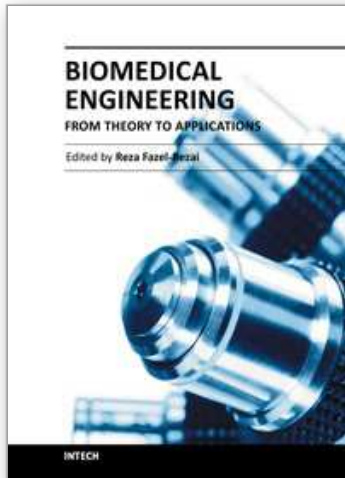
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In all different areas in biomedical engineering, the ultimate objectives in research and education are to improve the quality life, reduce the impact of disease on the everyday life of individuals, and provide an appropriate infrastructure to promote and enhance the interaction of biomedical engineering researchers. This book is prepared in two volumes to introduce a recent advances in different areas of biomedical engineering such as biomaterials, cellular engineering, biomedical devices, nanotechnology, and biomechanics. It is hoped that both of the volumes will bring more awareness about the biomedical engineering field and help in completing or establishing new research areas in biomedical engineering.

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