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High content analysis of the biocompatibility of nickel nanowires

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ABSTRACT

Nickel nanowires, 20 μm long and 200 nm in diameter, were fabricated by electrodeposition into alumina templates, and characterised by superconducting quantum interference device (SQUID) magnetometer, X-ray diffraction and scanning electron microscopy. Biocompatibility studies of nickel nanowires with differentiated THP-1 cell line-derived macrophages were carried out. From a multiparametric assay, using high content analysis (HCA), the critical time points and concentrations of nickel nanowires on THP-1 cellular response were identified. The nanowires displayed little or no toxic effects on THP-1 cells over short incubation times (10 h), and at low concentrations (<100 nanowires per cell). Our findings indicate the potential suitability of these wires for biological and clinical applications.

The interaction between nanomaterials and biological specimens has been attracting much interest recently due to their potential use in biomedical and industrial applications, but information regarding their toxicity is limited. The ability to use nanowires and nanoparticles as carriers for various antibodies and fluorescent labels, thanks to advances in surface coordination chemistry, has enabled their successful use in drug delivery and biosensing [1,2]. The magnetic properties of these nanomaterials have also allowed their application in cell separation and manipulation. Meyer et al. first demonstrated the potential use of magnetic nickel nanowires for these applications [3]. They observed that fluorescent and non-fluorescent wires orientated in a head-to-tail configuration when a small magnetic field was applied, whereas magnetic nanoparticles ordered in close packed arrays. The separation efficiency of ferromagnetic nickel nanowires compared to commercially available superparamagnetic beads has also been investigated [4]. It was shown that nanowires outperformed magnetic beads by a factor of two. This was attributed to the larger magnetic moment and larger surface area of the wires. The large remnant magnetisation exhibited by nanowires enables their use in low-field environments, thus reducing strain effects experienced by cells and maximising their possible applications [5].

On the other hand, with advances in fabrication, functionalisation and applications of nanowires and nanoparticles there is a pressing need to understand their possible cytotoxic effects. Previous studies have been carried out to examine the cytotoxicity of nickel nanowires *in vitro*. After a 24 h incubation period at a low number of nanowires to cells (ratio 1:3), Reich et al. reported a non-toxic effect on mouse fibroblast cells after internalisation of the wires [6–8]. This may have been due to the low number of nanowires binding to individual cells. Prina-Mello et al. demonstrated the internalisation of nickel nanowires by rat marrow stromal cells (MSC), MC3T3-E1 osteoblast cells and UMR-106 osteosarcoma cells [9]. It was reported that the cell survival rate was greater than 95% up to 5 days after internalisation. This indicated that nickel nanowires could be used for various biological applications with no disruption to the cellular growth cycle.

Fabricating metal wires by electrodeposition into templates with nanometre diameters was first demonstrated in the late 1960s [10]. It provides a method of producing large quantities of nanowires with desired dimensions. One of the advantages of using electrodeposition is that the wires are always continuous. Continuity is guaranteed because if any breakage in the nanowire occurs, then conductivity will be lost and growth of that particular nanowire will stop [11]. The fabrication of multisegmented nanowires composed of multiple materials along the wire with various segment lengths enables the specific functionalisation of different receptors of interest onto a single nanowire [12]. They have an advantage over magnetic beads, in that the precise location of molecular markers can be positioned on particular segments of the nanowire.

High content analysis (HCA) has proven to be a powerful tool for the evaluation of nanoparticle interactions with *in vitro* cellular systems of different origin. It is a fully automated system

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that allows for a quantitative and qualitative study of cellular properties in a fast and high-throughput manner. HCA approach enables cellular signaling and morphology of cells to be automatically analysed using fluorescent-based reagents.

Recently, HCA has been employed to study the cellular effect of silica-coated quantum dots (QDs) [13]. The use of this tool enabled a comprehensive analysis of the multiple cellular features indicating possible cytotoxicity of QDs. Recent data demonstrated the need to understand not only the possible toxic effects of QDs but also the mechanisms of their interaction with cells [14,15]. With the aid of HCA detailed studies of different QDs with various cell lineages provided a quantitative analysis of their toxic effects and localisation within cellular compartment [14,16].

In this paper, we report the synthesis and characterisation of magnetic nickel nanowires fabricated by electrodeposition. We also introduce the concept of HCA as a new technique to examine the possible cytotoxic effects of nickel nanowires *in vitro*. We have chosen the model of human phagocytic cells differentiated from the THP-1 cell line since macrophages represent the first line of immunodefence against artificially engineered nanomaterials in the human body. HCA is a reliable and unbiased system for examining the cytotoxicity of nanoparticles *in vitro* in a fast and standardised method. However, to date no study has been implemented using a high-throughput method to examine the multiple parameters indicating the possible cytotoxicity of nanowires *in vitro*. A unified screening approach is essential for the future role of nanomaterials in biomedical applications due to their varying size, shape, chemical composition and surface functionalisation.

1. Nanowire fabrication

The experimental set-up for electrodeposition involved an electrolyte bath containing 2 M $NiSO_4 \cdot 6H_2O$ with 0.6 M H_3BO_3 and a three-electrode cell with a platinum counter electrode and an Ag/AgCl (saturated KCl) reference electrode. Anopore inorganic alumina membranes (Anodisc 25; Whatman, UK), 20 mm in diameter and 60 μm thick, with 200 nm parallel pores served as the working electrode. Firstly, a gold layer, a few hundred nanometres in thickness, was sputtered onto the back of the membrane to provide a conducting substrate. Nickel was then deposited into the pores of the membrane at a potential of −0.9 to −1.0 V relative to the reference electrode. Electrons from the conducting layer reduced the nickel ions to metallic form resulting in the growth of nickel nanowires within the pores. The length of these wires could easily be controlled by altering the deposition time.

The nickel nanowires were removed from the template by dissolving the alumina membrane in 1 M NaOH. The solution was heated to 40 °C for 5 min and then sonicated in an ultrasonic bath (Grant XB3; UK) for 1 h. The NaOH solution was removed and replaced several times with fresh 1 M NaOH to ensure the membrane was completely dissolved and the nickel nanowires were liberated. Finally, the nickel nanowire stock sample was washed multiple times and resuspended in deionised water by centrifugation in order to remove the last traces of NaOH. The pH was checked to confirm NaOH had been completely removed before proceeding.

2. Sample characterisation

The nanowire samples were characterised using a variety of techniques in order to determine their overall dimensions, magnetic properties, crystallographic structure and concentration. Dimensions of the nanowires were examined using a scanning electron microscopy (SEM; Carl Zeiss EVO-50) operating at 10 kV. From Fig. 1 it can be seen that nickel nanowires had a diameter of 200 ± 20 nm with an average length of 20 ± 2 μm and were relatively uniform in length.

Powder X-ray diffraction (XRD) was used to determine the crystallographic structure of the sample. A Philips X'Pert Diffractometer system was used with a copper X-ray tube operating at 40 kV and 40 mA. The wavelength of the strongest Cu radiation (Kα) was 1.5406 Å with a corresponding energy of 8.047 keV. A few milligrams of dried nanowire powder were placed on a PW3064 sample spinner and data were collected in a step scan mode from 30° to 100° at a step size of 0.008 and a rate of 120 s per step using an X'Celerator detector. The scan data were then analysed by comparing the peak positions and relative intensities with standard data on nickel provided by the International Centre for Diffraction Data (ICDD). Fig. 2 shows the XRD pattern of the nickel nanowires. The sample had a face-centered cubic (FCC) structure with a lattice parameter a_0 of 0.353 nm. There was some evidence of (111) texture.

Magnetisation measurements on the nanowire sample were carried out at room temperature using a Quantum Design MPMS

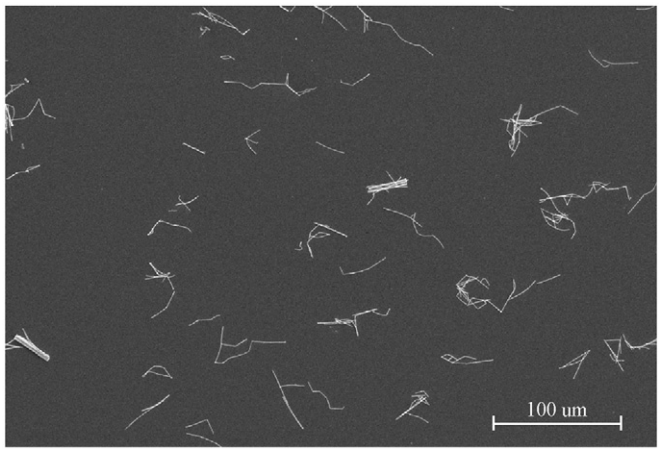

Fig. 1. SEM image of nickel nanowires with a diameter of 200 ± 20 nm and an average length of 20 ± 2 μm. Scale bar 100 μm.

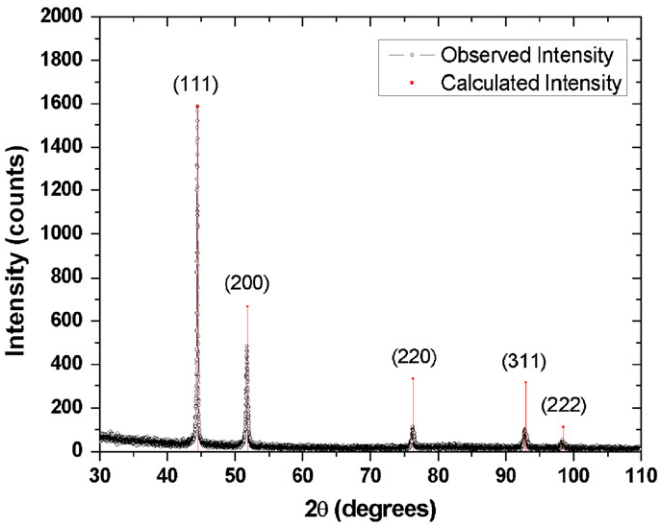

Fig. 2. X-ray diffraction pattern of nickel nanowire powder.

XL superconducting quantum interference device (SQUID) magnetometer with applied magnetic fields up to 5 T. From Fig. 3, the saturation magnetisation was found to be 40 $Am^2 kg^{-1}$, which is significantly less than the expected value for bulk nickel (55.4 $Am^2 kg^{-1}$). A likely source of this discrepancy is due to the oxidation of the surface layer of nickel when exposed to NaOH during removal of the nanowires from the alumina template. A surface coating of oxide, approximately 15 nm thick, can explain this reduced magnetisation. The nanowires also exhibited a coercive field of 48 mT and a remnant magnetisation of 0.26 $Am^2 kg^{-1}$. The average magnetic moment per wire was 2.2×10^{-13} Am^2.

A novel technique was developed using Nomarski imaging, also known as differential interference contrast (DIC) imaging, to determine the concentration of nanowires defined as number of nanowires per millilitre (NW mL^{-1}) rather then conventional method of nanowire mass per millilitre (ng mL^{-1}). This was performed in order to quantify the number of nanowires affecting cellular response in cytotoxicity experiments. Nomarski imaging is a type of phase contrast microscopy where an image is produced from refractive index inhomogenities in the sample rather than absorption inhomogenities. Light is passed through the sample, which is made up of elements whose refractive indices differ. As a result, the phase of the wavefront is altered. A contrast image is produced by converting these phase changes into amplitude changes. The procedure involved pipetting a drop of nickel nanowire sample onto an Si/SiO_2 wafer and visualising it under a microscope (Nikon Eclipse LV series; UK) containing a DIC prism positioned between two crossed polarisers. Fig. 4 shows the comparative analysis between counting the dispersed nickel nanowires on the wafer using a tally counter versus using a defined algorithm on the Image-Pro software (Media Cybernetics, UK). Data from the Image-Pro software, within a predefined object range, demonstrated the accuracy of nanowire measurement using a tally counter. Both methods enabled the total number of nanowires per millilitre (NW mL^{-1}) to be determined.

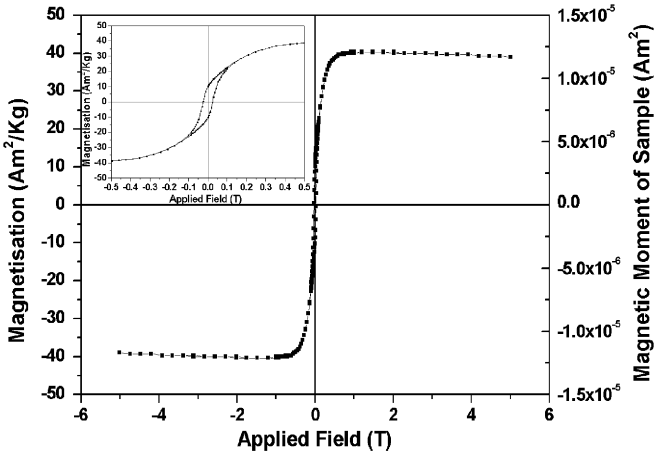

Fig. 3. SQUID magnetisation curve measured at room temperature for nickel nanowires in a diamagnetic sample holder.

3. Cell culture and high content analysis

To examine the effect of nickel nanowires on cellular response, THP-1 suspension cells were used. They are a human monocytic leukemia cell line with a diameter of 40 μm. These cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 2 mM L^{-1} L-glutamine and 100 μg mL^{-1} penicillin–streptomycin (Sigma-Aldrich), to inhibit bacterial contamination. Cells were cultured in T75 tissue culture flasks and incubated at 37 °C and 5% CO_2 until highly confluent. In order to seed THP-1 cells in 96-well, flat-bottom plates (Nunc, USA), they were first stimulated with phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich). This chemical induces cells to differentiate into adherent macrophages and stop their natural proliferation. THP-1 cells and 25 ng PMA mL^{-1} in RPMI media were dispensed at 200 μL/well into desired wells of the 96-well plate using a Matrix WellMate (Thermo Fisher Scientific, USA). This improved the accuracy of cell plating and ensured cell viability due to quick dispensing speed. Two hundred microlitre RPMI 1640 media were also dispensed into the outer wells of the plate to prevent edge effects occurring. Plates were incubated for 72 h in the above conditions to allow THP-1 cells to adhere to the bottom of the wells.

Once THP-1 cells had successfully adhered to the wells, nickel nanowire samples could be plated at various concentrations ranging from 10 nanowires to every cell (10:1) to 500 nanowires to every cell (500:1). To accurately plate the various ratios of nanowires to cells, an initial reference cell count was carried on specific wells on all 96-well plates. This was achieved by incubating 1 μg mL^{-1} Hoechst 33342 fluorescent dye (Sigma-Aldrich) for 30 min at 37 °C and 5% CO_2 that stained and labelled DNA and in turn made it possible to visualise the nuclei of the viable cells. Plates were then read on HCS KineticScan Reader (KSR) (Thermo Fisher Scientific, USA) where the cells were exposed to a UV lamp for approximately 30 ms in a controlled environment to maintain cell viability. The cell count was

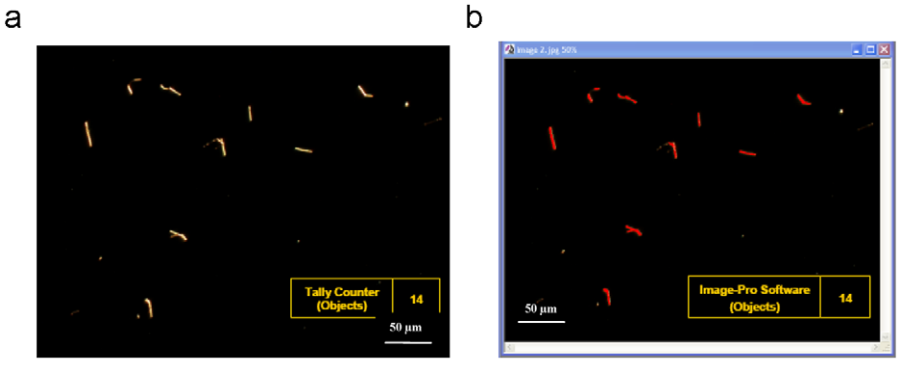

Fig. 4. Nomarski images of nickel nanowires taken to determine sample concentration using (a) a tally counter and (b) a defined algorithm on Image-Pro software. Scale bar 50 μm.

evaluated from the output data and thus the nanowire to cell concentration was calculated ahead of the plating step procedure.

The nickel nanowire solution was sonicated for approximately 1 h to obtain a uniform dispersion. The nanowire sample was pipetted multiple times with THP-1 cells in each of the wells to ensure a homogeneous mixture. Each of the nanowire to cell ratios was repeated in triplicate to improve the accuracy of the experiment. Negative controls of THP-1 cells plated with no nickel nanowires and a positive control of THP-1 cells plated with 1 M $NiSO_4$ solution were also included in triplicate. This concentration of $NiSO_4$ was chosen to sufficiently induce cell death. To thoroughly examine the effect of nickel nanowires on THP-1 cells a time course study was also implemented. Nickel nanowires were incubated with cells for a few hours up to a day in standard conditions to evaluate the time dependence of wires on cell viability.

An appropriate assay for high content analysis was selected to examine the main factors indicating toxicity. The multiparameter cytotoxicity 1 HitKit (Cellomics, PA, USA) is an *in vitro* assay that allows changes in many cellular properties to be examined simultaneously using a number of different fluorescent dyes. These properties include (1) cell viability, (2) nuclear morphology and size, (3) cell membrane permeability and (4) lysosomal mass–pH. After the desired exposure time of THP-1 cells to nickel nanowires, each well was stained appropriately with the HitKit, as indicated in our previous work with slight modifications [14–16]. Plates were then read on the HCS KSR using three detection channels with different excitation filters. These included a DAPI filter, which detected a blue fluorescence indicating nuclear intensity at a wavelength of 461 nm; FITC filter, which detected a green fluorescence indicating cell permeability at a wavelength of 509 nm and a TRITC filter, which detected lysosomal mass and pH changes with a red fluorescence at a wavelength of 599 nm. Once the scan was completed, data were retrieved and analysed to obtain information about the cellular properties.

A typical HCA output image can be seen in Fig. 5. Cell viability and nuclear size/morphology are indicated by a blue fluorescence, cell membrane permeability by a green fluorescence and lysosomal mass and pH with a red fluorescence. A specific algorithm was written in order to extract the quantitative data, shown in Fig. 6, from these images.

The results of the cytotoxicity studies are summarised in Fig. 6. Data are represented as mean values normalised relative to the untreated control cells ± standard deviation for three replicates ($n = 3$). THP-1 cell viability can be seen in Fig. 6a while cell membrane permeability is indicated in Fig. 6b. Nuclear morphology/size and changes in lysosomal mass–pH were also investigated (data not shown). Nickel nanowires appear to have no considerable effect on THP-1 cellular response after incubation times of 3 and 6 h regardless of the nanowire concentration. Cell viability decreased by less than 30% for 500 nanowires plated to every cell. Membrane permeability showed a slight increase in intensity after these time points. After incubation periods of 15 and 24 h, THP-1 cells remained viable with a marginal decrease in cell count for 100 nanowires per cell (Fig. 6a). However, the lethal dose concentration occurred at 500 nanowires per cell when there was a 50% loss in cell viability for both 15 and 24 h time points. This evidence was supported by a dramatic increase in membrane permeability intensity indicating the cell membrane had been compromised (Fig. 6b). The intensity increased due to a large volume of fluorescent dye entering the cell's nucleus, often associated with an ongoing apoptotic response. The natural phagocytic behaviour of THP-1 cells induced by prolonged exposure to the nanowires and their affinity to bind to oxidised

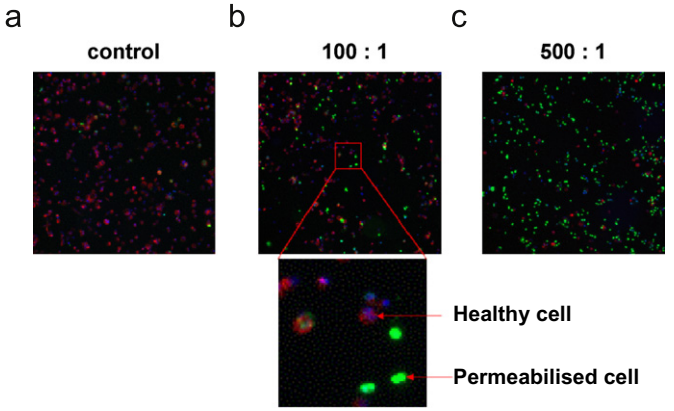

Fig. 5. HCA images of nickel nanowires plated with THP-1 cells for 24 h. Images show THP-1 cells plated with (a) no nickel nanowires i.e. negative control, (b) 100 nanowires per cell and (c) 500 nanowires per cell. An increase in cell membrane permeability, green fluorescence can be seen with increasing concentration of nanowires. This is accompanied by a decrease in both blue and red fluorescence, indicating cell viability and the number of lysosomes present, respectively. These changes in fluorescence intensity are indicative of cell death.

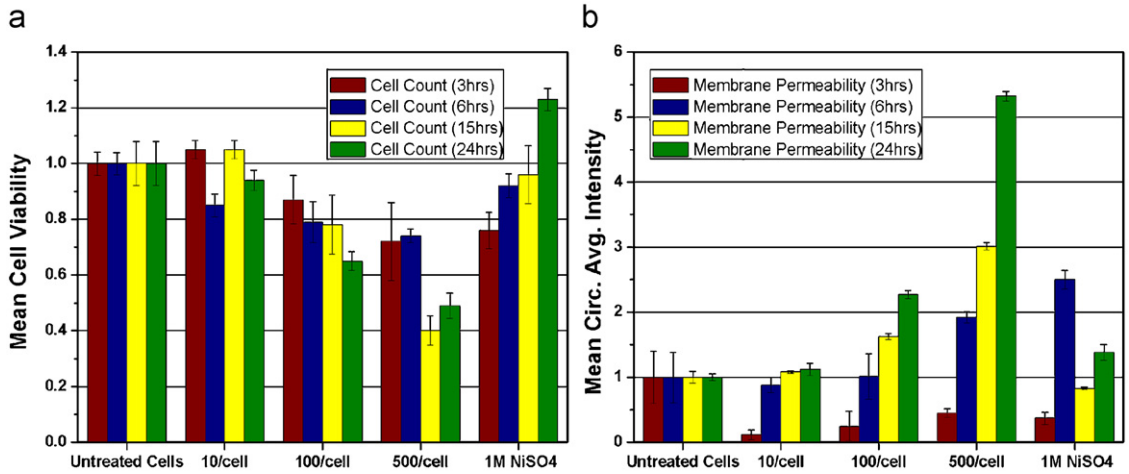

Fig. 6. Investigation of possible cytotoxicity effects of nickel nanowires on (a) cell viability and (b) cell membrane permeability. The dose and time dependence of nickel nanowires on THP-1 cellular response is shown.

hydrophilic surfaces, via integrins, resulted in reduced cell counts and membrane permeabilisation. Whereas, an increase in cell count for the 1 M $NiSO_4$ positive control is due to the possible fragmentation of the cells.

4. Conclusions

The results of this high content analysis study established that there was a time and concentration dependence of nickel nanowires on THP-1 cellular response. THP-1 cells remained viable for 24 h with no significant decrease in cell count or affect on cellular membrane for a concentration of 10 nanowires plated to every cell. It was also shown that THP-1 cells remain viable for periods in the order of 10 h even after ingesting surface-oxidised nickel wires at concentrations up to 100 nanowires per cell in the culture media. These results point the way to the application of nickel nanowires for *in vitro* magnetic manipulation and labelling of macrophage cells for future biological and clinical diagnostic applications, including cell manipulation and separation.

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References

[1] K.K. Jain, Drug Discovery Today 10 (2005) 1435.
[2] N.L. Rosi, C.A. Mirkin, Chem. Rev. 105 (2005) 1547.
[3] M. Tanase, L.A. Bauer, A. Hultgren, et al., Nano Lett. 1 (2001) 155.
[4] A. Hultgren, M. Tanase, C.S. Chen, et al., IEEE Trans. Mag. 40 (2004) 2988.
[5] D.H. Reich, M. Tanase, A. Hultgren, et al., J. Appl. Phys. 93 (2003) 7275.
[6] A. Hultgren, M. Tanase, C.S. Chen, et al., J. Appl. Phys. 93 (2003) 7554.
[7] A. Hultgren, M. Tanase, E.J. Felton, et al., Biotechnol. Prog. 21 (2005) 509.
[8] M. Tanase, E.J. Felton, D.S. Gray, et al., Lab Chip 5 (2005) 598.
[9] A. Prina-Mello, Z. Diao, J.M.D. Coey, J. Nanobiotechnol. 4 (2006).
[10] G.E. Possin, Rev. Sci. Instrum. 41 (1970) 772.
[11] K. Nielsch, F. Müller, A.P. Li, et al., Adv. Mater. 12 (2000) 582.
[12] L.A. Bauer, D.H. Reich, G.J. Meyer, ACS Langmuir 19 (2003) 7043.
[13] T. Zhang, J.L. Stilwell, D. Gerion, et al., Nano Lett. 6 (2006) 800.
[14] S.J. Byrne, Y. Williams, A. Davies, et al., Small 3 (2007) 1152.
[15] I. Nabiev, S. Mitchell, A. Davies, et al., Nano Lett. 7 (2007) 3452.
[16] E. Jan, S.J. Byrne, M. Cuddihy, et al., ACS Nano. 2 (2008) 928.