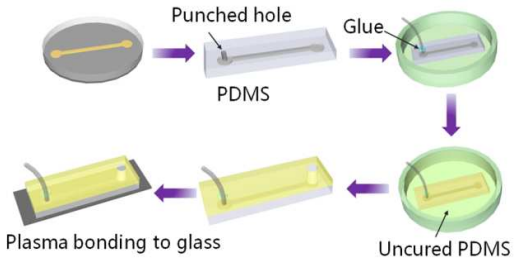
Lab on a Chip

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/loc

A simple but robust PDMS tubing method is used for controlled synthesis of polymeric nanoparticles.



Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

TECHNICAL INNOVATION

A Microfluidic Tubing Method and Its Application to Controlled Synthesis of Polymeric Nanoparticles

Jidong Wang,[‡a] Wenwen Chen,[‡a] Jiashu Sun,[*a] Chao Liu,[b] Qifang Yin,[b] Lu Zhang,[a] Yunlei Xianyu,[a] Xinghua Shi,[b] Guoqing Hu,[*b] and Xingyu Jiang[*a]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

This report describes a straightforward but robust tubing method for connecting polydimethylsiloxane (PDMS) microfluidic devices to external equipments. The interconnection is irreversible and can sustain a pressure up to 4.5 MPa that is characterized experimentally and theoretically. To demonstrate applications of this high-pressure tubing technique, we fabricate a semicircular microfluidic channel to implement a high-throughput, size-controlled synthesis of poly(lactic-co-glycolic acid) (PLGA) nanoparticles ranging from 55 to 135 nm in diameter. This microfluidic device allows for a total flow rate of 410 mL/hr, resulting in an enhanced convective mixing which can be utilized to precipitate small size nanoparticles with a good dispersion. We expect that this tubing technique would be widely used in microfluidic chips for nanoparticle synthesis, cell manipulation, and potentially nanofluidic applications.

Microfluidic systems are emerging as important tools for chemical and biological applications such as diagnostics and screening.[1-4] Since the invention of microfluidic technologies, polydimethylsiloxane (PDMS) has become one of the most used materials.[5] PDMS-based microfluidic devices provide many benefits, including rapid prototyping, low cost, optically transparent, and easy to bond with different substrates.[6, 7] To commercialize these microfluidic systems, most devices require a reliable packaging method. One of the major challenges for packaging is to establish multiple fluidic interconnections from chips to the macroscopic world.[8]

There are basically three kinds of approaches to make interconnections: 1) directly insert tubes into the ports of chip, 2) glue connectors such as tubes, tips or capillaries on the chip surface above the ports, and 3) use rubber O-rings, screws or port clamps to create press-fit connections.[9, 10] The first two methods are simple and straightforward, but suffer from some drawbacks like fluid leakage from interconnection at high pressure, channel clogging by adhesives, and large dead volume.[7] The press-fit interconnections always require additional components such as holders and clamps, and lack of flexible adoption.[11, 12] Recently, many efforts have been made to design and fabricate standardized, plug-and-play, and multichannel interfaces for microfluidic devices.[8, 10, 13] One aim of these chip-to-world interfaces is to automatically introduce different reagents and samples into microfluidic devices for chemical or biological anaylsis.[2]

Since most microfluidic systems take advantage of the small length scales and low Reynolds numbers, the interconnections only need to sustain a pressure of several atmospheres.[10] Typically, the press-fit connections can support a maximum pressure of around 1.5 Mpa.[14] The glued tubing interconnection can sustain an inlet pressure of 600 kPa.[6] Nevertheless, high-pressure or high-velocity operation inside microfluidic devices would lead to unique flow phenomena and can induce additional benefits, thus endowing microfluidics with new characteristics. In our previous studies, we demonstrated a rapid and continuous tumor cell separation from blood cells based on hydrodynamic inertial effect that is manifest only at a relatively high velocity.[15, 16] In addition, microfluidic-based reactions under high pressure may significantly increase the rates of thermal and mass transfer, thus enabling aggressive reaction conditions and high-throughput production. For example, the high inlet flow pressure and the high pressure capacity of tubing interconnection are required to increase the yield of nanoparticles synthesized in microfluidic platforms.

Here we present a simple and flexible tubing method to connect PDMS chips to external equipments like syringe pumps. Microfluidic channels are fabricated using standard soft-lithography techniques with SU8 master mold on a silicon substrate.[4] The microfluidic chip for leakage tests has a straight channel with dimensions of 50 μm wide (or 300 μm wide), 50 μm deep, and 6 cm long (Fig. 1a). The semicircular channel for preparing nanoparticles is 300 μm wide, 50 μm deep with a total length of 5 cm (Fig. 3). Degassed PDMS (mixed in a 10:1 ratio of PDMS base with curing agent, Sylgard 184, Dow Corning Inc.) is poured over the mold, and baked at 80 °C for 2 hr in an oven. The PDMS slab has a thickness of around 5 mm, which is removed from the silicon substrate by a razor blade (Fig. 1a).

After obtaining the PDMS slab, we use a flat-tipped needle (inner diameter of the needle is 0.5 mm, outer diameter is 0.8 mm) with sharpened outer edge to carefully core a hole of 0.5 mm in diameter at the channel inlet. We placed the PDMS chip onto a new Petri dish, and gently pressed it to ensure complete contact between embedded microchannel and dish surface. Before

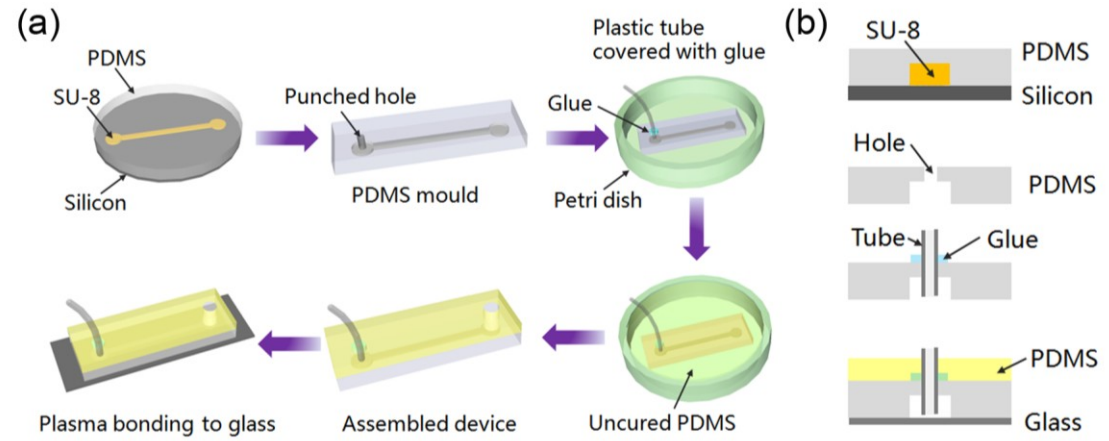


Fig.1 Schematics of fabricating tubing interconnection for PDMS microfluidic devices. (a) PDMS chip with a cored port is laid on a new Petri dish, followed by inserting a plastic tube covered with a small amount of glue at the end. An uncured layer of PDMS is poured on top of the PDMS, and baked to solidify. The assembled PDMS chip with tubing is bonded to a glass slide by plasma treatment. (b) The cross-sectional illustration of fabrication of tubing interconnection. The fabrication process of tubing interconnection for both the straight and the semicircular microchannel is the same.

inserting a plastic tube (inner diameter of 0.5 mm, outer diameter of 1 mm) into the hole, we smear a small amount of adhesive sealant (Dow Corning® 3145 RTV) that is 2mm above the beveled end of tube (Fig. 1b, and Fig. S1). To secure this tubing interconnection, we pour another layer of uncured PDMS on top of the PDMS chip to totally cover the interconnection, and bake the whole device inside the Petri dish at 80 °C for 2 hr. The thickness of this two-layer PDMS device is 8 mm. The assembled PDMS chip with tubing is finally bonded to a glass slide by plasma treatment (Fig. 2). Although we use the adhesive in our tubing system, the clogging of microchannels and contamination of sealant are not evolved because: 1) The smeared adhesive is above the beveled end of tube before inserting the tube into the PDMS port (Fig. S1); 2) The adhesive will remain on the PDMS surface after inserting the tubing into the port as the diameter of soft PDMS port is smaller than that of rigid tube (Fig. S1); 3) The adhesive used here is a non-flowing adhesive with high tensile strength that won't flow through the small gap between the plastic tube and the PDMS port during solidification.

To characterize the ability of this tubing interconnection to withstand pressure, we perform the leakage test. After fabrication of interconnection, a syringe pump (PHD Ultra, Harvard Apparatus) is applied to infuse colored water through a 5-mL syringe into the microfluidic channel (50 µm × 50 µm cross-section). The flow rates can be adjusted by the syringe pump, and we use six flow rates (10 mL/hr, 20mL/hr, 30 mL/hr, 40 mL/hr, 50mL/hr and 60 mL/hr) to characterize the performance of the interconnection. Each flow rate is kept constant until the 5 mL water is run out or the interconnection is burst. Six microfluidic chips with the same dimension are made for repetition. We also fabricate another straight microchannel that is 300 µm wide, 50 µm high, and 6 cm long to test the burst pressure of interconnection. The burst process is recorded by a digital camera for the straight channel of 300 µm wide × 50 µm high × 6 cm long (Nikon).

Because PDMS is an elastic material, a high flow rate will lead to channel deformation, thus affecting the pressure drop and flow profile inside the channel. The tendency of channel deformation increases with decreasing the aspect ratio of microchannel (height/width).[17] To characterize the performance of tubing interconnection, we use a straight channel (6 cm long) with square cross section of 50 µm × 50 µm to minimize the effect of channel deformation. The hydraulic resistance (R_{hyd}) for straight channel with square cross-sectional shape is given by:[18]

$$R_{hyd} = 28.4\eta L\frac{1}{h^4} \quad (1)$$

where η is the viscosity of the fluid, L and h are the length and height of microchannel, respectively. The hydraulic resistance of our channel is 2.73×10^{14} Pa·s/m^3. The pressure drop (Δp) across this channel at a constant flow rate (Q) is:

$$\Delta p = R_{hyd}Q \quad (2)$$

When we use a syringe pump to constantly inject colored water into the microchannel, the pressure at the interconnection can be calculated by Eq. (2). Up to a flow rate of 50 mL/hr (Re = 278), there is no leakage from the tubing interconnection or the bonding interface for 6 min. However, if we increase the flow rate to 60 mL/hr (Re = 333), the tubing interconnection is burst within a minute. This flow rate will impose a pressure of 4.5 Mpa at the inlet, which is also confirmed by numerical simulation (Fig. S2).

Unlike most chips where the leakage is either from the tubing interface or the bonding interface between PDMS and glass, the burst of our interconnection is from the interior of PDMS under a high pressure (Fig. 2). We make six microfluidic channels (50 µm wide × 50 µm high × 6 cm long) by the same tubing technique for testing, and all of them burst at 60 mL/hr. We also note that the explosion of PDMS takes place around the inlet PDMS port, and results in a tilted crack (Fig. 2). In comparison, we make a glued tubing interconnection without the second layer of PDMS for the same microchannel, and perform the leakage test. Experimental observation indicates that the colored water leaks from the gap between plastic tube and the PDMS port at a low flow rate of 10 mL/hr, corresponding to an imposed pressure of 0.75 Mpa (Fig. S3).

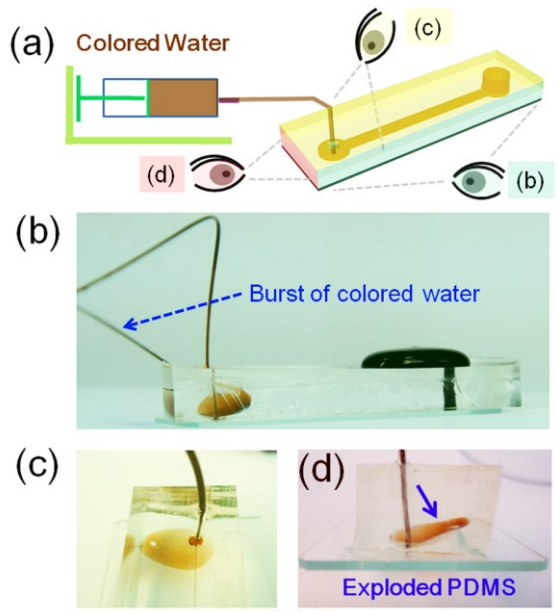


Fig.2 Leakage tests of tubing interconnection for the straight microchannel. (a) Schematics of experimental set-up. The microfluidic channel is connected to a syringe pump through tubing interconnection. (b) The snapshot of colored water bursting at the inlet interconnection of microchannel (300 μm wide × 50 μm high × 6 cm long) at 800 mL/hr. (c), (d) Photography of tubing interconnection after leakage at different camera angles. The small arrow indicates the interstice of exploded PDMS.

Another straight microfluidic channel of 300 μm wide, 50 μm high, and 6 cm long is also used to test the pressure capability of our tubing interconnection. The hydraulic resistance ($R_{hyd,\,r}$) for straight channel with rectangular cross section is:[18]

$$R_{hyd,r} = \frac{12\eta L}{1-0.63(h/w)}\frac{1}{h^3 w} \qquad (3)$$

where w is the width of microchannel. By using this equation, we can obtain the channel resistance of 2.15×10^{13} Pa·s/m^3. This interconnection can support an inlet flow rate of 750 mL/hr for 1 min, and get burst from the interior of PDMS as we increase the flow rate to 800 mL/hr, yielding an inlet pressure of 4.77 Mpa (Fig. 2, and Movie S1-up, in the Supporting Information). We also fabricate a simple glued interconnection for this channel, which leaks at 150 mL/hr, corresponding to a pressure of 0.9 Mpa (Movie S1-down in the Supporting Information). To expand the current pressure limit of our two-layered tubing system, other materials like Teflon with better mechanical properties should be used to fabricate microfluidic chips.

Nanoprecipitation is a simple and widely used method to prepare nanoparticles. This method typically uses water-miscible solvents to dissolve polymer, and dips the polymer solution slowly into water with sonication or stirring. The interfacial deposition of polymeric nanoparticles occurs as soon as the solvent mixes with water. Therefore, rapid and sufficient mixing is vital to generate small-sized and well-dispersed nanoparticles.[19] Microfluidic-based nanoprecipitation enables the precise control of mixing in small length scale, thus becoming a promising platform for controlled synthesis of nanoparticles.[20] A major challenge for microfluidic-based synthesis, however, lies in the low productivity of nanoparticles, which is limited by the low flow rate ranging from hundreds of μL to several mL per hour inside microfluidic devices. With our tubing interconnection technique for chips, we can dramatically increase the productivity by increasing the flow rates of polymer solution and water (Fig. S4).

We first investigate the mixing at low flow rate with different FRs inside the three-inlet semicircular microchannel of 300 μm wide × 50 μm high × 5 cm long. The middle fluorescein stream at 1 to 4 mL/hr could be hydrodynamically focused into a narrow band by two water sheaths of 20 mL/hr each, which is confirmed by a three-dimensional (3D) model using Fluent (Fig S5, and Fig. S6). The width of focused stream becomes wider with increasing the flow rate of middle stream, indicated by the reduced FR from 40 to 10 (Fig. S6). At the total flow rate of 41 to 44 mL/hr, the Reynolds number inside the microchannel is around 65, and the mixing mainly relies on diffusion. In comparison, by employing our tubing method, we can significantly increase the total flow rate by ten times to 410 mL/hr (Re = 650). The inlet pressure is estimated to be 4.2 Mpa by using numerical simulation. With a significant increase of the Reynolds number inside the semicircular microchannel, four microvortices perpendicular to the flow direction are generated at the junction of three inlets due to the stronger Dean flow, resulting in a more efficient convective mixing at the FR of 10 to 40 (Fig. S6, and Fig. S7). The 3D confocal images also confirm that at a low Re number, the diffusional mixing has a constant profile in the z direction after three streams enter into the main channel (Fig. S5). In comparison the convective mixing under a high Re number results in a chaotic mixing in the z direction, which occurs at the junction of three inlet streams (Fig. S5).

Based on the above investigation, we synthesize PLGA nanoparticles at low and high flow rates with varying FRs (Supporting Information). PLGA nanoparticles of a small size of approximately 55 nm are obtained at a high flow rate (410 mL/hr, Re = 650) and a large FR (40). The size of nanoparticles increases to around 70 nm by adjusting the FR to 20. If we further decrease the FR to 10, a larger size of approximately 135 nm is achieved (Fig. 3). Both TEM and DLS results indicate a good dispersion of PLGA nanoparticles (Fig. 3). We also characterize PLGA nanoparticles synthesized at a low flow rate (41 to 44 mL/hr, Re = 65) with FR from 10 to 40. The size of nanoparticles is 110 nm at FR of 40, and increases to 180 nm at FR of 10 (Fig. 3). For the same FR, the nanoparticles obtained at the high flow rate (410 mL/hr) are approximately 50 nm smaller than those from the low flow rate (41 to 44 mL/hr) (Table 1). We speculate that this size difference is caused by diffusion mixing versus convective mixing. The convective mixing enables a more efficient and rapid interfacial deposition of small-sized PLGA nanoparticles. In addition, high-flow-rate synthesis produces nanoparticles with a narrow size distribution and a low polydispersity (PDI) of 0.1, while nanoparticles prepared by low-flow-rate synthesis have a higher PDI of around 0.2.

In addition, we precipitate PLGA nanoparticles by conventional bulk method as follows: 2 % PLGA organic solutions (250, 500, 750, and 1000 μL) are added dropwise into four flasks of 10 mL water, and stirred magnetically for 3 min at room temperature. The mass concentrations of PLGA

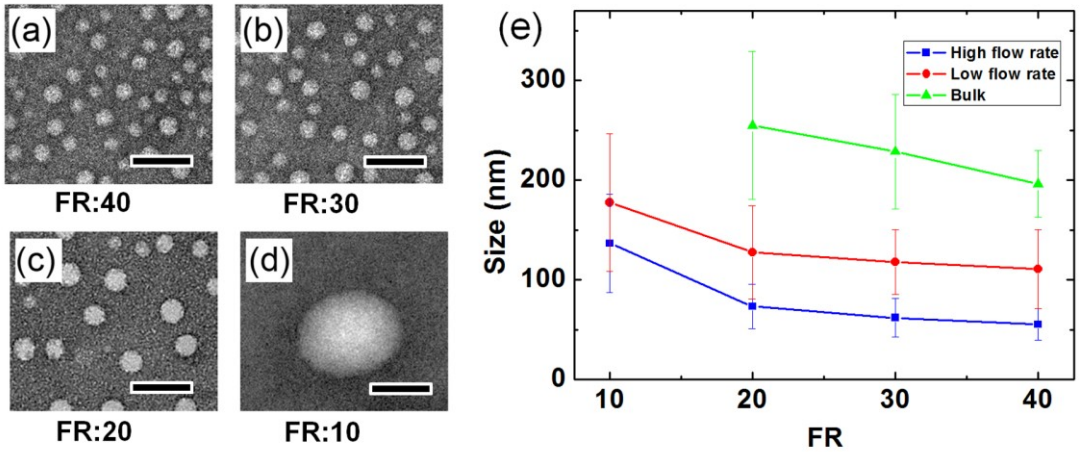


Fig.3 TEM images of PLGA nanoparticles precipitated under different FRs. (a) middle channel: 10 mL/hr, each side channel: 200 mL/hr, and FR: 40, (b) middle channel: 13 mL/hr, each side channel: 198.5 mL/hr, and FR: 30, (c) middle channel: 20 mL/hr, each side channel: 195 mL/hr, and FR: 20, and (d) middle channel: 38 mL/hr, each side channel: 186 mL/hr, and FR: 10. The scale bar is 100 nm. (e) Size distribution of PLGA nanoparticles as a function of FR by different approaches.

Table 1. The comparsion of precipiated PLGA nanoparticles by microfluidics (high and low flow rates) and bulk.

	Total flow rate (mL/hr)	Mass production (g/hr)	Minimum Size (nm)	PDI
Microfluidics	41–44	0.02–0.08	110	~0.2
Microfluidics	410	0.2–0.8	55	~0.1
Bulk		0.1–0.4	200	~0.4

nanoparticles are equal to those from microfluidic approach with FR between 40 to 10. For 1000 µL 2 % PLGA into 10 mL water, visible white PLGA particles are precipitated, and exceed the size limits of DLS measurements. DLS results from the other three samples indicate that PLGA nanoparticles by bulk exhibit large sizes (200 - 250 nm) and wide distribution (PDI ~ 0.4) (Fig. 3 and Table 1). The mass production of PLGA nanoparticles by bulk is from 0.1 to 0.4 g/hr, 2 times lower than high-flow-rate microfluidic method (Table 1).

X. J. and J. S. acknowledge financial support from MOST (2011CB933201, 2012AA030308, 2012AA022703 and 2013AA032204), NSFC (51105086, 91213305, 21025520, and 51073045), Chinese Academy of Sciences and Beijing Municipal Science & Technology Commission (Z131100002713024).

Notes and references

[a] *Beijing Engineering Research Center for BioNanotechnology & Key Lab for Biological Effects of Nanomaterials and Nanosafety, National Center for NanoScience and Technology, Beijing, 100190, China.*
E-mail: xingyujiang@nanoctr.cn; sunjs@nanoctr.cn
[b] *Institute of Mechanics, Chinese Academy of Sciences, 100190, China.*
E-mail: guoqing.hu@imech.ac.cn
† *Electronic supplementary information (ESI) available.*
‡ These authors contributed equally to this work.

1. Y. Y. Liu, Y. Sun, K. Sun, L. S. Song and X. Y. Jiang, *J Mater Chem*, 2010, **20**, 7305-7311.
2. X. Mu, W. F. Zheng, J. S. Sun, W. Zhang and X. Y. Jiang, *Small*, 2013, **9**, 9-21.
3. J. Sun, Y. Kang, E. Boczko and X. Jiang, *Electroanal*, 2013, **25**, 1023-1028.
4. J. Sun, C. C. Stowers, E. M. Boczko and D. Li, *Lab Chip*, 2010, **10**, 2986-2993.
5. E. Sollier, C. Murray, P. Maoddi and D. Di Carlo, *Lab Chip*, 2011, **11**, 3752-3765.
6. A. M. Christensen, D. A. Chang-Yen and B. K. Gale, *Journal of Micromechanics and Microengineering*, 2005, **15**, 928-934.
7. C. K. Fredrickson and Z. H. Fan, *Lab Chip*, 2004, **4**, 526-533.
8. E. Wilhelm, C. Neumann, T. Duttenhofer, L. Pires and B. E. Rapp, *Lab Chip*, 2013, **13**, 4343-4351.
9. S. Haeberle and R. Zengerle, *Lab Chip*, 2007, **7**, 1094-1110.
10. A. Scott, A. K. Au, E. Vinckenbosch and A. Folch, *Lab Chip*, 2013, **13**, 2036-2039.
11. H. Kortmann, L. M. Blank and A. Schmid, *Lab Chip*, 2009, **9**, 1455-1460.
12. A. Arora, G. Simone, G. B. Salieb-Beugelaar, J. T. Kim and A. Manz, *Anal Chem*, 2010, **82**, 4830-4847.
13. A. Chen and T. R. Pan, *Lab Chip*, 2011, **11**, 727-732.
14. A. A. S. Bhagat, P. Jothimuthu, A. Pais and I. Papautsky, *Journal of Micromechanics and Microengineering*, 2007, **17**, 42-49.
15. J. S. Sun, C. Liu, M. M. Li, J. D. Wang, Y. L. Xianyu, G. Q. Hu and X. Y. Jiang, *Biomicrofluidics*, 2013, **7**, 011802.
16. J. Sun, M. Li, C. Liu, Y. Zhang, D. Liu, W. Liu, G. Hu and X. Jiang, *Lab Chip*, 2012, **12**, 3952-3960.
17. T. Gervais, J. El-Ali, A. Gunther and K. F. Jensen, *Lab Chip*, 2006, **6**, 500-507.
18. B. Kirby, *Micro- and nanoscale fluid mechanics : transport in microfluidic devices*, Cambridge University Press, New York, 2010.
19. J. Sun, Y. Xianyu, M. Li, W. Liu, L. Zhang, D. Liu, C. Liu, G. Hu and X. Jiang, *Nanoscale*, 2013, **5**, 5262-5265.
20. R. Karnik, F. Gu, P. Basto, C. Cannizzaro, L. Dean, W. Kyei-Manu, R. Langer and O. C. Farokhzad, *Nano Lett*, 2008, **8**, 2906-2912.