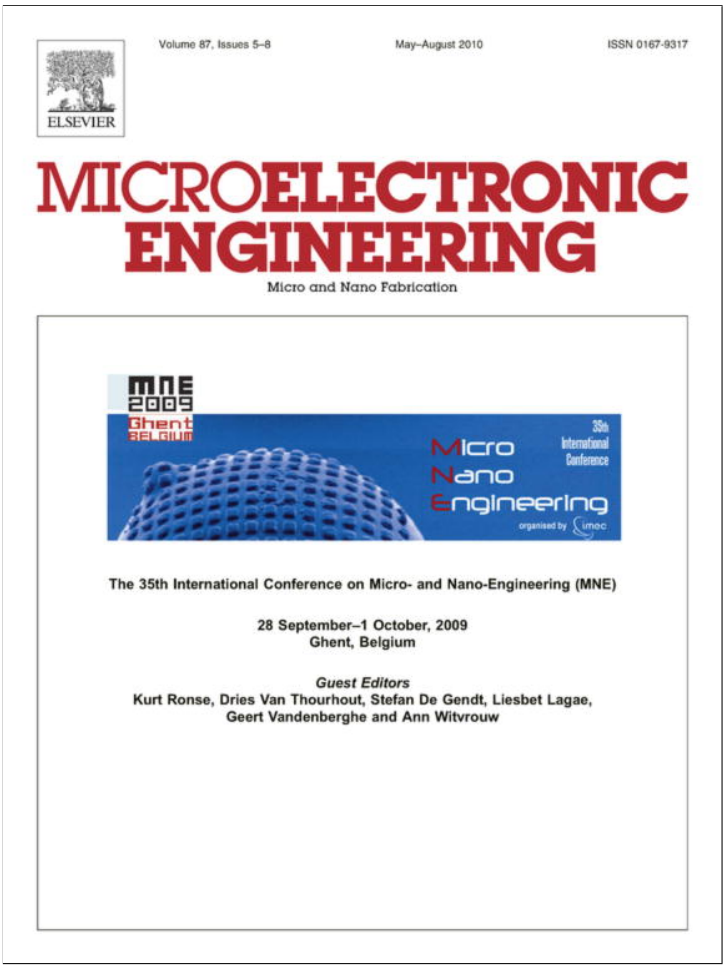

Microelectronic Engineering 87 (2010) 760–764

Contents lists available at ScienceDirect

Microelectronic Engineering

journal homepage: www.elsevier.com/locate/mee

Microfabricated magnetic bead polydimethylsiloxane microarrays

Jenny Aveyard [a], Tobias Hedegaard [b], Brian Bilenberg [b], Dan V. Nicolau [a,*]

[a] Department of Electrical Engineering and Electronics, University of Liverpool, Brownlow Hill, L69 3GJ, UK
[b] NIL Technology ApS, Diplomvej 381, DK-2800 Kongens Lyngby, Denmark

ARTICLE INFO

Article history:
Received 12 September 2009
Received in revised form 23 November 2009
Accepted 25 November 2009
Available online 29 November 2009

Keywords:
Microarrays
Self assembly
Bead arrays
Microfabrication
Magnetic microbeads

ABSTRACT

Bead-based technologies are an attractive alternative to classical microarrays due to the high surface area of the beads which enables the immobilization of large numbers of probe molecules leading to increased kinetics and improved signal/noise ratios. A simple, versatile and inexpensive method for the fabrication of magnetic bead arrays is presented. The beads are first assembled into silicon pyramidal wells that have been fabricated by established microfabrication techniques and then mounted onto the apex of PDMS pyramids that replicate the silicon substrates by replica moulding. The bead arrays were used to detect oligonucleotide target sequences for the pathogenic avian H5N1 flu virus and results indicated that superior signal/noise ratios could be achieved compared to those from a classical microarray format.

1. Introduction

Classical microarrays are ordered arrays of microscopic elements on a planar substrate that allow fast, specific high-throughput analysis of target molecules [1]. They are used in many applications including genetic screening, diagnostics, drug discovery and protein analysis. Despite their popularity, microarrays often suffer from slow kinetics and are expensive to fabricate. As a result there have been many attempts to improve their sensitivity, multiplexing capability and cost-effectiveness without losing any of the attributes of the classical microarray format. One possibility that has been investigated recently is the use of microbeads for probe immobilization. Microbeads exhibit high surface areas and as a result increased densities of probe molecules are immobilized in a given area in comparison to mechanically spotted probe molecules on a classical planar microarray surface. In addition, the attachment to a microbead lifts the probe molecules a few μm from the surface resulting in improved kinetics and an increased signal to noise ratio on the array [2–4].

In this work, we attempted to combine the benefits of planar and bead-based array in a single platform and amplify this synergy by using beads with multiple operational functionalities, i.e., chemical and magnetic.

2. Materials and methods

N-hydroxysuccinimidyl 3-(2-pyridyldithio)propionate (SPDP), bovine serum albumin (BSA), 6-[Fluorescein-5(6)-carboxamido]hexanoic acid *N*-hydroxysuccinimide ester (FITC-NHS) were purchased from Sigma Aldrich, the aminosilanized slides (Gap II) from Corning Life Sciences, the amine-terminated magnetic beads (2.9 μm) from Invitrogen, the Sephadex G-25 columns from Pierce, the disulfide all oligonucleotides were from Eurogentec, and the polydimethylsiloxane (PDMS, Sylgard elastomer kit) was purchased from Dow Corning.

Buffers and solutions have been prepared as follows. PBS comprises 15 mM sodium phosphate, 0.15 M NaCl, pH 7.4, bicarbonate buffer comprises 0.1 M sodium bicarbonate, pH 8.2; wash buffer 1 has a composition of 0.1% sodium dodecyl sulfate (SDS); blocking buffer has 300 mM sodium citrate, 750 mM NaCl, 0.1% SDS containing 5 mg ml^{-1} BSA, pH 7. The hybridization solution is similar to the blocking buffer, but with 1 mg ml^{-1} BSA. Finally, wash buffer 2 comprises 120 mM sodium citrate, 300 mM NaCl, 0.1% SDS, pH 7; the wash buffer 3 is as wash buffers except without SDS; and wash buffer 4 comprises 12 mM sodium citrate, 30 mM NaCl, pH 7.

2.1. Fabrication of pyramidal microstructures

Established microfabrication techniques were used to produce pyramidal wells of a defined size on silicon masters. After beads were assembled in the wells, they were transferred to the apex of PDMS pyramids by PDMS replica moulding (Fig. 1). For the fabrication of pyramidal microstructures a 1 μm SiO_2 layer was grown

* Corresponding author. Tel.: +44 151 794 4537; fax: +44 151 794 4540.
E-mail address: d.nicolau@liverpool.ac.uk (D.V. Nicolau).

0167-9317/$ - see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.mee.2009.11.136

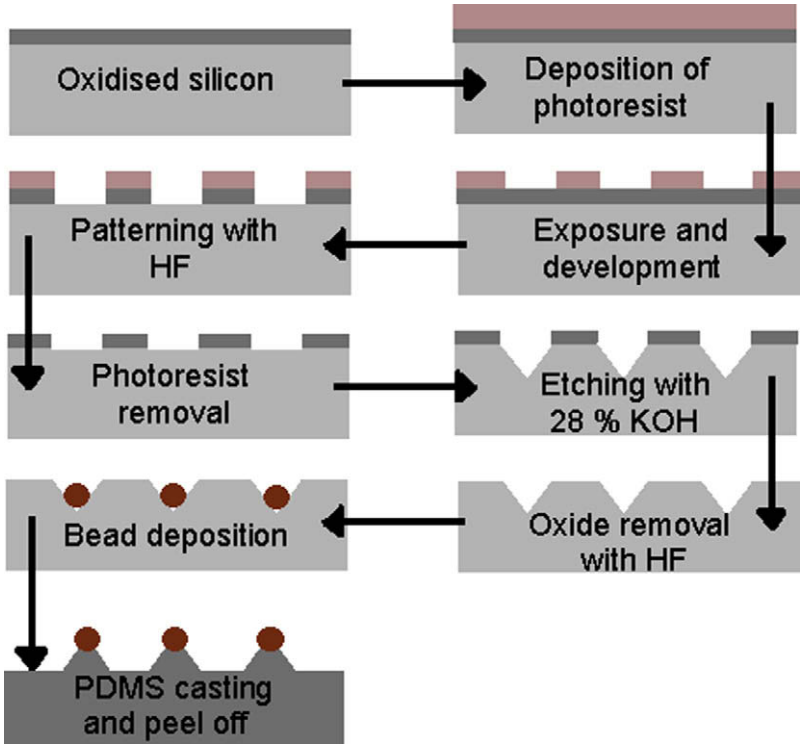

Fig. 1. Scheme used for the fabrication of PDMS magnetic bead arrays. Photoresist coated silicon wafers were exposed and developed. The underlying SiO_2 layer was then patterned using hydrofluoric acid and the resist was removed with acetone. The wafer was then anisotropically etched with 28% KOH to produce pyramidal wells. After functionalized magnetic beads were deposited, PDMS replica moulding under mild conditions was performed to transfer the beads from the wells to the PDMS array.

on a {1 0 0} silicon wafer and classical lithography was performed comprising of spin coating the wafer with positive resist followed by pattern exposure and development. The underlying SiO_2 layer of the Si wafers was then patterned using hydrofluoric acid before the photoresist was stripped with acetone. The wafer was then etched anisotropically with a 28% KOH solution to produce pyramidal wells. The etched silicon wafer consisted of 50 × 50 arrays of pyramidal wells with widths of 10 μm and depths of 5.7 μm. The wafer was cut into 1 cm × 1 cm chips, each comprising of 12 microarrays, which were used as masters for the production of bead-PDMS microarrays.

2.2. Functionalization of magnetic beads

For oligonucleotide probe attachment; amine-terminated magnetic beads were incubated with SPDP. SPDP is a heterobifunctional reagent which has an amine-reactive NHS ester on one terminus and a pyridyl disulfide moiety on the other. The reaction with this compound results in the formation of a disulfide end-group on the beads. 100 μl of washed amine-terminated magnetic beads were slow-tilt rotated with 1 ml of 5 mM SPDP (in PBS) for 2 h at room temperature. At the end of this time, the un-reacted SPDP was removed by magnetic precipitation of the beads 3 times in PBS. 3 μM of disulfide-terminated oligonucleotide was reduced by slow-tilt rotating at room temperature with 1 ml of 1 mM dithiothreitol (DTT; in bicarbonate buffer). At the end of this time excess DTT was removed by gel filtration on Sephadex G-25 equilibrated with the same buffer. The eluent fraction containing the oligonucleotide was immediately added to 20 μl of disulfide functionalized beads and slow-tilt rotated at room temperature for 12 h. At the end of this time, the un-reacted oligonucleotide was removed by magnetic precipitation of the beads 3 times in PBS.

2.3. Fabrication of magnetic bead arrays

To assemble the magnetic beads in the pyramidal wells, silicon masters were mounted on top of a magnet and incubated on a rocker plate with 1 ml of PBS containing 10 μl of functionalized beads (2×10^6 beads) for 1 h at room temperature. At the end of this time, silicon masters were washed 3 times with distilled water and air dried. The masters were then characterized by confocal microscopy to determine the location of the beads before PDMS replica moulding. PDMS was prepared by mixing 10 parts prepolymer with 1 part curing agent. After de-gassing for 1 h under vacuum, the solution was poured over the master and cured at 60 °C for 2 h. Before the reaction with the fluorescently labelled complementary (or non-complementary control) oligonucleotide, the PDMS replica was cut from the master and blocked by incubating with 1 ml of a 1% w/v BSA solution on a rocker plate for 1 h at room temperature.

2.4. Testing the functionality of assembled beads

To determine whether the beads could be functionalized after assembly onto PDMS posts, a PDMS replica with amine-functionalized beads was incubated on a rocker plate with 1 ml of 2 mM of FITC-NHS for 2 h at room temperature. At the end of this time, the un-reacted FITC-NHS was removed by washing the PDMS bead array 3 times in PBS. The images were obtained using a Zeiss confocal LSM 510 microscope with Zen analysis software.

2.5. Microarrays

Disulfide moieties were introduced into Corning Gap II aminosilane slides by incubating the slides with 13 ml of 1 mM SPDP (in PBS) on a rocker plate for 2 h at room temperature. At the end of this time, slides were washed 2 times with PBS containing

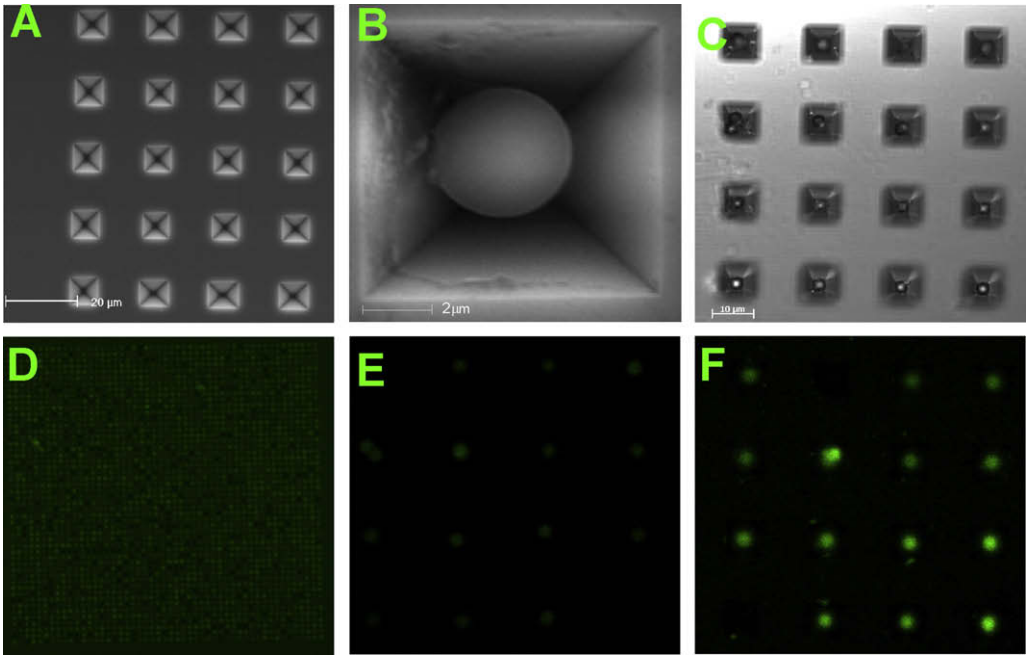

Fig. 2. (A) Scanning electron micrograph (SEM) of an array of pyramidal wells on a silicon master. (B) SEM of microbead in a pyramidal well. (C) Differential interference contrast (DIC) image of magnetic beads mounted on PDMS pyramids. (D) Fluorescence micrograph of microbead array showing the auto-fluorescence exhibited by the beads when imaged with high laser intensity (100%). (E) Close-up of bead array showing the auto-fluorescent beads. (F) Close-up of bead array after reaction with amine-reactive fluorescent dye (FITC-NHS). After reaction with FITC-NHS, the beads exhibit a fourfold increase in fluorescent signal. E and F were obtained under the same conditions, using the 488 nm laser at 5% and the excitation and emission filters for FITC.

10% ethanol and 2 times with PBS before drying in a centrifuge. For oligonucleotide conjugation, 3 μM disulfide functionalized oligonucleotide was reduced as in Section 2.2, except that excess DTT was removed using a Sephadex G-25 column equilibrated with bicarbonate buffer. The reduced oligonucleotides were printed onto SPDP modified microscope slides using a MicroGrid II compact microarraying robot. After standing overnight, arrays were washed once with 15 ml of wash buffer 1 and blocked by incubating on a rocker plate with 15 ml of blocking buffer for 2 h at room temperature. At the end of this time, 0.5 ml of complementary (or non-complementary control) Cy5 labelled target (2.5 μM in hybridization buffer), was hybridized to the arrays for 2 h at 50 °C. The hybridization of Cy5 labelled target to PDMS bead arrays was performed in the same way. After hybridization, the arrays were washed once for 5 min in wash buffer 2, once for 5 min in wash buffers 3 and 4 before drying in a centrifuge. The images were obtained as described in Section 2.4.

3. Results and discussion

3.1. Bead array fabrication and determining the chemical stability of functionalized beads

PDMS is an ideal material for the production of bead arrays, as it is simple to use, inexpensive, flexible, biocompatible, gas permeable and optically transparent. Fig. 2A–C presents the silicon pyramidal wells and the assembled beads. Fig. 2D is an image of a bead array illustrating the typical density of the assembled beads. To assess the chemical robustness of the amine functionalities on the beads, PDMS mounted beads were reacted with an amine-reactive fluorescent dye. Fig. 2E and F show the bead arrays before and after the reaction with FITC-NHS. Despite the slight auto-fluorescence exhibited by the beads, a fourfold increase in the fluorescence intensity (over the auto-fluorescent background) was observed after reaction with the dye. The control arrays (images not shown) did not present this amplification. The high fluorescent signal observed in Fig. 2F demonstrates that the mild conditions of assembly and PDMS curing/mounting do not affect the chemical reactivity of the beads. This is an important finding, as some biological molecules, such as antibodies can be denatured in harsh chemical conditions or high temperatures.

3.2. Detection of H5N1 specific target oligonucleotide sequences using PDMS magnetic bead arrays

Mounted beads functionalized with specific probe oligonucleotide sequences for the highly pathogenic avian H5N1 influenza virus were exposed to the fluorescently labelled complementary target or non-complementary (control) sequence. In order to eliminate the auto-fluorescence, Cy5 dye was used in these experiments. This is excited and emits at longer wavelengths (650 and 670 nm) where the auto-fluorescence is minimal. Fig. 3A–C shows the results from these experiments. When the bead arrays were exposed to the complementary target sequence, hybridization occurred and the beads fluoresced. If the beads were exposed to the non-complementary sequence (Fig. 3A) no hybridization occurred and no fluorescence was observed. The signal to noise ratio (SNR) obtained from the fully complementary sequence was 24 whereas the non-complementary sequences present SNRs of 1. Future work will entail an investigation into how probe concentration affects the SNR and whether this method can be used for the detection of single-base mismatches in probe sequences.

3.3. Comparison with classical microarray

Regular contact-robot printed microarrays performed on microscope slides were compared to PDMS bead arrays. Commercially available silanized microscope slides were chosen for this work as these substrates are reported to exhibit low background fluorescence and high SNRs. Fig. 4A and B shows the results from these

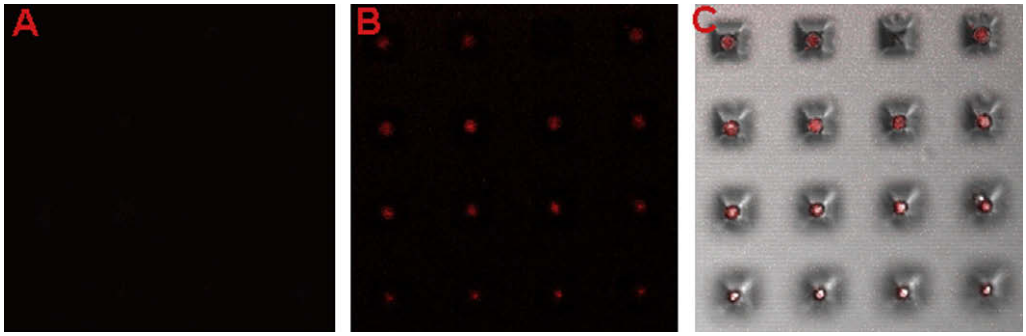

Fig. 3. Images showing specific detection of pathogenic avian H5N1 influenza virus. (A) Fluorescence micrograph of microbeads on pyramidal posts after reaction with Cy5 labelled non-complementary (control) sequence. (B) Fluorescence micrograph after reaction with complementary fluorescently labelled oligonucleotide. (C) Corresponding DIC image. Images were obtained under the same conditions using the 633 laser and the excitation and emission filters for Cy5.

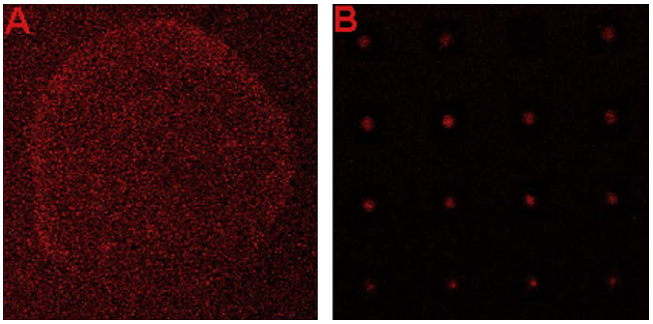

Fig. 4. Comparison of robotically printed microarray spot and PDMS magnetic bead array (not to scale). (A) Fluorescence micrograph showing specific detection of avian flu probe sequences in a microarray spot on modified corning gap slide. (B) Corresponding bead array image. Both images were obtained under the same conditions.

experiments. Fig. 4A shows a single printed microarray spot that is approximately 200 μm in diameter. A very high background signal can be observed in this image due to non-specific binding of labelled oligonucleotide onto the functionalized surface. The SNR for the microarray spot was 2.4, which is 10 times lower than the bead arrays. It is thought that the increased SNR exhibited by the bead arrays is due to the raised bead position. This should increase the kinetics of the reaction and reduce the steric hindrance between the probe and target molecules. In addition the high surface area exhibited by the beads results in an increased density of immobilized probe molecules compared to the microarray spot. Although we have not yet performed experiments to determine the number of oligonucleotides, our calculations indicate that a microarray spot of 200 μm diameter could contain a maximum of 157×10^6 oligonucleotides (based on the assumption that each oligonucleotide is standing up and will occupy 0.2 nm^2). These calculations indicate that 4.75×10^3 of densely packed beads could occupy the same area. Each individual bead would have a surface area of 26.42 μm^2 and therefore this would result in the immobilization of 4 times more oligonucleotide. All of these factors are thought to contribute to an overall increase in sensitivity.

3.4. Technology overview

The proposed technology uses anisotropically etched pyramidal wells on silicon masters as templates for the PDMS magnetic microbead arrays. Superparamagnetic microbeads functionalized with either chemical attachment groups (amines) or recognition molecules (oligonucleotides) were deposited into arrays of pyramidal wells on a silicon substrate. The number of beads immobilized in each well is controlled by the dimension and shape of the well relative to the diameter of the bead [5,6]. The technology benefits from a large yield of the technological steps, i.e., nearly 90% for the allocation of beads in wells, and essentially for all subsequent steps (transfer of the beads on the PDMS pyramids, functionalization of the beads and biomolecular recognition).

This method of fabrication and assembly is simple and versatile. Magnetic microbeads are commercially available in an assortment of sizes and there are a wide variety of different surface chemistries available. They can be purchased pre-coated with the protein of choice or can simply be modified in-house to display the desired functionality enabling complete flexibility over the chemistry of probe attachment. In addition, the mild conditions of both the assembly of beads and the fabrication of the PDMS array allowed the functionalisation with probe molecules before or after bead assembly on the PDMS pyramids. Moreover, these magnetic bead microarrays are simple, chemically and mechanically robust, inexpensive, spatially addressable and allow the analysis of multiple targets. This work has demonstrated the potential to create high-density arrays with better readout capabilities and superior signal to noise ratios than those achievable with classical microarrays.

4. Conclusion

Classical microarrays are expensive to produce, suffer from slow-reaction kinetics and often entail lengthy standardization procedures to improve printed spot morphology, reproducibility and increase signal/noise ratios. Herein we have presented a method for the construction of PDMS magnetic bead arrays based on an established photolithography microfabrication and replica moulding technique. The method of fabrication is simple, reliable and inexpensive and the bead arrays are chemically and physically robust. This technology has demonstrated the potential to produce image signals, signal/noise ratios and reaction kinetics superior to classical microarrays.

Acknowledgements

This work was supported by European funded FP7 grant (BISNES). Thanks to Carmel Pinnington from the Department of Earth and Ocean Sciences for the SEM images and Professor Andrew Cossins from the School of Biological Sciences at the University of Liverpool for the use of the Microarraying facilities.

References

[1] M. Schena, Microarray Analysis, John Wiley and Sons, New Jersey, 2003.
[2] T.L. Jennings, K.S. Rahman, S. Fournier-Bioloz, Analytical Chemistry 80 (2008) 2849.

[3] L. Filipponi, P.D. Sawant, F. Fulga, D.V. Nicolau, Biosensors and Bioelectronics 24 (2008) 1850.
[4] C.A. Marquette, M. Cretich, L.J. Blum, M. Chiari, Talanta 71 (2007) 1312.
[5] I. Lee, H.P. Zhang, M.F. Rubner, P.T. Hammond, Advanced Materials 14 (2002) 572.
[6] D.Y. Xia, A. Biswas, D. Li, S.R.J. Brueck, Advanced Materials 16 (2004) 1427.