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Sol-gel Material Optimization for Aptamer Biosensors

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Abstract

Biochips are a powerful emerging technology for biomedical, environmental applications. Especially, making use of bioseonors in the evaluation of toxicity becomes increasingly important. For biosensor as a toxicity detection, biomolecules like antibodies or aptamers have been developed to specifically capture the toxic target molecules. In addition, the development of optimal chip materials capable of maintaining the activity of embedded biomolecules such as proteins or aptamers has proven challenging. Here, using sol-gel materials, new chip material, whose ability for immobilizing the embedded aptamers and maintaining the ability of embedded aptamers is optimal, was searched. We used sol-gel formulation screening methods previously developed and found the best formulation which shows high sensitive and specific interactions of aptamers. This study results will support the technological advancement for diagnosis and environmental sensor.

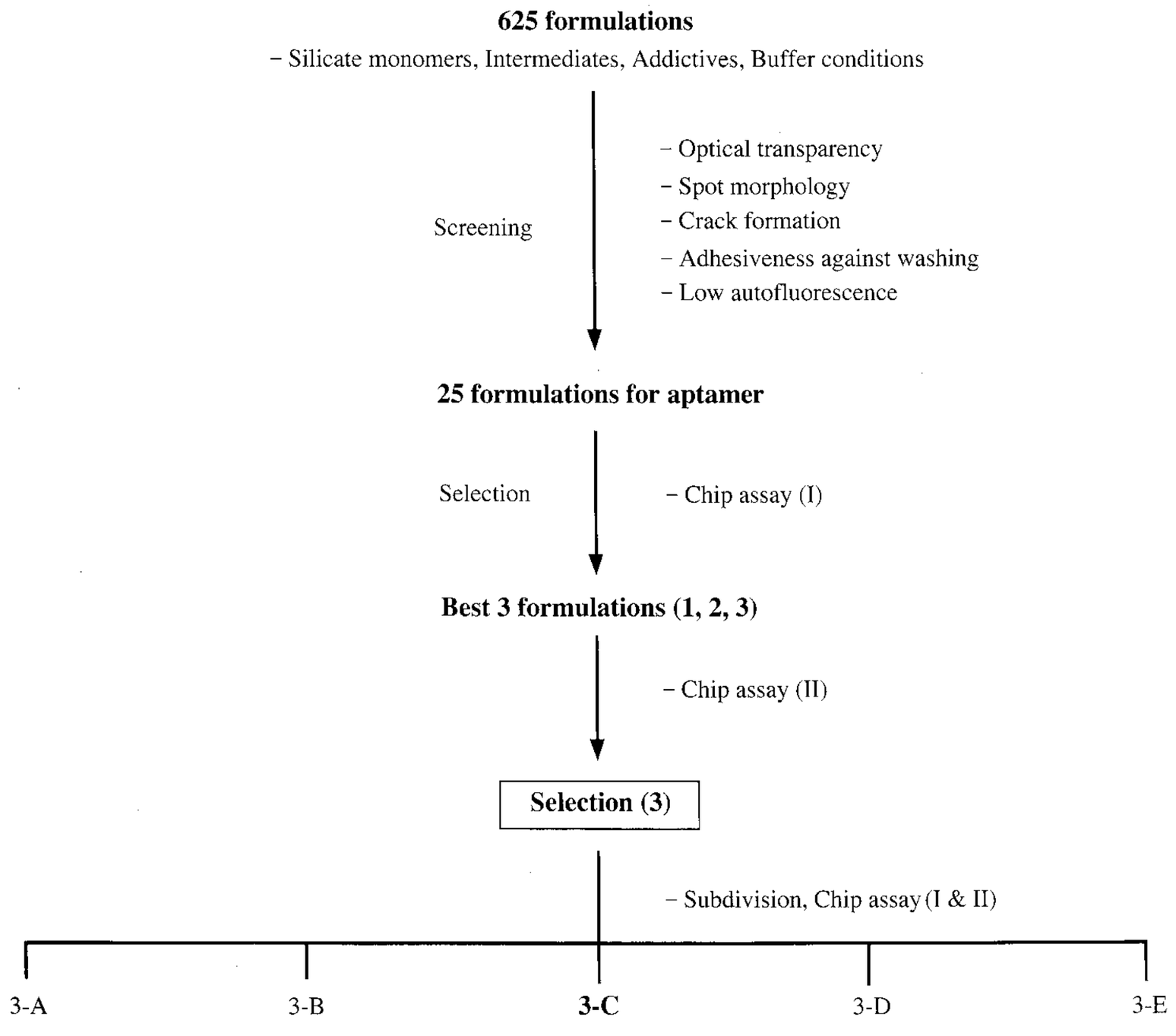
Keywords: Sol-gel chemistry, Biosensor, Aptamer, Environmental hazardous detection, Material screening, Aptamer chip

Biochip technology provides a novel tool for analysis of various biomolecules immobilized on the surface of chips[1]. In addition to biomedical applications such as disease diagnosis and drug development, biochip is more and more being used for detection of small molecules-toxic chemicals or pollutants[2,3].

To develop the biochips or biosensor for detecting or evaluating toxicity, there are two more factors we should consider comparing to the development of medical application. Firstly, since toxic chemicals are hard to make good antibodies as capturing agents, optimal capturing agents for detections of toxic molecules should be developed. Secondly, the design of the optimal chip materials that is able to maintain the nature of the embedded capturing biomolecules should be done. Biomolecules tend to lose their activity on the surface of bio-chips when immobilized in the biocompatible surface or where often causes loss of their binding sites that are oriented in wrong direction[4].

As a capturing agent for evaluating toxicity, "Aptamer", single stranded RNA or DNA can be used[5,6]. Aptamer was originally developed for finding molecules that have a good affinity and selectivity to target ligand or molecules[7]. Aptamers are similar to antibodies, but, it can be more favorable to biochip application because of following reasons[8]. Through SELEX, a process to find suitable single strand DNA for aptamer, aptamers that has higher affinity and selectivity can be produced, and also mass production is feasible[9]. Aptamers can be produced very pure and transformative by chemical modifications[10]. In addition, it can be maintained for long time and especially, doesn't trigger immune reaction in organism since it is selected in vitro. These advantages make aptamers more attractive as a capturing agent for toxic molecules, which cannot normally produce antibodies because of in vivo toxicity of target molecules.

Sol-gel-based chips do not require affinity to captured agent, so various molecules, such as proteins or aptamers can be captured on the surface of biochips without any modification of biomolecules[11]. The variety of formulations of silicate or additives can produce diverse physical properties, so sol-gel can be



Scheme 1. Experimental scheme of the screening and selection strategy for aptamer. A total of 625 materials were screened using 5 screening assay described in the Materials and Methods section, resulting in 25 materials. The immobilization efficiency assay (Chip assay I) resulted in the selection of three materials and the number 3 material was suitable for aptamer chip. To optimize the condition of the selected formulation, No. 3 was subdivided into five different formulations (3A-3E) and the best formulation of aptamer chip for nucleic acids was found newly.

applicable to wide variety of biomolecules. In addition, sol-gel material is inexpensive to other materials that were used as a biochip[12]. While a protein immobilized on the biochip surfaces usually loses their activity, in sol-gel material, proteins can maintain their activity. Previously, we showed that sol-gel based protein chip showed high sensitive and specific activity for disease diagnosis chips[6]. Therefore, we made use of sol-gel process to develop and find optimal formulation for aptamer chips, since, unlike normal nucleic acids, aptamers have 3-dimensional structures similar to proteins.

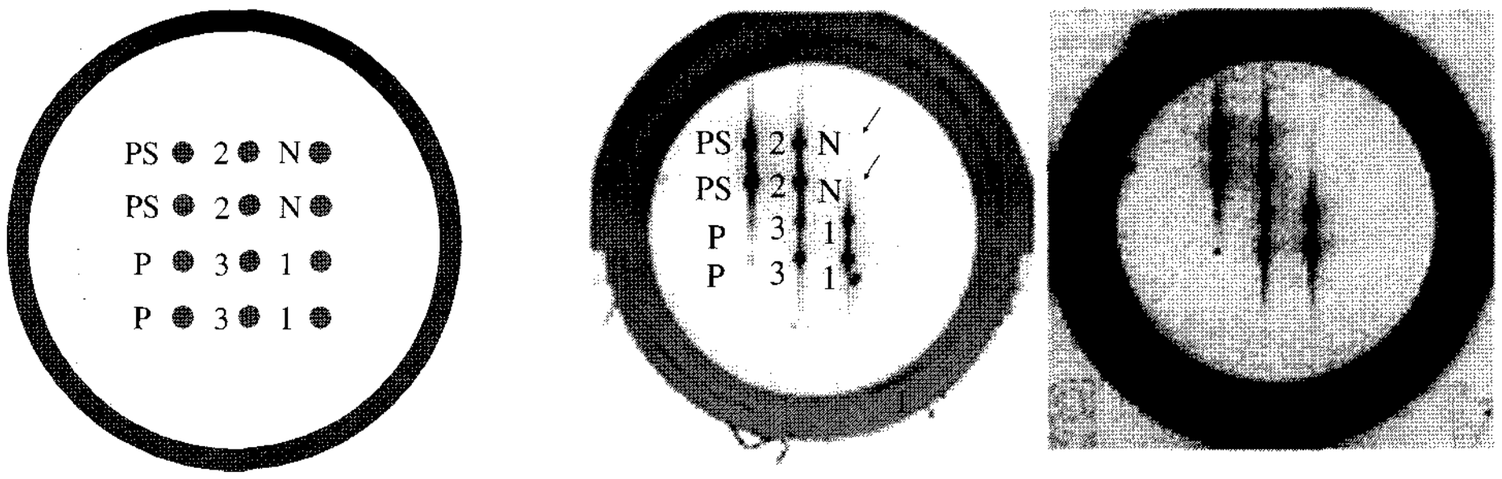
In this study, based on sol-gel formulation for protein chips previously developed, the optimal sol-gel materials for the nucleic acids were screened by screening and selection methods. The selected formulation was optimum to detect aptamer-antigen, and aptamer-ligand interaction in microarrays.

Screening for Optimal Sol-gel Chip Materials

Based on protein chip materials previously selected[13], we tried to optimize the formulation for nucleic acids or aptamers. Here, we used TATA ssDNA as a model single strand nucleic acid representing for aptamers. This TATA DNA is originally double strand complementary DNA which is known to bind the TBP proteins[14]. We first made 625 formulations derived from sol-gel protein chip formulation and tested these formulation with five assays described in the materials and methods session (Scheme 1). After these assays, adhesion to the surface after washing, morphology, gelation time, transparency and autofluorescence, we chose spots with strong attachment and good shapes after washing with long gelation time (>4 hours) and low auto fluorescence. In addition, if there were cracks determined by microscope on the spots, we excluded those formulations. From these assays, we founded 25 good formulations out of 625 formulations.

After the screening, 25 formulations were tested more for selecting the chip materials optimal for aptamers. As described in Scheme 1, we performed two consecutive chip assays using TATA DNA as a model aptamer. To test the immobilization efficiency (Chip assay I) of 25 formulations, we immobilized the Cy3-labeled ssDNA (TATA-fwd cy3) in each formulation, washed and compared the fluorescent intensity before and after washing. From these chips assay I, we could select 3 formulations for immobilizing nucleic acids (Scheme 1).

(a) Chip assay I



[Spot position] [Unwashed] [Washed]

(b) Chip assay II

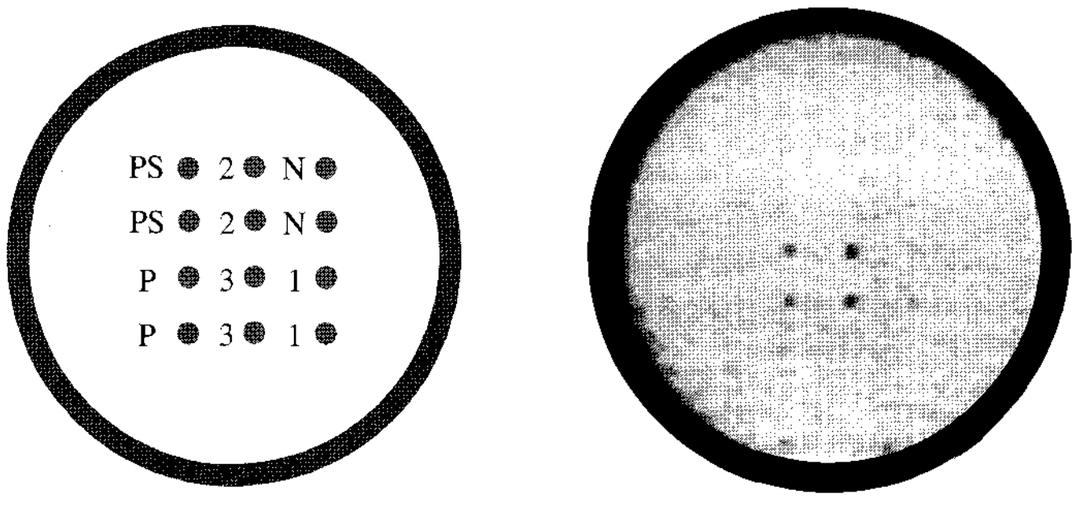


Figure 1. Characterizations of selected formulation for nucleic acids. (a) Chip assay I. Each formulation (1, 2, and 3) was spotted with TATA-fwd-cy3 oligo and compared with the formulation previously selected for protein chip (PS). After washing step, the fluorescent intensity of spots was compared before (unwashed) and after washing (washed). (b) Chip assay II. For activity test, the TATA reverse oligos were immobilized within the spots from three formulations (1-3) and PS formulation. P: positive control (Cy-3), N: negative control (Distilled Water).

Further Characterizations of Selected Formulation for Nucleic Acids

From the screening, Screened three formulations show the different percentage of silicate monomers, additives and buffer composition. Selected formulation 1 shows 40.40% of TMOS, MTMS and GPTMOS, 12.50% of 10 mM HCl, and 47.10% of buffer solution with nucleic acids (10 μM). Selected formulation 2 shows 43.70% of TMOS, MTMS and GPTMOS, 12.60% of 10 mM HCl, and 43.70% of buffer solution with nucleic acids (10 μM). Selected formulation 3 shows 43.40% of TMOS, MTMS and GPTMOS, 12.50% of 10 mM HCl, and 44.10% of buffer solution with nucleic acids (10 μM). Using these selected formulation with good immobilization efficiency, we further confirmed the immobilization efficiency by spotting these three formulations parallel with the formulation previously selected for the protein chip[13]. The previously developed protein chip material showed a large spot signal, no loss of activity for the reserved proteins, and low background noise after incubation with the antibody. As shown in Figure 1a, the scanned images of the resulting spots with TATA-fwd -cy3 aptamer immobilized after harsh washing showed that TATA-fwd cy3 aptamers can be well immobilized in the three sol-gel formulations comparing to the protein chip material previously selected.

In addition to the three formulations showing high immobilization efficiency, we further tested the activity (Chip assay II) of the selected three materials (Scheme 1). The TATA reverse oligos were immobilized within the spots from three formulations, and then, the Cy-3 labeled TATA fwd oligos were incubated with sol-gel spots immobilized with the TATA reverse oligos which is complementary to TATA fwd oligos. After incubation and washing step, we found that formulation 3 shows better activity among three selected formulations (Figure 1b).

Optimization of the Selected Formulation

To obtain more optimized materials from the selected formulation 3, we subdivided formulation 3 into the five formulations (3A-3E) with different ratios of silicate monomers and additives (Scheme 1). Then we further tested the spots spotted from these five formulations along with positive and negative controls. Five formulations show the different percentage of silicate monomers, additives, and same percentage of buffer solution with nucleic acids as follows. Five formulations (3A-3E) shows 43.9%, 43.9%, 43.8%,

Figure 2. Optimization of the selected three formulations. Formulation 3 was subdivided into the five formulations (3A-3E). Chip assay I (2a), II (2b), and spot intensity analysis were carried out as described in the Materials and Methods. Formulation 3C showed the highest activity for aptamer chip.

43.8%, and 43.8% of TMOS, MTMS and GPTMOS respectively, 12.50%, 12.4%, 12.5%, 12.4%, and 12.5% of 10 mM HCl respectively, and 43.7% of buffer solution with nucleic acids (10 μM). As shown in Figure 2a, we can measure the strong fluorescent signals from the five spots before and after washing, which means immobilization efficiency is good with five materials. However, when we tested activity (Chip assay II), we could not observe the signals from the subdivided 5 spots except 3C spot (Figure 2b). Therefore, selected 3C spot shows the best material for aptamer chip.

Discussion

In this work, we tried to develop an optimal material for aptamer chip. First, an intensive material screening and selection protocol was used to isolate 25 sol-gel materials with the desired physical properties. They might have stability and resisting ability as an aptamer chip for nucleic acids. To optimize the sol-gel materials for aptamer chip, we could select the best three formulations among 25 sol-gel materials using TATA-fwd-cy3 oligo as an aptamer model. The physical properties of selected materials were tested and compared between the newly selected formulations (1-3) and previously confirmed sol-gel material for protein chips. They all showed the high immobilization efficiency. However, in the case of sol-gel material for protein chip, they showed the low spot intensity in the activity test. This indicates that this formulation is suitable for immobilizing of the protein or nucleic acid on the chip surface, but not optimal materials for aptamer chip. To optimize the sol-gel properties for aptamer chip, we subdivided formulation 3 into the five sub-formulations. One of these formulations, formulation 3C, has the most sensitive and exact properties to detect the interaction between TATA-fwd and TATA-rev oligomer. Finally, we could select the best sol-gel formulation for nucleic acid immobilization and hybridization. Comparing to the protein chips previously developed, this work showed that nucleic acids such as aptamers should be immobilized in different sol-gel formulation for biochip purpose[15,16].

The sol-gels produce an optically transparent 3D silica matrix that forms around the biomolecule of interest, thus stabilizing their nanoporous structure and functionality. Especially, the pore sizes of sol-gel

can be affected by the molar ratio between TMSO and water, and by pH due to the hydrolysis reaction[17]. Therefore, optimal size of nanoporous structure should fit to the size of the encapsulated biomolecules[18,19] and the specific binding reactions through the nanoporous channel should happen in sol-gel matrix[20].

The novel formulation from this study shows the possibility of biochip technology more applicable to diverse areas, especially for toxicity evaluation.

Materials and Methods

Oligo Preparations

The TATA fwd-cy3 (ssDNA: 5′-Cy3-GGGAA TTCGG GCTAT AAAAG GGGGA TCCGG-3′) was purchased (Bioneer, Korea). The TATA reverse oligo (ssDNA: 5′-CCGGA TCCCC CTTTT ATAGC CCGAA TTCCC-3′) were purchased (Bioneer, Korea).

Material Preparations

Solution containing 5-25% silicate monomers (tetramethy orthosilicate (TMOS; Gelest), n-methyltrimethoxysilane (MTMOS; Aldrich)), 2.5-15% of additives (3-glycidoxpropyltrimethoxysilane (GPTMOS; Sigma)) were used previously described[13].

Screening Assays

For the 625 formulations, we spotted each formulation to the surface of the chips using microarrayer (Accent, Gene machine) for the following assays previously described.

Adhesiveness Assays

The spots were tested for strong attachment on the chip surface after 10 min washing with 1X PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) at 25°C The spot formulations which did not show strong attachment were excluded.

Spot Morphology Assays

The shape and diameter of the spots were determined by the scanner (FLA-5100, Fuzi) in optical mode. The broken, oversized, oval-shaped spots were excluded.

Gelation Time Assay

The gelation time was measured by scratching the spots with a pipet tip every minute. We chose the spots with >1 hr gelation time.

Optical Transparency and Autofluorescence

The spots were scanned by fluorescent scanner (FLA -5100, Fuzi). The spots with fluorescent signals lower than that of background were selected.

Immobilization Efficiency (Chip Assay I)

To measure the efficiency of aptamer immobilization within the spots, we encapsulated Cy3 labeled TATA fwd oligos with screened sol-gel materials. The spot intensity both from before and after washing was measured by fluorescent scanner and compared by analysis program (Multi Gauge V2.2).

Activity (Chip Assay II)

To test the nucleic acid activity immobilized in the sol-gel materials, the interaction activity between complementary nucleic acids, TATA fwd and TAT rev oligomers was measured with each selected formulation from chip assay I. TATA rev oligos (150 pmole) complementary to TATA fwd were immobilized with screened formulations. Those spots with TATA rev were incubated with Cy3 labeled TATA fwd oligos (100 pmole) in binding buffer (25 mM Tris-HCl (pH 8), 100 mM NaCl, 25 mM KCl, 10 mM $MgCl_2$) for 1 hour at room temperature as previously described[6]. The sol-gel spots were washed 3 times with 200 μL washing solution for 5 minutes. The resultant spots were scanned and analyzed for activity.

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References

1. Yi, C. Q., Li, C. W., Ji, S. L. & Yang, M. S. Microfluidics technology for manipulation and analysis of biological cells. *Anal Chim Acta* **560**:1-23 (2006).
2. Song, M., Kim, Y. J. & Ryu, J. C. Toxicogenomic study of toxic mechanism on methotrexate, amiodarone, nitrofurantoin and carbamazepine in human bronchial epithelial (BEAS-2B). *Molecular & Cellular Toxicology* **3**:51-59 (2007).
3. Kim, Y. J., Kim, M. S. & Ryu, J. C. Genotoxicity and identification of differentially expressed genes of formaldehyde in human Jurkat cells. *Molecular & Cellular Toxicology* **1**:230-236 (2005).

4. Kasemo, B. & Hook, F. Protein and vesicle interaction with surfaces. *Abstr Pap Am Chem S* **223**:U444-U444 (2002).
5. Brody, E. N. *et al.* The use of aptamers in large arrays for molecular diagnostics. *Mol Diagn* **4**:381-388 (1999).
6. Lee, S. *et al.* Chip-based detection of hepatitis C virus using RNA aptamers that specifically bind to HCV core antigen. *Biochem Bioph Res Co* **358**:47-52 (2007).
7. Liao, W., Guo, S. & Zhao, X. S. Novel probes for protein chip applications. *Front Biosci* **11**:186-197 (2006).
8. Wang, H. *et al.* Identification of proteins bound to a thioaptamer probe on a proteomics array. *Biochem Bioph Res Co* **347**:586-593 (2006).
9. Wilson, D. S. & Szostak, J. W. In vitro selection of functional nucleic acids. *Annu Rev Biochem* **68**:611-647 (1999).
10. Sacca, B., Lacroix, L. & Mergny, J. L. The effect of chemical modifications on the thermal stability of different G-quadruplex-forming oligonucleotides. *Nucleic Acids Res* **33**:1182-1192 (2005).
11. Park, C. B. & Clark, D. S. Sol-gel encapsulated enzyme arrays for high-throughput screening of biocatalytic activity. *Biotechnol Bioeng* **78**:229-235 (2002).
12. Gill, I. Bio-doped nanocomposite polymers: Sol-gel bioencapsulates. *Chem Mater* **13**:3404-3421 (2001).
13. Kim, S. *et al.* Improved sensitivity and physical properties of sol-gel protein chips using large-scale material screening and selection. *Anal Chem* **78**:7392-7396 (2006).
14. Langelier, M. F. *et al.* Structural and functional interactions of transcription factor (TF) IIA with TFIIE and TFIIF in transcription initiation by RNA polymerase II. *J Biol Chem* **276**:38652-38657 (2001).
15. Livage, J., Coradin, T. & Roux, C. Encapsulation of biomolecules in silica gels. *J Phys-Condens Mat* **13**: R673-R691 (2001).
16. Ekins, R. & Chu, F.W. Microarrays: their origins and applications. *Trends Biotechnol* **17**:217-218 (1999).
17. Boury, B. & Corriu, R. J. P. Adjusting the porosity of a silica-based hybrid material. *Adv Mater* **12**:989-992 (2000).
18. Fujii, T., Yano, T., Nakamura, K. & Miyawaki, O. The sol-gel preparation and characterization of nanoporous silica membrane with controlled pore size. *J Membrane Sci* **187**:171-180 (2001).
19. Gogotsi, Y. *et al.* Nanoporous carbide-derived carbon with tunable pore size. *Nat Mater* **2**:591-594 (2003).
20. Gill, I. & Ballesteros, A. Bioencapsulation within synthetic polymers (Part 1): sol-gel encapsulated biologicals. *Trends Biotechnol* **18**:282-296 (2000).