Porous Silicon Microparticles as an Alternative Support for Solid Phase DNA Synthesis

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ABSTRACT

Current methods to produce short DNA strands (oligonucleotides) involve the stepwise coupling of phosphoramidites onto a solid support, typically controlled pore glass. The full-length oligonucleotide is then cleaved from the solid support using a suitable aqueous or organic base and the oligonucleotide is subsequently separated from the spent support. This final step, albeit seemingly easy, invariably leads to increased production costs due to increased synthesis time and reduced yields. This paper describes the preparation of a dissolvable support for DNA synthesis based on porous silicon (pSi). Initially it was thought that the pSi support would undergo dissolution by hydrolysis upon cleavage of the freshly synthesised oligonucleotide strands with ammonium hydroxide. The ability to dissolve the solid support after completion of the synthesis cycle would eliminate the separation step required in current DNA synthesis protocols, leading to simpler and faster synthesis as well as increased yields, however it was found that the functionalisation of the pSi imparted a stability that impeded the dissolution. This strategy may also find applications for drug delivery where the controlled release of carrier-immobilised short antisense DNA is desired. The approach taken involves the fabrication of porous silicon (pSi) microparticles and films. Subsequently, the pSi is oxidised and functionalised with a dimethoxytrityl protected propanediol to facilitate the stepwise solid phase synthesis of DNA oligonucleotides. The functionalisation of the pSi is monitored by diffuse reflectance infrared spectroscopy and the successful trityl labelling of the pSi is detected by UV-Vis spectroscopy after release of the dimethoxytrityl cation in the presence of trichloroacetic acid (TCA). Oligonucleotide yields can be quantified by UV-Vis spectroscopy.

Keywords: Porous silicon, solid phase DNA synthesis, microparticles

1. INTRODUCTION

Single stranded deoxyribose nucleic acid (DNA) can be produced in a controlled, stepwise manner[1] on commercial DNA synthesisers[1, 2]. This allows the manufacture and purchase of specific DNA strands on demand. Commonly the stepwise synthesis of DNA is performed on controlled pore glass (CPG) supports[2]. Because CPG is insoluble, it needs to be mechanically separated from the newly synthesised DNA. This separation step can result in reduced yields and add to the synthesis time, hence it is important for solid supports to facilitate fast straightforward separation of the DNA strand[3]. A dissolvable support would offer an interesting alternative to the conventional synthesis format. We have chosen to investigate porous silicon (pSi) as a candidate to replace common CPG, as it should readily dissolve during the final DNA cleavage and deprotection step typically carried out in ammonium hydroxide.

The conversion of bulk silicon into its high surface area, biocompatible porous counterpart is readily achieved by electrochemical etching in hydrofluoric acid (HF). This method allows pore sizes ranging from as small as 2 nm and greater than 100 nm to be achieved[4]. It has been observed that pSi undergoes degradation in aqueous environment, for example, biological fluids[5, 6]. Exposure of pSi to biological fluids facilitates its breakdown into silicic acid ($Si(OH)_4$) which is non-toxic and comprises nearly 95% of naturally occurring silicon in the environment[4], furthermore $Si(OH)_4$ is important in optimal bone growth[5]. This highlights porous silicon suitability as a scaffold for a wide range of biomedical applications such as drug delivery systems and tissue engineering scaffolds. pSi can be easily functionalised with many different chemical entities and the modified pSi could be useful for a range of applications from biosensors[7] to biomaterials[4, 5]. This functionalisation can be performed on either the hydride-terminated surface or the hydroxyl-terminated surface. Recent reviews give a comprehensive account of a range of surface chemistry

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modification techniques to introduce small molecules to pSi surfaces[8, 9]. Some recent uses of functionalized pSi films include vapor sensor chips[10, 11] and the creation of interfaces between biological and semiconductor surfaces[12] among others[8, 13-19].

pSi possesses a number of distinct advantages over conventional CPG. The most significant being the ease of controlled manufacture of pSi with different porosities, pore sizes and structures to meet a specific application[12, 16, 20]. This is achieved by varying the dopant type and amount, current, charge densities and the etching solutions components and concentrations. Furthermore, the surface functionalisation of pSi can be carried out with relatively mild reaction conditions and with readily available chemicals. Another distinct advantage that pSi has is its ability to be produced in structures such as microparticles. The size of pSi microparticles can be controlled by sonication to fragment the larger pSi film into smaller particles[21]. The ability to produce pSi in various forms such as 2 dimensional films or 3 dimensional particles may also give rise to new DNA synthesis formats. The focus of this study was to take advantage of pSi's ability to be solubilised with aqueous base[22] which is used during the final cleavage step of DNA synthesis. This approach utilises a pSi solid support with a covalently attached linker which is terminally functionalised with a dimethoxytrityl (DMT) protected primary alcohol, which can be utilised as a novel solid phase for DNA synthesis.

Azhayev[3] reported the synthesis of seven new solid phase supports derivatised from long chain alkylamino CPG (lcaaCPG). These new supports allow for the preparation of oligonucleotides in conventional DNA synthesizers with no modification to the existing equipment. These solid supports are designed to facilitate faster cleavage of the 3'-terminal phosphodiester group, which can be performed in 33% NH_3/H_2O at room temperature for 2 hours, which is a vast improvement from the usual 60°C and 6 hour treatment. Cleavage is facilitated by deprotection of the 2° hydroxyl group before it assists in the attack on the 3' phosphate group releasing the synthesised oligonucleotide with a terminal 3' hydroxyl group. Azhayev produced supports with 100μmol of DMT per mg of CPG before successfully synthesising and cleaving DNA from the supports with a yield of 42% and purity of 82%. These attributes make Azhayev's linker appealing for use on pSi as the synthesis and functionalisation conditions are compatible with pSi. Furthermore the fast cleavage combined with the ability of the underlying pSi support to degrade should lead to the creation of volatile supports that facilitate the release of functional DNA possessing a 3' hydroxyl group.

The synthesis of DNA has previously been attempted on porous silicon surfaces as well as Si(111) and pSi silicon nanoparticles[23, 24]. The previous synthesis work used the surface hydrosilation of hydrogen terminated pSi surfaces with 1,ω-dimethoxytritylundecenol. This produced a surface with DMT groups that could be used as a scaffold for the automated synthesis of DNA. To create functional nanoparticles the pSi films were broken up by the necessary reflux used during the hydrosilation reaction.

2. METHODOLOGY

2.1 Microparticle Fabrication

Microparticles were electrochemically etched from boron doped p^{++} wafers (3", boron doped, <1-0-0>, 500±25μm single sided polished wafers with resistivity <0.001Ωcm^{-1}) supplied by Virginia Semiconductors. The etching solution consisted of a 3:1 v/v mixture of aqueous 49% HF and ethanol. Etching was carried out in an 18 cm^2 etching cell using a two-electrode configuration with a Pt mesh counter electrode. A current density of 222 mAcm^{-2} was applied for 2 minutes and the pSi film was then removed from the Si substrate by applying a current density of 500 mAcm^{-2} for 30 seconds. Dichloromethane was then added, which resulted in a free-standing porous film lifting off of the Si substrate, this film broke up into smaller pieces. The film pieces were then briefly sonicated to create pSi microparticles. The pSi microparticle suspension was transferred to a glass reaction vessel that contained a fine glass frit. The solvent was removed by aspiration. Subsequent reactions were performed in the same vessel to exclude loss of the pSi microparticles due to transfer.

2.2 Synthesis of (±)-3-amino-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol (3)

All reactions were carried out under inert conditions using argon supplied through a septum by a syringe needle and a balloon. Dichloromethane (Merck) and pyridine (BDH) were distilled over CaH_2 and stored under molecular sieves away from light. Tetrahydrofuran (Ajax) was distilled over sodium and benzophenone.

^1H and ^{13}C spectra were obtained on a Varian Gemini Spectrometer operating at either 200 or 300MHz. All spectra were recorded in deuterated chloroform with 1% tetramethylsilane (TMS) unless noted otherwise. The spectra were referenced to the $CDCl_3$ peak or the TMS peak. Electrospray Ionisation (ESI) spectra were recorded using a Water/Micromass Quattro micro triple quadrupole mass spectrometer fitted with an electrospray source. The spectra were scanned in positive ion mode from 100 m/z to 700 m/z, in 2 seconds. The cone voltage was set at 60 V while the ESI capillary voltage was 3.0 kV and the source temperature was 80 °C. The solvent flow was 50% aqueous acetonitrile at a flow rate of 10 μL/min provided by an integral syringe pump.

(1) (±)-3-Trifluoroacetamido-1,2-propanediol

3-amino-1,2-propanediol (10mmol, 0.91g, Aldrich) was dissolved and stirred in methyltrifluoroacetate (10ml, 100mmol, Fluka) overnight at 20°C. The resulting colourless oil was co-evaporated 3 times with 30ml of toluene to yield **(1)** as a viscous colourless oil, yield 1.42g, 76.1%. ^1H NMR δ were consistent with those reported by Asheyev et al.[3]. ^{13}C NMR; δ 45.0, 66.3, 72.5, 118.7 and 138.2ppm.

(2) (±)-3-Trifluoroacetamido-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol

Dimethoxytrityl-chloride (DMT-Cl) (5mmol, 1.7g, Fluka) was added to a solution of compound **(1)** (5mmol, 0.94g) in 20ml of anhydrous pyridine and was stirred overnight at 20°C before being quenched with 2ml of methanol. The solvents were evaporated and the residue was dissolved in 50ml of ethyl acetate. The organic solution was washed with saturated sodium bicarbonate (3 x 30ml) and water (3 x 30ml), dried with Na_2SO_4 and evaporated to dryness. Flash chromatography of the crude reaction mixture using a mixture of ether and hexane as the solvent system (gradient was stepped from 25% to 50% and finally 75% ether) provided compound **(2)** as a viscous yellow oil (0.6g, 16.2%). ^1H NMR δ were consistent with those reported by Asheyev et al.[3]. ^{13}C NMR; δ 42.4, 55.5, 64.2, 69.1, 113.4, 127.2, 128.0, 129.2, 130.1, 139.7 and 158.8ppm.

(3) (±)-3-amino-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol

Compound **(2)** (0.6g, 1.2mmol) was dissolved in 9M NH_3 (20mL) solution (prepared by diluting 16M aqueous NH_3 with methanol), was stirred overnight at 20°C. The reaction mixture was then evaporated to dryness to provide compound **(3)**, 0.55g (100%) of colourless oil. ^1H NMR δ were consistent with those reported by Asheyev et al.[3]. ^{13}C NMR; δ 43.9, 55.4, 65.5, 69.3, 113.4, 127.1, 128.1, 128.2, 130.1, 135.9 and 158.8. LCMS (ESI) calcd for $C_{24}H_{27}NNaO_4^+$ $(M + Na)^+ = 416.2$. Found 416.2 (100), 303.2 (60), 112.2 (10).

2.3 Microparticle Functionalisation

During microparticle functionalisation all reaction mixtures were shaken by a wrist action shaker. The pSi microparticles were suspended in 5ml of 100mM 3-aminopropyl-dimethyl-ethoxysilane in toluene and allowed to react at 60°C for 19h. The microparticles were then washed with acetonitrile (3 x 1ml) and dried under N_2. The newly generated surface amine groups were converted to a carboxylic acid functional surface by adding a mixture of succinic anhydride 7.5mg (7.5μmol) in pyridine (0.5ml) and 1-methylimidazole:THF (4:21, 0.5ml). The mixture was shaken overnight at 20°C. The pSi microparticles were filtered and washed with pyridine (3 x 1ml) and THF (3 x 1ml) and then dried under N_2. Subsequently, all un-reacted NH_2 groups were capped using a mixture of 1-methylimidazole:THF (4:21, 0.5ml) and acetic anhydride:2,6-lutidine:THF (1:1:8, 0.5ml). The mixture was agitated for 2h at 20°C. The microparticles were filtered and washed with THF (1ml), pyridine (1ml) and acetonitrile (3 x 1ml) and dried under N_2. The thus derivatised microparticles were mixed with **(3)** (0.012g, 30μmol) in a pyridine solution (0.75ml) containing 1-hydroxy-benzotriazole (4.6mg, 30μmol) and N,N'-diisopropylcarbodiimide (4.7μL, 30μmol). The mixture was left to agitate overnight at 20°C after which the microparticles were filtered and washed with acetonitrile (3 x 1ml) before being dried under N_2. The microparticles were then suspended in a mixture of 2,6-lutidine:THF (1:2.6, 1ml) and formic acid:acetic anhydride (1:1.68, 0.27ml) was added over a 3h period. The mixture was left agitating overnight at 20°C. The microparticles were then washed with acetonitrile (3 x 1ml) before being dried under N_2. Finally, the microparticles were collected from the reaction vessel and transferred to a sealed glass vial.

To facilitate the collection of infrared (IR) spectral data the complete pSi functionalisation was simultaneously carried out on a pSi film and both surfaces were monitored. All IR spectra were obtained using a Nicolet Avatar 370MCT from Thermo Electron Corporation equipped with a Smart Diffuse Reflectance Accessory. Diffuse Reflectance Infra-red Fourier Transform (DRIFT) spectra were recorded and analysed using OMNIC version 7.0 software. Spectra were obtained in the range of 650-4000cm^{-1} at a resolution of 1 cm^{-1} or 4 cm^{-1}. All spectra were blanked using a flat silicon wafer.

The derivitisation of the pSi microparticles with the DMT protected linker was assayed by performing the acidic cleavage of the DMT cation from the pSi microparticles surface. The cleavage was performed in 2% trichloroacetic acid (TCA) in DCM. The absorbance spectrum (λ_{max} = 498 nm) was measured on a Hewlett Packard 8452A diode array spectrophotometer and analysed using Agilent 8452 UV-Visible Chemstation Software.

SEM was performed on a Philips XL30 field emission scanning electron microscope, with an acceleration voltage of 10kV. The samples were imaged without coating as the low resistivity and semi-conducting nature of the pSi facilitates the dissipation of charge build up.

2.4 DNA Synthesis and Characterisation

DNA synthesis was carried out on an Applied Biosystems 391 DNA Synthesiser PCR Mate EP with an extra round of de-tritylation steps and an extra 20-second hold step to extend the time allowed for the phosphoramidite to couple. The synthesis of a polyG-6mer (5'-GGG GGG-3') single-strand of DNA on the microparticles was performed using an empty Applied Biosystems column filled with functionalized pSi particles. A polyG-6mer was also synthesised on a regular CPG column for comparison. Further DNA synthesis (polyG-6mer and polyC-6mer) was conducted on an ABI 394 with a 45 second coupling step to encourage a higher yield.

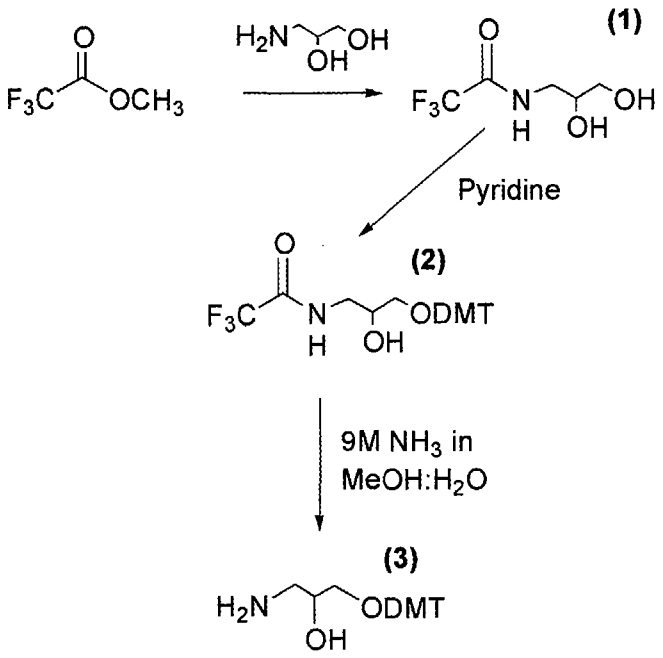
After the completion of the DNA synthesis, ammonium hydroxide was added to the microparticles and heated at 50°C overnight to dissolve the microparticles and deprotect the DNA strand. pSi microparticles that had not completely solubilised into solution after DNA cleavage required filtration. This could be carried out simultaneously with the required DNA gel-filtration step. This was performed using microspin^{TM} G-25 columns (Amersham Pharmacia Biotech) by following the manufacturer's instructions without any modifications.

The concentration of the synthesised DNA was determined by UV-Vis spectroscopy. The characteristic 260nm absorbance of the synthesised DNA strands was measured in a quartz cuvette using the Hewlett Packard 8452A diode array spectrophotometer. The measured absorbance value was fitted to the Beer-Lambert law assuming a molar extinction coefficient of 62000M^{-1}cm^{-1} for polyG-6mer.

3. RESULTS AND DISCUSSION

3.1 Linker preparation

3-Trifluoroacetamido-1,2-propanediol **(1)** was obtained in 76% by amidation of 3-amino-1,2-propanediol (scheme 1) following a procedure described by Asheyev et al.[3]. (±)-3-Trifluoroacetamido-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol **(2)** was then produced and isolated by flash chromatography to yield 16.2%. Subsequently (±)-3-amino-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol **(3)** was produced by the cleavage of the trifluoroacetate protecting group by exposure to 9M ammonia overnight and yielded 100%. The successful synthesis of **(3)** was confirmed by mass spectrometry and NMR.

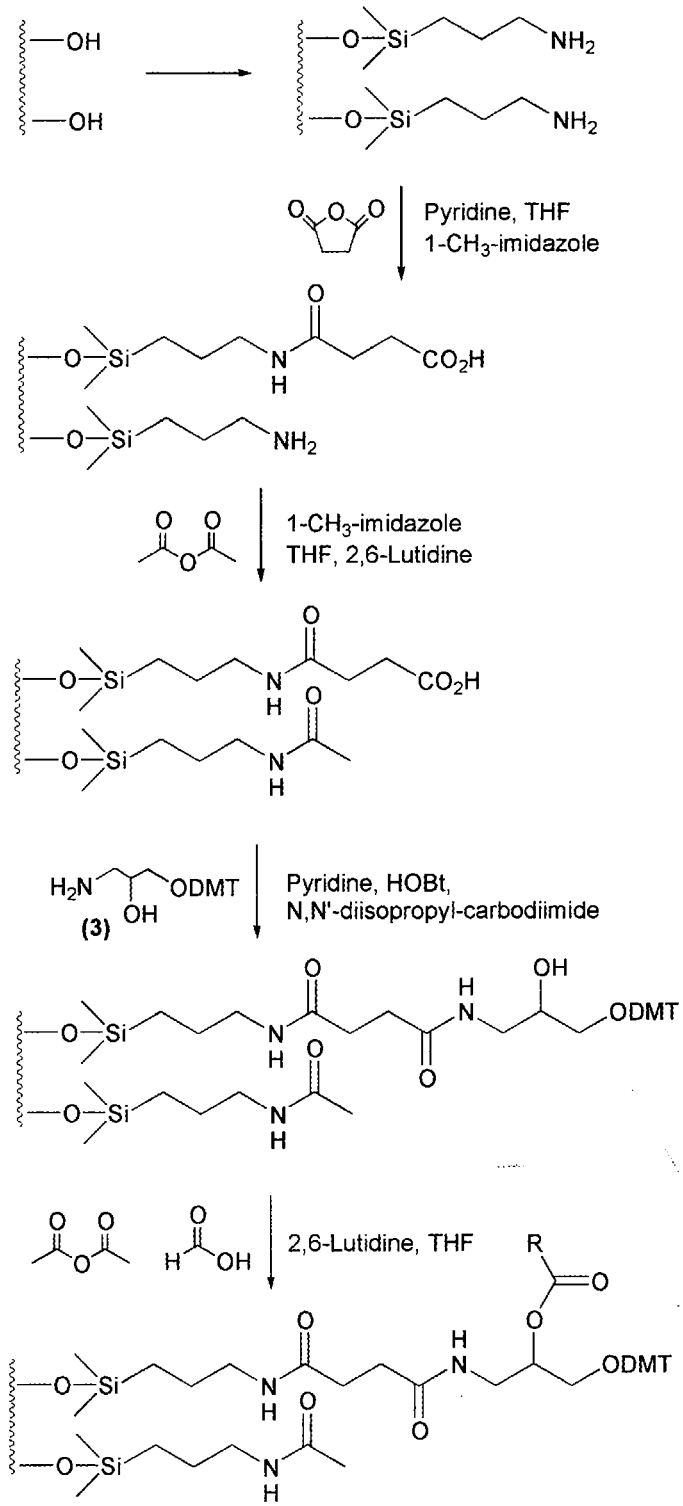


Scheme 1: Synthesis of (±)-3-amino-1-(4,4'-dimethoxytriphenylmethyl)-2-propanediol **(3)**

3.2 Microparticle Functionalisation

The microparticle functionalisation was monitored by IR spectroscopy. To ensure that the IR spectra obtained provided as much information about the surface chemistry as possible the reaction was carried out on a pSi film and monitored by IR spectroscopy.

The pSi microparticle functionalisation depicted in Scheme 2 adapted from Azheyev et al. [3] involved the formation of the hydroxyl functionalised surface followed by silanisation with 3-aminopropyl-dimethyl-ethoxysilane. The NH_2 surface was then converted to a carboxyl surface by exposure to succinic anhydride in the presence of pyridine and imidazole. As a precautionary step all unreacted NH_2 groups were capped by acetylation with acetic anhydride. The microparticles were then coupled to **(3)** and then formylated to inhibit the secondary alcohol reacting in the DNA synthesiser.



Scheme 2: Generation of the DMT functional pSi surface

Reactions carried out on a pSi film were monitored by IR spectroscopy and are shown below in Fig. 1.

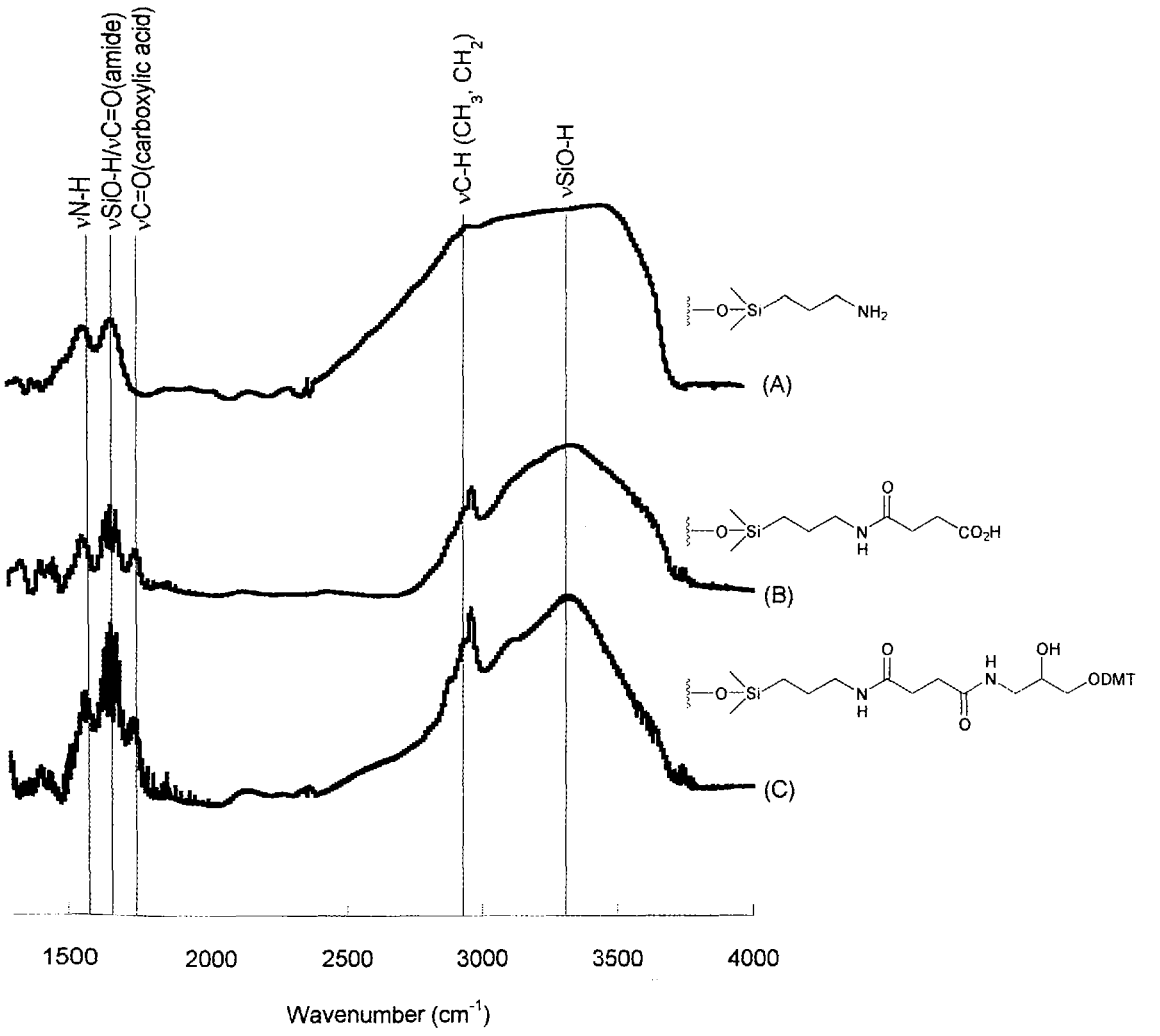


Figure 1: DRIFT spectra of functionalised pSi film. (A) Silanised (amine-terminated) surface; (B) Silanised surface capped with succinic anhydride (carboxylic acid terminated surface with uncapped amino groups); (C) DMT-terminated surface. Spectra (A) was obtained at a resolution of 4 cm^{-1}, while (B) and (C) were obtained at a resolution of 1 cm^{-1}. The spectral width has been narrowed for comparison.

The amine-terminated surface showed Si-CH_3 bending at 1260 cm^{-1} and CH stretching in CH_2 and CH_3 arising from the short propyl chain and the two methyl groups of the silane tether (Not shown). No significant stretch at 1000-1350 cm^{-1} for C-N stretching was observed, however this may be buried in the large Si-O stretch at 1200 cm^{-1}(Not shown). The N-H bend for the primary amine is at 1571 cm^{-1} while the peaks at 1654 cm^{-1} and 3400 cm^{-1} may be attributed to the SiO-H bending mode[16].

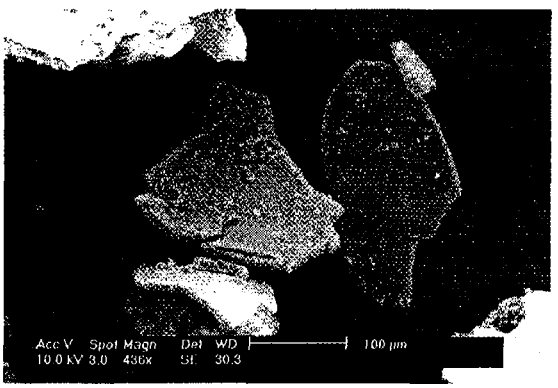
The conversion of the amine functional surface to the carboxylic acid derivative occurs via the ring opening of succinic anhydride to create an amide bond. The DRIFT spectra now shows peaks at 1561 cm^{-1} for N-H bending and 1737 cm^{-1} for the carboxylic acid (Fig. 1(B)). The peak at 1656 cm^{-1} may be due to the SiO-H bending mode as well as for the C=O stretch in the amide linkage.

Conjugation of the DMT protected linker **(3)** to the terminal carboxylic acid surface produced no new peaks in the DRIFT spectra (Fig. 1(C)). Due to the prominent OH peak from 3000 – 3600 cm^{-1} the normally weak aromatic C-H stretch at 3000-3100 cm^{-1} could not be detected in the spectra.

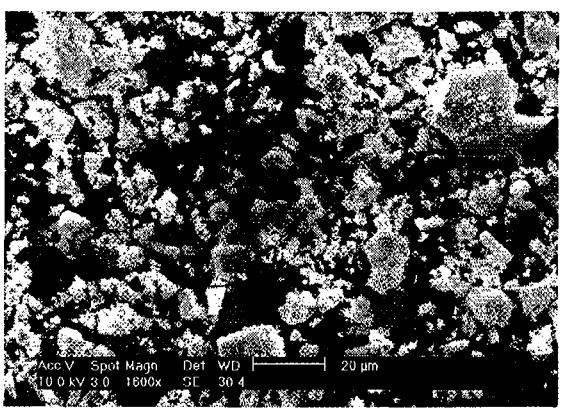
The DMT functionalisation of the microparticles was assayed via the addition of trichloroacetic acid (TCA) and subsequent UV-Vis analysis. For both functionalisation pSi microparticles and films the DMT cation was detected, demonstrating that functionalisation was successful.

3.3 Scanning Electron Microscopy (SEM)

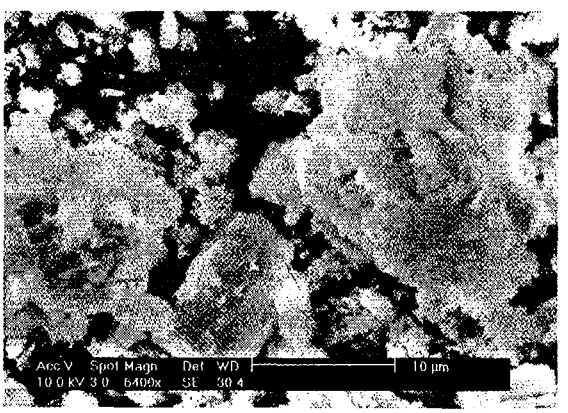
SEM was used to characterise the microparticles size and morphology. The SEM of the microparticles revealed particles of varying sizes, from over 100μm to just a few μm (Fig. 2). SEM of the ozone oxidised microparticles before sonication showed an irregular shape with sizes typically over 100μm (Fig. 2(A)). Prolonged sonication can be used as one method to control the size of the microparticles[21]. The SEM of sonicated microparticles showed that the typical size of the microparticles had decreased significantly, from 100 μm to less than 20 μm (data not shown). The size of the microparticles after the functionalisation ranges from a few μm to about 20μm (Fig. 2(B) and (C)), this suggests that the agitation applied during functionalisation has a similar effect on the pSi microparticles as sonication does.



(A)



(B)



(C)

Figure 2: SEM micrographs of (A) oxidised pSi microparticles before sonication, (B) functionalised oxidised pSi microparticles. (C) High resolution SEM micrograph showing functionalised oxidised pSi microparticles.

3.4 DNA Synthesis on pSi Supports

Short DNA oligonucleotides (polyG-6mer, G6) were synthesized using DMT functionalized pSi and CPG as solid supports. After completion of the synthesis cycle, the solid supports were incubated in aqueous ammonium hydroxide to cleave the oligonucleotides off the support. In the case of pSi microparticles, the pSi scaffold was expected to dissolve within minutes, however it was found that both the CPG and pSi microparticles had to be removed by filtration. The

amount of DNA produced was quantified by measuring the absorbance at 260 nm. Table 1 shows the nano-moles of oligonucleotide per milligram of solid support used.

Table 1: Quantification of oligonucleotide synthesized on pSi microparticles and standard CPG

Sample	DNA produced (nmol/mg)
pSi microparticle G6	4.36
CPG G6	6.77

The amount of DNA produced on each support (Table 1) shows that 35% less oligonucleotide was synthesized per mg of functionalised pSi support. This result indicates that pSi is a suitable support for DNA synthesis. It is envisioned that the yield may be increased by optimising the particle size and distribution of the pSi scaffold. Furthermore, the influence of the pSi pore size needs to be investigated as does the ability of the scaffold to remain intact during the synthesis of longer DNA oligonucleotides.

5. CONCLUSIONS

The functionalisation of both pSi films and pSi microparticles with a DMT protected linker is described. Short DNA oligonucleotides were synthesized using the functionalized pSi particles as solid support. Notably, DNA synthesis could be achieved without modification to the automated DNA synthesizer or synthesis protocols. Upon cleavage and deprotection of oligonucleotides on pSi in ammonium hydroxide solution, the solid support did not dissolve as anticipated. Oligonucleotides thus prepared were quantified by UV-Vis spectroscopy. Although the determined yields for the oligonucleotides synthesized on pSi particles were lower than on commercial CPG, it is anticipated that similar or even superior yields could be obtained after optimization of the pSi particle size and its distribution as well as the pore size.

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REFERENCES

1. Efcavitch, J., *Automated System for the Optimized Chemical Synthesis of Oligodeoxyribosenucleotides.* Macromolecular Sequencing and Synthesis, Selected Methods and Applications, 1988: p. 221-234.
2. Applied-Biosystems, *Section 6: Chemistry for Automated DNA Synthesis*, in *Applied Biosystems 391 DNA Synthesiser PCR Mate EP Users Guide.* 1989, Applied Biosystems. p. 6-1 to 6-36.
3. Azhayev, A., Antopolsky, M., *Amide group assisted 3'-dephosphorylayion of oligonucleotides synthesized on universal A-supports.* Tetrahedron, 2001. **57**: p. 4977-4986.
4. Canham, L., Aston, R., *Will a chip everyday keep the doctor away?* Physics World, 2001: p. 27-31.
5. Anderson, S., Elliott, H., Walli , D., Canham, L., Powell, J., *Dissolution of different forms of porous silicon wafers under simulated physiological conditions.* phys. stat. sol., 2003. **197**(2): p. 331-335.
6. Canham, L., *Bioactive Silicon Structure Fabrication Through Nanoetching Techniques.* Adv. Mater., 1995. **7**(12): p. 1033-1037.
7. Steinem, C., Janshoff, A., Lin, V., Voelcker, N., Reza Ghadiri, M., *DNA hybridisation-enhanced porous silicon corrosion: mechanistic investigations and prospect for optical interferometric biosensing.* Tetrahedron, 2004. **60**: p. 11259-11267.
8. Buriak, J., *Silicon-Carbon Bonds on Porous Silicon Surfaces.* Adv. Mater., 1999. **11**: p. 3.
9. Schmeltzer, J., Buriak, J., *Recent Developments in the Chemistry and Chemical Applications of Porous Silicon*, in *The Chemistry of Nanomaterials: Synthesis, Properties and Applications*, C. Rao, Muller, A., Cheetham, A., Editor. 2004, Wiley. p. 518-550.
10. Gao, T., Gao, J., Sailor, M., *Tuning the Response and Stability of Thin Film Mesoporous Silicon Vapor Sensors by Surface Modification.* Langmuir, 2002. **18**: p. 9953-9957.
11. Bakker, J., Arwin, H., Wang, G., Järrendahl, K., *Improvement of porous silicon based gas sensors by polymer modification.* Phys. Stat. Sol., 2003. **197**(2): p. 378– 381.

12. Wojtyk, J., Morin, K., Boukherroub, R., Wayner, D., *Modification of Porous Silicon Surfaces with Activated Ester Monolayer.* Langmuir, 2002. **18**: p. 6081-6087.
13. Boukherroub, R., Morin, S., Wayner,. D., Lockwood, D., *Thermal route for Chemical Modification and Photoluminescence Stabilisation of Porous Silicon.* Phys. Stat. Sol., 2000. **182**: p. 117-121.
14. Boukherroub, R., Petit, A., Loupy, A., Chazalviel, J., Ozanam, F., *Microwave assisted Chemical Functionalisation of Hydrogen-Terminated Porous Silicon Surfaces.* J. Phys. Chem. B, 2003. **107**: p. 13459-13462.
15. Boukherroub, R., Wayner,. D., Lockwood, D., Zargarian, D., *Photoluminescence quenching of chemically functionalised porous silicon by a ruthenium cluster.* Phys. Stat. Sol., 2003. **197**(2): p. 476-481.
16. Xu, D., Sun, L., Li, H., Zhang, L., Guo, G., Zhao, X., Gui, L., *Hydrolysis and silanisation of the hydrosilicon surface of freshly prepared porous silicon by an amine catalytic reaction.* New J. Chem., 2003. **27**: p. 300-306.
17. Singh, P., Lakshmikumar, S., *Quenching and Recovery of Photoluminescence Intensity of Silicon Nanoparticles Embedded in Optically Transparent Polymers.* Semicond. Sci. Technol., 2002. **17**: p. 1123-1127.
18. Robins, E., Stewart, M., Buriak, J., *Anodic and Cathodic electrografting of alkynes on porous silicon.* Chem. Commun., 1999: p. 2479-2480.
19. Lees, I., Lin, H., Canaria, C., Gurtner, C., Sailor, M., Miskelly, G., *Chemical Stability of Porous Silicon Surfaces Electrochemically Modified with Functional Alkyl Species.* Langmuir, 2003. **19**: p. 9812-9817.
20. Canham, L., Reeves, C., Newey, J., Houlton, M., Cox, T., Buriak, J., Stewart, M., *Derivatised Mesoporous Silicon with Dramatically Improved Stability in Simulated Human Blood Plasma.* Adv. Mater., 1999. **11**(18): p. 1505-1507.
21. Cunin, F., Schmedake, T., Link, J., Li, Y., Koh, J., Bhatia, S., Sailor, M., *Biomolecular screening with encoded porous-silicon photonic crystals.* Nat. Mater., 2002. **1**: p. 39-41.
22. Allongue, P., Costa-Kieling, V., Gerischer, H., *Etching of Silicon in NaOH solutions II. Electrochemical Studies of n-Si(111) and (100) and Mechanism of the Dissolution.* J. Electrochem. Soc., 1993. **140**(4): p. 1018-1026.
23. Lie, L., Patole, S., Pike, A., Ryder, L., Connolly, B., Ward, A., Tuite, E., Houlton, A., Horrocks, B., *Immobilisation and synthesis of DNA on Si(111), nanocrystalline porous silicon and silicon nanoparticles.* Faraday Discuss., 2004. **125**: p. 235-249.
24. Pike, A., Lie, L., Eagling, R., Ryder, L., Patole, S., Connolly, B., Horrocks, B., Houlton, A., *DNA on silicon devices: On-chip synthesis, Hybridisation, and Charge Transfer.* Angew. Chem. Int. Ed, 2002. **41**(4): p. 615-617.