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A NEW SOLID-PHASE SUPPORT FOR OLIGONUCLEOTIDE SYNTHESIS

Rodrigo V. Devivar,[1]* Steve L. Koontz, William J. Peltier, James E. Pearson,
Todd A. Guillory, and Jill D. Fabricant
FlowGenix Corporation, 100 E. NASA Road 1, Suite 102, Webster, TX, 77598, *U.S.A.*

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Abstract. An alternative solid support for oligonucleotide synthesis was developed by coupling a polymer colloid to a modified polyethylene filter disc. The functions on the polymer colloid not used for attachment to the surface were derivatized with a Jeffamine® diamine and loaded with appropriate deoxynucleoside succinates. The performance of this support system was evaluated and compared to existing resins. © 1999 Elsevier Science Ltd. All rights reserved.

Over the years, many solid support systems have been studied for DNA synthesis, including Sephadex,[2] silica gel,[3] polydimethylacrylamide resins,[4] polyacrylmorpholide,[5] Kieselguhr-polydimethylacrylamide composites,[6] cellulose,[7] glass beads of controlled pore size (CPG),[8] a Teflon© laminated membrane system (MemSyn™)[9], and various polystyrene resins.[10-12] The development of different solid support systems has resulted in improvements in various loading methodologies,[13] improved coupling reagents,[14] more efficient oxidation solutions[15] and capping reagents,[16] and more efficient cleavage and deprotection conditions.[17] Despite these advances, the scope of automated DNA synthesis has been somewhat limited by the apparent lack of an ideal solid-support system.

We set out to develop a new type of solid-phase support system for DNA synthesis that would use inexpensive and widely available macroporous polymeric materials and overcome some of the drawbacks encountered in existing solid supports such as fragility, scalability, and handling complications. This type of approach would require the use of a gaseous, low-pressure, remote plasma process[18] for the surface modification of polyolefins. The remote plasma process affords an efficient introduction of covalently bound functional groups that are uniformly distributed throughout the interstitial pore surfaces of polymeric materials without producing significant degradation of the bulk polymer. Hence, the flow characteristics, mechanical properties, and chemical resistance of the starting porous material are essentially unchanged. When ammonia is the gas source for this process, amino functions are introduced on the surface of polyolefins.

To create a functional synthesis support, we loaded the aminated material (**1a**, in the shape of a disc: 0.25 inch diameter, 0.0625 inch thick) with 5'-DMT-C^{Bz}-succinate according to established procedures.[13a] This treatment generated support **1b**, which produced quality DNA. Our initial evaluation was performed on two types of sintered polyethylene (Porex® X-4920, nominal pore size of 30 μm, and Porex® T^3, nominal pore size of 8 μm). The major drawback of these materials was the low capacity due to their low surface areas.

We reasoned that the best approach to increase the synthesis capacity would be to introduce a scaffold so as to increase the number of usable functional groups. This type of system could be achieved by coupling a

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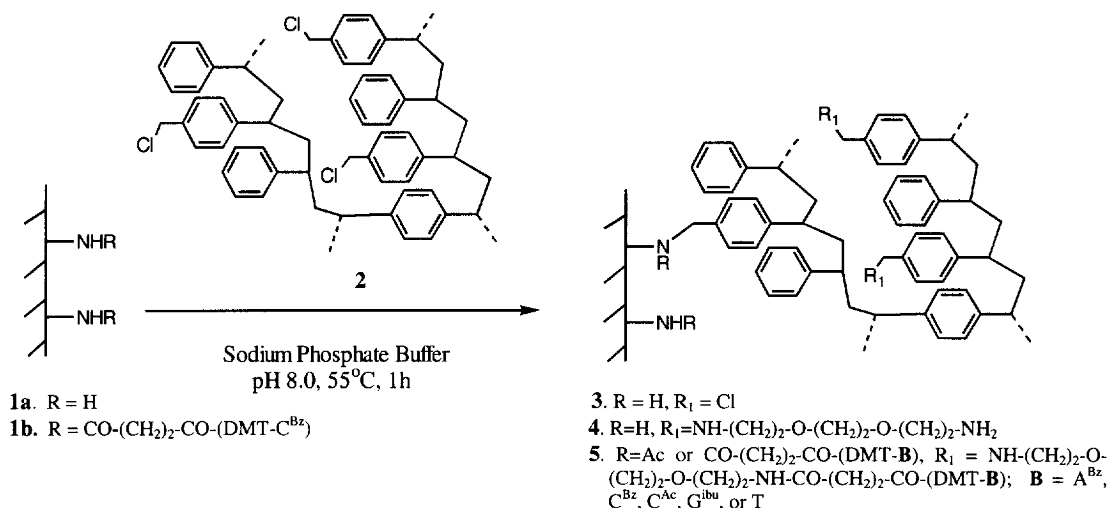
polymer colloid (**2**) to the pore surfaces of the amino-modified macroporous material (**1a**). Polymer colloids are latex particles of uniform size[19] that can be efficiently coupled to modified surfaces via a covalent linkage and can be engineered so as to produce the desired increase in usable functions. One of our requirements for a suitable polymer colloid was a low level of internal cross-linking to permit swelling in organic solvents, thereby allowing access to incoming reagents and permitting efficient wash cycles. The colloid we used for this investigation (**2**)[20] had a diameter of 0.46 μm and an internal composition of styrene (50%), chloromethylstyrene (49.8%), and divinylbenzene (0.2%). The relatively high content of chloromethylstyrene gave us ample functions for further synthetic manipulation. We chose Porex® X-4920, the material with larger pores for this study.



Scheme 1

Coupling of the polymer colloid (**2**) to the aminated surface of Porex® X-4920 (**1a**) was performed in sodium phosphate buffer, pH 8 at 55 °C for 1 h. Uncoupled colloid was washed away with several water rinses. The resulting surface structure (**3**) was then treated with a solution of triethylene glycol diamine (Jeffamine®[21] XTJ-504) to afford a pore-surface gel phase with structure **4**. The amino functions of **4** were then loaded for DNA synthesis by using established methods.[13a] After capping any underivatized amino functions with acetic anhydride/1-methylimidazole,[16] a solid support having structure **5** was achieved. The measured loading of solid support **5** (Table 1) was almost 200 times higher than solid support **1b**, thereby realizing the advantage of coupling a polymer colloid to the modified surface (**1a**). The DNA synthesis performance of **5** was evaluated[22] and compared with several other solid supports; the results are summarized in Table 1, and Figures 1 and 2.

Overall, the synthesis performance of **5** was comparable to CPG and polystyrene and superior to TentaGel and ArgoGel. The MALDI-TOF spectrum of an oligonucleotide prepared on support **5** (loaded with C^{Bz}) was devoid of extensive failure sequences (Figure 1). To ensure that **5** had applicability for routine DNA

synthesis, it was loaded with the common deoxynucleoside succinates and evaluated for DNA synthesis performance (Table 1). The preparation of a 15-base long phosphorothioate on support **5** using the Beaucage reagent afforded a full-length oligonucleotide with a 98.1% overall coupling efficiency (data not shown).

Table 1. Performance of Solid Phase Supports at 200 nmol in Preparing a 15-base Long Oligonucleotide.

Material	Loaded Base	Loading, μmol/g	Final OD units	Final Yield	% Purity-HPLC
1b (Porex® X4920)	C^{Bz}	0.07	0.16	64.0%	ND
5	C^{Bz}	11.41	23.33	76.5	77.9
5	C^{Ac}	9.35	18.88	61.9	82.3
5	A^{Bz}	12.27	18.52	60.7	75.8
5	G^{ibu}	9.19	12.87	41.1	82.3
5	T	10.84	17.50	56.9	83.4
TentaGel	C^{Bz}	45.8	7.90	25.9	27.7
ArgoGel	C^{Bz}	116.9	17.61	57.8	6.7
Polystyrene	C^{Bz}	12.2	14.50	47.2	80.3
CPG	C^{Bz}	35.3	18.22	59.2	74.3

All syntheses were performed on crimp-style columns. ND = Not determined. Polystyrene LV200 (PE-BioSystems) was used. ArgoGel (NH_2) was obtained from Argonaut Technologies; TentaGel (S NH_2) was obtained from Advanced Chemtech; both were loaded according to methods described in reference 13a. The loading levels were determined according to the procedure described in reference 23. Data for ArgoGel, TentaGel and polystyrene represent the mean of four or more experiments. Data for all other materials represent the mean of 20 or more experiments. The HPLC conditions reported in reference 25 were used along with a Dionex HPLC system equipped with a Gen-Pak™ Fax column (Waters).

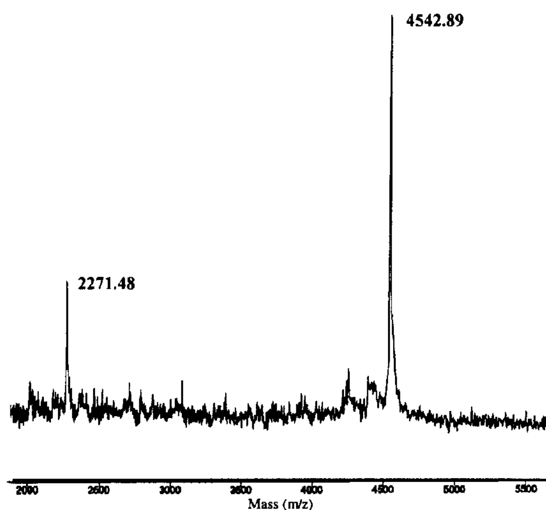


Figure 1. MALDI-TOF Spectrum of a 15-mer prepared on support **5** (**X** =C). Calculated m/z = 4544, measured m/z = 4542.89

Figure 2. 20% Bisacrylamide Gel of 15-mers prepared on support **5** (lane 1), TentaGel (lane 2), ArgoGel (lane 3) and CPG (lane 4).

Our initial experiments indicate that support **5** may be used for the preparation of synthetic oligonucleotides to afford quality DNA in good yield using instruments and processes optimized for particulate resins like CPG and polystyrene. The DNA synthesis performance of **5** was comparable to the commonly used solid supports, yet support **5** offers certain advantages. It may be cut to almost any shape and can therefore be used with most synthesis column designs. The rigidity of the bulk polyethylene material

offers dimensional stability. The one-piece design offers certain handling advantages and essentially eliminates problems associated with particulate fines. The mean pore size of the polyethylene material allows complete reagent flow through and ample space for the preparation of polymeric molecules such as DNA.

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20. Polymer colloid (**2**) was prepared by Bangs Laboratories, Inc., Fishers, Indiana.
21. Jeffamine® diamines are products of the Huntsman Corporation, Houston, Texas.
22. An Expedite 8909 DNA synthesis instrument (PerSeptive Biosystems) was used for all syntheses using phosphoramidite chemistry[24]. The sequence used in this study was: 5'- TCT-AGC-TAG-CTA-GC**X**-3', **X** = A, C, G, or T. The instrument software programs designed for CPG were used without modification. Oligonucleotides were cleaved and deprotected with ammonium hydroxide at 85 °C for 30 min. Before HPLC or gel analysis, the oligonucleotides were desalted on a Sephadex® G-25 column.
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