A New High Efficiency Hybrid Solid Support for Oligonucleotide Synthesis

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Introduction

As more oligonucleotide (oligo)-based drugs progress towards commercialization, reduction of production costs is a critical consideration. A recent analysis (1) shows that price and performance of the solid support can be major factors in reducing synthesis cost. For a given reactor column volume, increasing solid support nucleoside loading, dimensional stability during synthesis, and synthesis efficiency, can significantly enhance synthesis economy.

Controlled Porosity Glass (CPG) is a popular support for oligonucleotide synthesis due to its unique combination of rigidity, well defined pore structure, and dimensional stability in organic solvents. Commercial, low cross linked polystyrene (PS) supports can be loaded with initial nucleoside higher than conventional CPG supports and are often used for large-scale production of smaller oligos.

This paper describes a proprietary "hybrid" solid support design (2) that combines the strengths of the above materials to lower synthesis costs for a wide range of oligos. *Hyb*CPG consists of conventional CPG particles that are completely coated with a highly functionalized, thin, continuous, and conformal polymer layer. This provides the well defined, dimensionally stable pore structure and rigidity of CPG with the higher loading capacity and chemical resistance to alkali solutions provided by polystyrene.

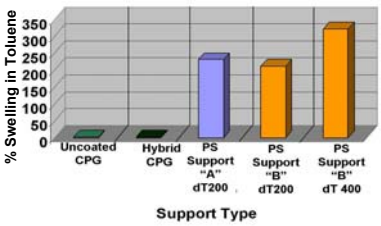
Background

CPG's loading capacity is limited by the density and distribution of surface silanol groups. Furthermore, surface area and number of anchoring silanol groups varies inversely with pore size.

While low level cross-linked PS supports can accommodate high ligand loadings on a dry weight basis, they expand when solvated, and column loadings must be limited to accommodate the swelling. Also, column backpressure can increase throughout the synthesis as the growing oligo causes further support expansion. Finally, column heights are limited due to the compressibility of the polymer supports. These drawbacks can limit the utility of such supports.

To address these deficiencies, a hybrid support based on CPG has been developed. Through a very thin, highly functionalized polymer layer, much higher ligand loadings (<2-5x) than conventional CPG can be achieved. The pore size/loading trade-off of conventional CPG is removed, since the number of unhindered ligands no longer depends on surface silanol distributions. Through careful design of the polymer structure, a uniform, sterically unhindered distribution of ligands is obtained. Since the polymer coating is very thin (~50 angstroms) and conformal, the well defined, stable pore structure and low backpressure, typical of CPG, is retained. The thin polymer layer also avoids expansion of the bulk support in synthesis solvents and protects the underlying glass from attack by alkali or fluoro-acid reagents.

A



B

Support Type	Nucleoside Loading (µmol/g)	MAX 1.2 ml Column Loading (µmol)	MAX 6.3 ml Column Loading (µmol)
Uncoated CPG (1000 Å)	60	22	110
Hybrid CPG (1000 Å)	192	88	440
PS Support "A"	200	50	250
PS Support "B"	400	58.6	308

Figure 3: Effect Support Swelling on Column Loading (Synthesis Scale)

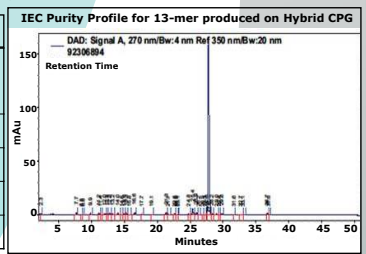
A. Both uncoated and hybrid CPG have ZERO % swelling in synthesis solvents, while polystyrene supports can swell up to 3.5 times in the same conditions.

B. Swelling limitations on column loading are significant in polystyrene based supports: Increased nucleoside loading capacity of non-swelling Hybrid CPG can translate into significant increases in column loading.

Figure 4: Representative DNA Synthesis Results

13-mer Sequence: 5′ GGG CCC AAA TTT T 3′				
Solid Support	**dT Loading (µmol/g)**	**Synthesis Scale (µmol)**	**Crude Yield OD's (A260) (µmol/g)**	**IEC Yield (% FLP)**
Uncoated CPG (500Å)	**99**	**27**	**94.4**	**86.3**
Hybrid CPG (500Å)	**163**	**48**	**97**	**84.4**
Polystyrene Support "A"	**200**	**51**	**104**	**86**
Polystyrene Support "B"	**400**	**55**	**94.9**	**88.5**

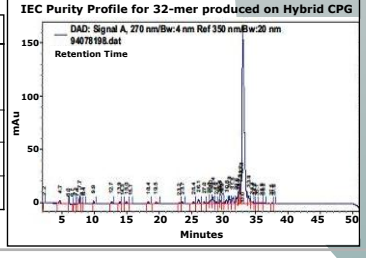
IEC Purity Profile for 13-mer produced on Hybrid CPG



32-mer Sequence: 5′ CAG TGG ATC TGC GCA CTT CAG GCT CCT GGG CT 3′				
Solid Support	**dT Loading (µmol/g)**	**Synthesis Scale (µmol)**	**Crude Yield OD's (A260) (µmol/g)**	**IEC Yield (% FLP)**
Uncoated CPG (1000Å)	**30**	**12**	**169**	**68**
Hybrid CPG (1600Å)	**88**	**27**	**152**	**69**
Polystyrene Support "A"	**80**	**20**	**172**	**57**

Synthesis conditions: AKTA OligoPilot 10 Plus, 1.2 ml column, amidite 0.1M, 3.0 eq.,

IEC Purity Profile for 32-mer produced on Hybrid CPG



Methods

- Conventional CPG of a given pore size and volume was coated with a cross-linked polystyrene-like polymer, using a patented application process (2)
- Polymer coating *uniformity* was evaluated using SEM and optical microscopy (Fig. 1).
- The *thickness* of the polymer coating was estimated by mercury porosimetry performed on the CPG before and after coating (Fig. 2).
- The *conformal nature* of the coating was inferred with mercury porosimetry, which showed the preservation of the mono-pore size distribution curve (Fig. 2).
- Coating *continuity* was verified through chemical resistance tests using concentrated ammonium hydroxide at 60° C for periods up to 5 hours by re-verification of the amine loading by non-aqueous perchloric acid titrations (Fig. 2).
- Bulk swelling of various solid supports was determined in acetonitrile AND toluene (a solvent commonly used as a deblocking reagent) (Fig. 3).

  Swelling data was measured after 24 hours of soaking in the solvent at room temperature.
- Oligo synthesis testing was performed using a GE Healthcare AKTA 10 with a 1.2 ml synthesis column. Over 400 DNA oligos (13, 25, and 32-mers) were made.
- Purity evaluations included IEC and CE and were performed by Integrated DNA Technologies (IDT), Coralville, IA.

Results

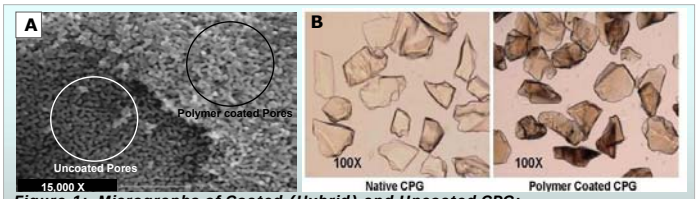


Figure 1: Micrographs of Coated (Hybrid) and Uncoated CPG:

A. An Environmental Scanning Electron Microscope(ESEM) photos of partially coated CPG particle to illustrate, by contrast, the conformal coating in the pores of the native CPG. The ESEM can operate in a partial vacuum, under moderate accelerating voltages to avoid charging effects and polymer damage from the electron beam.

B. Optical micrographs of CPG particles show thin coating that still allows light transmission

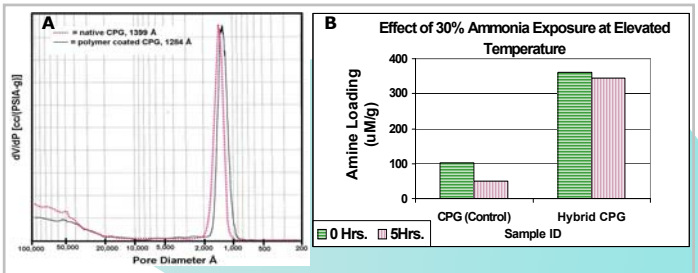


Figure 2: Effect of Polymer Coating on Critical CPG Properties:

A. Porosimetry Scan for Uncoated and Polymer Coated Hybrid CPG Support: The pore size of the coated sample is reduced by ~115 Å AND the uniform controlled pore size is preserved. A thickness of ~60 Å is inferred, indicating a multi-layered polymer coating.

B. The conformal polymer coating on hybrid CPG is not degraded by exposure to ammonium hydroxide, and therefore amine levels remain essentially unchanged whereas uncoated amino functionalized CPG looses up to ½ of it's amine functionality.

Conclusions

- A polymer coated hybrid CPG solid support with superior mechanical properties and chemical loading has been successfully developed. Through scanning electron and optical microscopy, mercury porosimetry, and chemical resistance tests, this coating has been shown to be completely conformal, thus, uniform pore characteristics, critical to maximum oligo synthesis efficiency and yields, are preserved.
- Observed bulk density and pore volume changes versus conventional CPG were negligible
- Due to the nano-dimensional thickness of the coating, the support exhibits NO swelling in any synthesis solvent (acetonitrile, toluene, dichloromethane).
- The disadvantage of inverse surface area/pore size correlation is removed because the conformal coating is highly chemically functionalized, and completely independent of the availability of surface silanol anchoring points. As a result, 13, 25 and 32- mer DNA oligonucleotides with purity levels comparable to CPG and polystyrene reference materials have been made on hybrid CPG with nucleoside loadings 2-3 x greater than conventional CPG.

Future Work

- Optimize physical parameters and polymer configurations to maximize synthesis efficiencies for both small oligo syntheses (i.e., short DNA sequences) and bulkier oligos such as RNA and longer DNA sequences.
- Manufacturing scale-up
- Field testing at pilot and production synthesis scales through collaboration with various end-users. End use testing will also include several oligo modifications such as phosphorothiates, MOE protection, lipid delivery moieties, LNA chemistry, and Spiegelmers.
- Evaluate hybrid CPG with several of the more promising universal linker designs.

1. Carter, G., Oligo Theraputics Market Landscape, in Tides. 2007: Las Vegas, NV.
2. Rothstein, M., D. Rothstein, and D. Lee, Hybrid Solid Supports Useful for Oligonucleotide Production, in US Patent & Trademark Office. 2009, Prime Synthesis, Inc. : United States.

We would like to gratefully acknowledge the following:

- Stefanie Rothstein for her dedication to this project, and skillful execution of laboratory experiments

- Integrated DNA Technologies for their expert assistance in oligo evaluations

*****This work was partially funded by a Grant from NIH National Institute of General Medical Sciences (NIGMS)*****