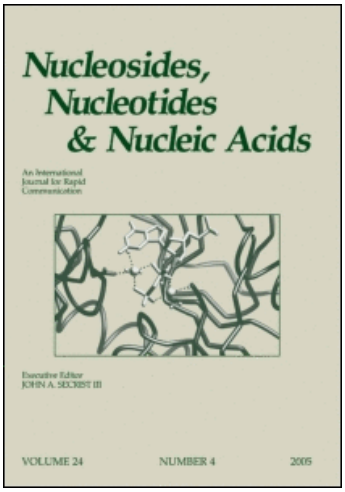

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OLIGONUCLEOTIDE SYNTHESIS ON LINEAR REACTIVE POLYMER GRAFTED ONTO SOLID SUPPORT, MEDICAL DIAGNOSTICS APPLICATIONS

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ABSTRACT : A new strategy has been developed to obtain polymer-ODN conjugates. However, free ODN appeared to contaminate synthesis. Various hypotheses are described herein to explain this side reaction.

The emergence of new infectious diseases (Hepatitis, HIV....) has favoured the development of genetic diagnosis, based on the detection of viral nucleic acid material via the ELOSA technique (Enzyme Linked OligoSorbent Assay). In this context, we have focused on the improvement of the sensitivity of the test using polymer-oligonucleotide conjugates for the capture and the detection of the nucleic acid target. For that, we have envisioned to develop direct synthesis of ODN from a linear polymer (poly(maleic anhydride-*alt*-ethylene) (I), Mn= 27000 g/mol) previously grafted on a solid support (Controlled Pore Glass, 2000) (1), see **FIG. 1**. First of all, the 5'-dimethoxytritylthymidine 3'-(6-aminohexylphosphate) (II) initiator of oligonucleotide synthesis was coupled to the anhydride functions of the polymer via an amide bond. This nucleotide activated polymer was then grafted on hydroxyl functionalized solid support via an alkali-labile ester bond. After grafting, a polyThymidine 26mer (26mer T) was synthesized from this functionalized CPG. The polymer-ODN conjugate was then cleaved from CPG by ammoniacal treatment. The crude material was analyzed by Size Exclusion Chromatography coupled to a MultiAngle Laser Light Scattering detector (SEC-

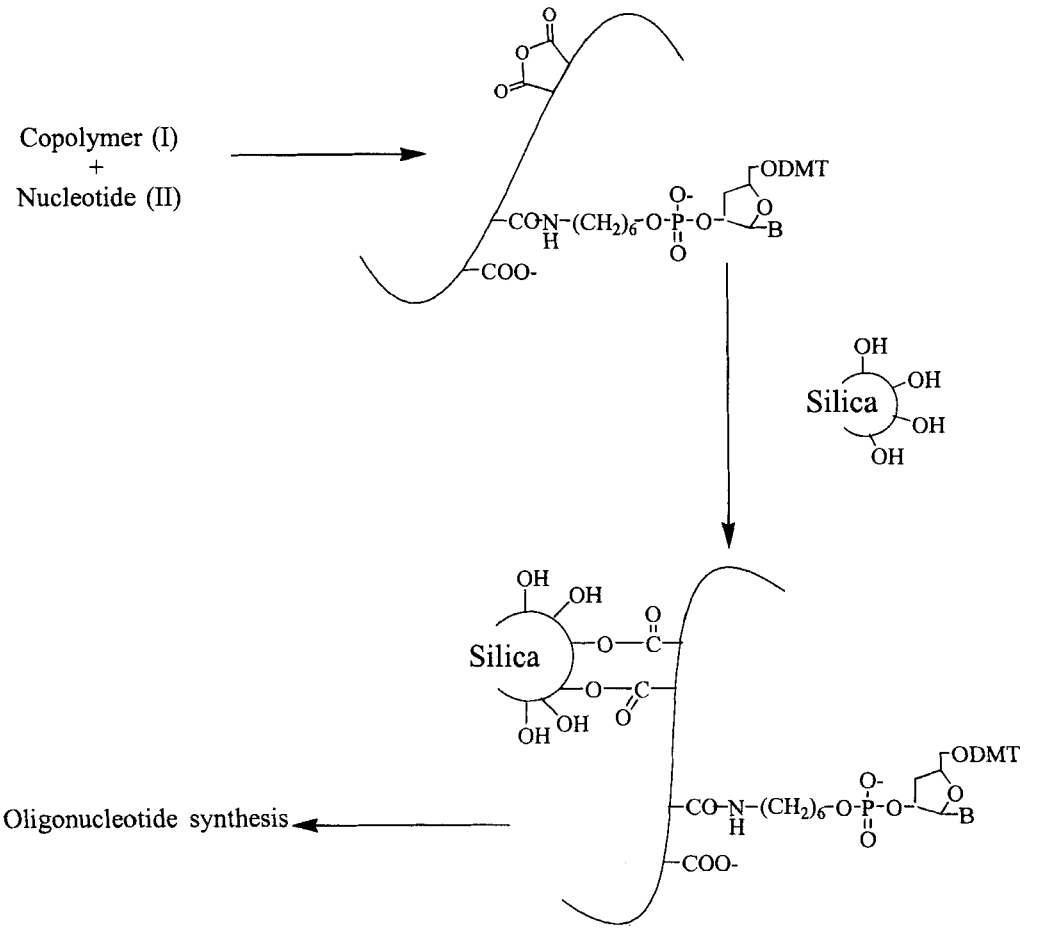

FIG. 1 : Synthesis strategy

MALLS). The presence of the expected conjugate was observed, and the analysis also showed a parasite population suspected to be free nucleic acid material (A).

To corroborate this result, a 26merT synthesis was attempted on a polymer functionalized CPG without any nucleotide (II). Like the other supports, these beads were classically capped with acetic anhydride just before synthesis. In the latter case, we also observed a parasite ODN population (B). Free ODN materials (A and B) were analyzed on PAGE after ^{32}P labeling. In the B case, one major strip appeared to correspond to a 25merT. This population was attributed to adsorption onto CPG surface of thymidine phosphoramidite during the first cycle of the synthesis. Mass spectroscopy analysis confirmed this

hypothesis as the molecular weight (7,622.20 g/mol) corresponded to polyThymidine 25mer 3'-phosphate. In the A case, the gel autoradiography revealed two strips corresponding to a 25merT and a more intense 26merT. This latter spot was attributed to the adsorption on the CPG surface of nucleotide (II) during the preparation of functionalized CPG support. This phenomenon was confirmed by an adsorption study. The nucleotide (II) was brought into contact with an hydroxyl functionalized CPG. The adsorption was followed by dimethoxytrityl cations measurement. An increase of adsorbed nucleotide (II) concentration versus time was observed. An other study by capillary electrophoresis, permitted to show that 10% of the nucleotide (II) was bound to the polymer by the exocyclic amine of the thymine. Hence, this more labile tether was rapidly cleaved during ammoniacal treatment, releasing free 26merT in solution with conjugate. In conclusion, the strategy propose herein permitted to synthesize polymer-ODN conjugate of good quality. However, our protocol leaded to parasite ODN synthesis, mainly due to both adsorption of nucleotide (II) and thymidine phosphoramidite. With the aim of limiting this phenomenon, different capping processes could be envisioned that would modify the surface polarity of CPG particles. Finally, this strategy could be apply to different supports, like chips or other beads.

1- Chaix, C. ; Minard, C. ; Delair, T. ; Pichot, C. ; Mandrand, B. *J. Appl. Polym. Sc.*, **1998**, in press.