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A Poly(cyclopentadithiophene) Matrix Suitable for Electrochemically Controlled DNA Delivery

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A conducting polymer is tested for DNA delivery trials. The conducting matrix used is successful for electrochemical delivery of DNA accumulated by covalent immobilization. The electrochemical process consists of the reduction of arylsulfonamide moieties, which occur as linker groups. The specific design of the polymer allows the electronic properties to be promoted, making available the cleavage potential in physiological media. The amount of DNA released from a modified platinum electrode is investigated by quartz crystal microbalance. The released species used to prove the system performance are long sequences of DNA strands, which are amplified by PCR after liberation and identified by electrophoresis migration.

To diagnose genetic alteration and for redress defective genes responsible for proteins deficiencies, simple sequence-specific tools are needed to detect genetic disorder and to release the normal gene at a specific location without being hindered by the extracellular barriers. To improve the efficiency of the genetically mediated therapy, different methods to replace targeted genes have been described. These methods differ by the vector employed to introduce the good gene and can be classified in two categories: viral and nonviral methods.[1]

Among the viral approaches, viruses, retroviruses, and adenoviruses are the major vectors used, showing better promise for the development of gene therapies. However, major problems are that the genetic material can be integrated in an arbitrary position in the genome, leading to insertional mutagenesis or cancer.[2] Add to this that even inactivated versions of a viral vector can stimulate an immune response,[3] so the development in nonviral methods is of great interest.

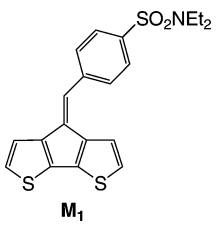
The main advantage of the nonviral method over viral approaches is the low host immunogenicity.[4] Among of these, injection of naked DNA plasmid or ds-ODN are the simplest methods of nonviral transfection. Nevertheless, the expression of genetic material is less important in comparison with other classical approaches of transfection, due to alteration of the extra DNA molecule. To improve the efficiency of nonviral gene therapy, lipoplexes or polyplexes are tested.[5] In these complex structures, DNA is protected by a shell of lipids or polymers, respectively.

Much previous work concerned the development of new active supports allowing potent drug release in a pulsatile manner. Different strategies have been examined based on progress in material chemistry.[6] The electrochemical probe as controlled delivery systems appears to be a promising approach that mimics better the way in which some biological compounds are released in the human body.[7]

Herein, an electrochemical probe modified by a specific conducting matrix obtained from oxidation of cyclopentadithiophene (CPDT) units is tested for DNA delivery applications. The released species used to prove the system performance are long sequences of DNA strands, which are easy to detect and to amplify after liberation.

MATERIALS AND METHODS

Materials. Tetrabutylammonium hexafluorophosphate, employed as an electrolyte support, was purchased from Fluka and used as received (electrochemical grade). Salt was stored in desiccators over silica gel. Acetonitrile purchased from SDS was stored under inert atmosphere and used without any further purification. Monomer 4-cyclopenta[1,2-*b*; 3,4-*b*′]dithiophene-4-ylidenemethyl-*N*,*N*-diethylbenzenesulfonamide, $\mathbf{M_1}$, was synthesized as described previously.[8]



$\mathbf{M_1}$

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(1) (a) Crystal, R. G. *Science* **1995**, *270*, 404−410. (b) Taneja, S. S.; Pang, S.; Cohan, P.; Belldegrun, A. *Cancer Surv.* **1995**, *23*, 247−266. (c) Ferber, D. *Science* **2001**, *294*, 1638−1642.

(2) (a) Kaiser, J. *Science* **2003**, *299*, 495. (b) Marshall, E. *Science* **2003**, *299*, 320.

(3) Lehrman, S. *Nature* **1999**, *401*, 517−518.

(4) Sedegah, M.; Charoenvit, Y.; Minh, L.; Belmonte, M.; Majam, V. F.; Abot, S.; Ganeshan, H.; Kumar, S.; Bacon, D. J.; Stowers, A.; Narum, D. L.; Carucci, D. J.; Rogers, W. O. *Gene Ther.* **2004**, *11*, 448−456.

(5) (a) Elouahabi, A.; Ruysschaert, J.-M. *Mol. Ther.* **2005**, *11*, 336−347. (b) Kono, K.; Torikoshi, Y.; Mitsutomi, M.; Itoh, T.; Emi, N.; Yanagie, H.; Takagishi, T. *Gene Ther.* **2001**, *8*, 5−12.

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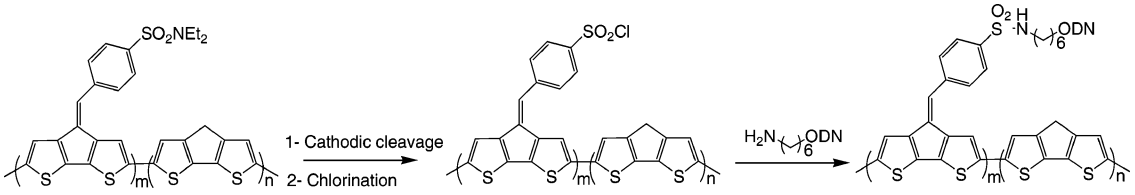
Scheme 1. Accumulation of ODN by Covalent Immobilization with a Sulfonyl Chloride Function as Linker Group



Experiments with DNA-modified substrates consist of hybridizing a 42-base oligonucleotide (ODN) probe immobilized on the surface of the conducting matrix with a 675-base-long target. The probe consists of a synthetic 5′-amino-terminated, single-stranded 42-base ODN with the sequence NH_2-C_6-(5′) TT GTA AAA TAC GAG GGG CGG TCA GAA AGT TAT GTA ATT CTG T (3′) (Biofidal; $FW_{probe} = 13\ 252$ g mol^{-1}). The 3′-extremity of the 675-base, single-stranded target was complementary to the probe. The 675-base-long target was produced in our laboratory as described previously,[9] using the DNA sequence of a transposable element mariner (Accession number AJ496134) cloned in the pGEM-T plasmid (pGEM-T easy vector system I, Promega).

Preparation of Copolymer Film. Preparation of poly(CPDT-*co*-$\mathbf{M_1}$) films was performed under dried argon atmosphere in three-electrode cells connected to a VMP PAR model VMP2/Z-40 potentiostat driven by the EC-Lab Software version 6.70. Electrolyte consisted of an acetonitrile solution containing 0.1 M Bu_4NPF_6 as support salt. AT-cut 9-MHz platinum-coated quartz crystal oscillator (supplied by BioLogic; i.d. = 5 mm) was used as the working electrode. The counter electrode consisted of a platinum wire and a silver wire immersed in a 0.1 M $AgNO_3$ acetonitrile solution was used as reference electrode.

For preparation of poly(CPDT-*co*-$\mathbf{M_1}$), the working electrode was submitted to cycling of potential from −0.4 to +0.75 V versus $Ag|Ag^+$ with a scan rate of 100 mV s^{-1} in a 1/500 molar ratio mixture of $\mathbf{M_1}$ and CPDT (10 mM). A total of 10.4 μg of copolymer was deposited with a positive charge consumed at the neutral state equal to 180 mC cm^{-2}. It must be noted that oxidation potentials of CPDT and $\mathbf{M_1}$ are very close ($\Delta E = 40$ mV), and it is reasonable to do the approximation that the ratio of $\mathbf{M_1}$/CPDT in the copolymer film was near than in solution. For better copolymerization efficiency, neutral alumina, previously activated at 300 °C under vacuum for 5 h, was added in the electrolytic solution.

Electrochemical investigations of DNA-modified films were performed in a TE/0.6 M NaCl buffer solution (TE denotes Tris−EDTA solution containing Tris-HCl 10 mM, EDTA 1 mM, pH 7.6) with potential referred from saturated calomel electrode (SCE).

DNA Probe Immobilization and Hybridization. In order to activate the arylsulfonamide groups for ODN immobilization, poly(CPDT-*co*-$\mathbf{M_1}$) film was chemically modified according to a process already published elsewhere.[10] Arylsulfonamide terminal groups of the starting copolymer film were electrochemically cleaved by four sweeps of potential between −0.5 to −2.0 V versus $Ag|Ag^+$ at 100 mV s^{-1} in CH_3CN containing 0.1 M Bu_4NPF_6 and next exposed to an acetonitrile solution containing 3.4×10^{-2} M *N*-chlorosuccinimide (NCS) for 8 min, leading to sulfonyl chloride groups requisite for the single-stranded DNA probe immobilization as illustrated in Scheme 1. When the linker group was activated, the copolymer film was immersed in a TE/0.6 M NaCl buffer solution and five cyclic voltammograms were performed over the −0.3 to +0.5 V range versus SCE at a scan rate of 100 mV s^{-1}, in order to achieve an ionic exchange. For DNA probe immobilization, the working electrode was immersed overnight at room temperature in TE/0.6 M NaCl buffer solution containing 1 μM DNA probe. Then, electrode was removed from the immobilization cell and rinsed with Milli-Q water at 50 °C for 1 h under stirring in order to remove the unreacted probes. For DNA hybridization, electrode was incubated during 4h at 35 °C in a 0.5M/NaCl phosphate buffer solution (NaH_2PO_4/Na_2HPO_4 0.1 M, pH 7.4) containing the 675-base DNA whose the 3′-extremity was complementary to the immobilized probe.

Electrochemical Investigations. For quartz crystal microbalance (QCM) experiments, microgravimetric measurements were performed by using AT-cut, 9-MHz platinum-coated quartz crystal oscillators connected to a PAR quartz crystal analyzer model QCM922 driven by the WinEchem Seiko EG&G software. QCM investigations were carried out in a one-compartment cell in Teflon in order to follow the in situ mass fluctuations.

RESULTS AND DISCUSSION

In many advanced drug delivery systems, the drug release is triggered by hydrolysis or corrosion of the polymer shell that encapsulates the potent drug.[11] Nevertheless, not many delivery systems involve an electrochemical cleavage in a physiological environment according to the narrow electroinactivity domain in aqueous media.

To overcome this problem, we propose to use a conducting polymer that possesses an activated linker group, making the electrochemical cleavage available in aqueous solution. Preliminary study[12] shows that the specific design of the $\mathbf{M_1}$ unit promotes the electronic communication between the main chain

(6) (a) Chirila, T. V.; Rakoczy, P. E.; Garrett, K. L.; Lou, X.; Constable; I. J. *Biomaterial* **2002**, *23*, 321−342. (b) Stiriba, S. E.; Frey, H.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 1329−1334.

(7) (a) Miller, L. L.; Lau, A. N. K.; Miller, E. K. *J. Am. Chem. Soc.* **1982**, *104*, 5242−5244. (b) Lau, A. N. K.; Miller, L. L. *J. Am. Chem. Soc.* **1983**, *105*, 5271−5277. (c) Wang, J.; Rivas, G.; Jiang, M.; Zhang, X. *Langmuir* **1999**, *15*, 6541−6545. (d) Moore, E.; Curtin, M.; Ionita, J.; Maguire, A.R.; Ceccone, G.; Galvin, P. *Anal. Chem.* **2007**, *79*, 2050−2057.

(8) Pilard, J. F.; Cougnon, C.; Rault-Berthelot, J.; Berthelot, A.; Hubert, C.; Tran, K. J. *Electroanal. Chem.* **2004**, *568*, 195−201.

(9) Gautier, C.; Cougnon, C.; Pilard, J.-F.; Casse, N.; Chénais, B.; Laulier, M. *Biosens. Bioelectron.* **2007**, *22*, 2025−2031.

(10) Gautier, C.; Cougnon, C.; Pilard, J. F.; Casse, N. J. *Electroanal. Chem.* **2006**, *587*, 276−283.

(11) (a) Wuthrich, P.; Ng, S. Y.; Fritzinger, B. K.; Roskos, K. V.; Heller, J. *J. Controlled Release* **1992**, *21*, 191−200. (b) Santini, J. T.; Richards, A. C.; Scheidt, R.; Cima, M. J.; Langer, R. *Angew. Chem., Int. Ed.* **2000**, *39*, 2396−2407.

(12) Gautier, C.; Cougnon, C.; Pilard, J.-F. *Electrochem. Commun.* **2006**, *8*, 1045−1054.

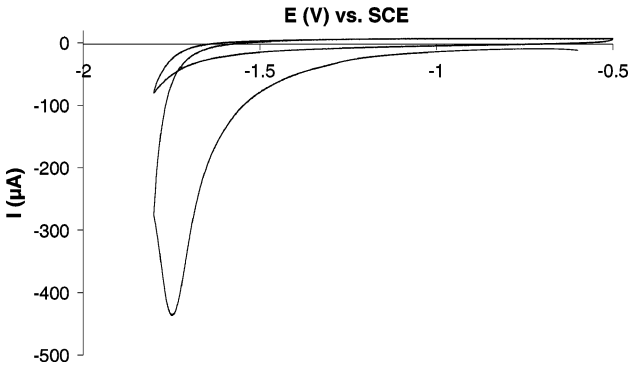


Figure 1. CVs recorded in aqueous medium containing 0.5 M $LiClO_4$ on poly($\mathbf{M_1}$)-coated carbon vitreous electrode. The scan rate is 100 mV s^{-1}.

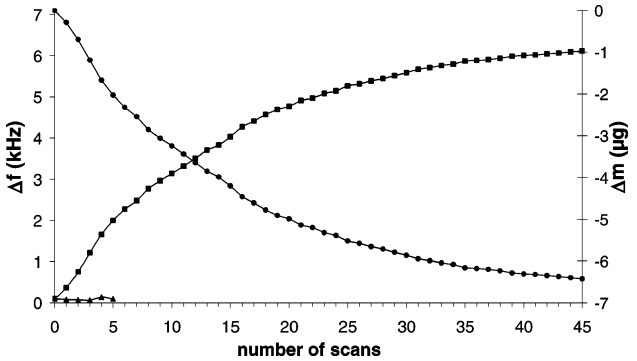


Figure 2. In situ frequency change recorded with successive scans of potential over the −0.5 to −1.5 V range (triangles) and the −0.5 to −1.7 V range (squares) at 100 mV s^{-1}. Circles represent the changes of mass calculated with the Sauerbrey relation from the frequency changes recorded over the −0.5 to −1.7 V range. The working electrode consists of copolymer film modified by exposure to ssDNA probe and, next, incubated at 35 °C for 4 h in a 0.5 M/NaCl phosphate buffer solution containing the 675-base DNA whose the 3′-extremity is complementary to the probe.

of polymer and the side chain through a π-conjugated spacer. So, the reduction of arylsulfonamide groups becomes available in physiological media, which constitutes an interesting approach for the elaboration of matrixes able to deliver potent drugs under external electrical stimulus.

Figure 1 shows the cathodic behavior of the poly($\mathbf{M_1}$) film, performed in aqueous solution.

CVs show an irreversible peak located at −1.7 V, which disappears from the second sweep, consistent with the bielectronic cleavage of the SN bond.[13] This result indicates that the poly($\mathbf{M_1}$) platform is a good candidate for the component of the electrochemical drug delivery system operating in physiological media, since the potential of cleavage can be reached in water.

For DNA delivery trials, a copolymer film, poly(CPDT-*co*-$\mathbf{M_1}$), is deposited onto an AT-cut, 9-MHz platinum-coated quartz crystal oscillator as described in Materials and Methods, and the frequency *f* versus number-of-scan profile is monitored by a QCM technique. Figure 2 shows the frequency changes upon reduction of the film performed in TE/0.6 M NaCl buffer solution after ssDNA probe immobilization and hybridization with the 675-base target DNA. The experimental procedure consists of recording

(13) Dubey, S.; Fabre, B.; Marchand, G.; Pilard, J.-F.; Simonet, J. J. *Electroanal. Chem.* **1999**, *477*, 121−129.

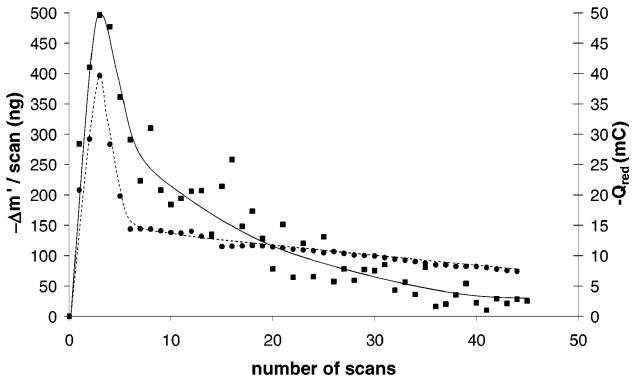


Figure 3. Change in mass per individual CV (squares), superimposed with the change in cathodic charge obtained from individual CV (circles).

the frequency change in relation with cyclic voltammetry experiments. The cathodic behavior is investigated for two reverse potential values at which the cathodic cleavage occurs or not (i.e., −1.5 and −1.7 V vs SCE, respectively). When the sweep of potential is recorded over the −0.5 to −1.5 V range, no frequency change is observed (Figure 2, triangles). In contrast, when the reverse potential is equal to −1.7 V, gradual frequency increase is recorded during the first 30 sweeps (Figure 2, squares). Such a QCM profile indicates that the cathodic treatment leads to a concomitant loss of mass, in accordance with the DNA delivery upon reduction. The changes in mass, calculated from the Sauerbrey relation, is superimposed on the frequency change (Figure 2, circles).

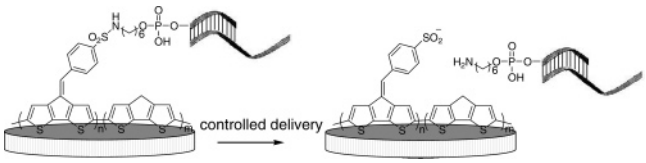
The loss of mass correlates well with the amount of biological material immobilized, contrary to what is observed with other polythiophene matrixes,[9] for which the loss of mass is larger, in absolute value, than the sum of mass increases recorded upon immobilization and hybridization. A previous study[9] shows that, when the cathodically sensitive group is not activated, the DNA is delivered by cathodic degradation of the copolymer, due to water reduction generating hydrogen evolution that disrupts the film deposited on the electrode surface.

The change in mass per individual CV is very instructive about the way in which the DNA is liberated. Results shown at Figure 3 correspond to the successive charge (circles) and mass (squares) changes in relation with the individual CVs obtained during the first 45 scans over the −0.5 to −1.7 V range. Mass profile reported in Figure 3 is consistent with the derivative, $\Delta m'$, of the Δm versus number-of-scan pattern shown at Figure 2 and corresponds to the conventional profile for DNA delivery. These results indicate that the biological material is liberated in a pulsatile manner.

The DNA delivery profile is marked by a rapid increase during the three first CVs and next, by a gradual decrease, implying that the amount of DNA liberated is higher at the beginning of the electrochemical treatment than at the end. Furthermore, the good correlation between the charge and frequency changes reported in Figure 3 indicates that the use of the poly(CPDT-*co*-$\mathbf{M_1}$) film allows the DNA delivery "on demand", in accordance with the cathodic cleavage of the SN bond as illustrated in Scheme 2.

The released DNA is precipitated at −80 °C for 1 h after having addition of 25 μL of 3 M sodium acetate and 700 μL of absolute ethanol. After washing with ethanol and drying under vacuum,

Scheme 2. Electrochemically Controlled DNA Delivery



DNA released fragments are dissolved in 15 μL of Milli-Q water and amplified by PCR. The PCR product is deposed on agarose gel (Figure 4) for identification by electrophoresis migration. Compared to the 100-bp DNA marker, the delivery of 675-base DNA is valid and it appears that the integrity of biological material is preserved during the electrochemical controlled delivery.

The use of a polymer-coated electrode in such a conventional drug delivery system is of great interest, since it is possible to conceive a multielectrode system, which can allow us to program different DNA delivery profiles by a series of timed pulses of potential applied to the different biologically active spots. The result reported here constitutes an unprecedented result and could be an interesting alternative for local drug delivery at applied potential in physiological media. Furthermore, the copolymer backbone can be reused in a new anchorage/delivery sequence by only a new exposure to NCS after cleavage.

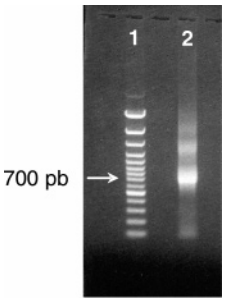


Figure 4. Agarose gel picture of single-stranded 675-base DNA target obtained after electrochemically controlled delivery and amplified by PCR (lane 2) compared to DNA marker O'GeneRuler 100-bp DNA Ladder Plus (lane 1).

CONCLUSION

The use of a polythiophene matrix for DNA delivery triggered by an electrical stimulus is very successful. The results reported demonstrate the ability to electrochemically liberate DNA from a conducting polymer by cleavage of a covalent bond. This approach constitutes an interesting alternative to classical gene delivery systems. The amount of biological material delivered is followed by QCM investigation in order to determinate the delivery profile. A multielectrode system to obtain more complex release profiles is under investigation in our laboratory in connection with the development of therapeutic tools.

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