



Feature Article

Surface-initiated controlled radical polymerization enhanced DNA biosensing



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ABSTRACT

The surface chemistry at the DNA recognition and binding interface plays an important role in determining the performance of DNA microarrays and DNA biosensors. Surface-initiated controlled radical polymerization (SI-CRP) reactions represent a powerful toolbox to generate microarray and biosensor surfaces with enhanced DNA recognition and binding properties or to amplify and transduce these events. Surface-initiated polymerizations generate thin films in which all polymer chains are tethered with one chain end to the underlying surface and are also referred to as polymer brushes. SI-CRP reactions possess a number of features that make them highly attractive to engineer the properties of biosensor interfaces. First of all, the thickness of the films can be precisely adjusted to match the requirements of the specific biosensor format. Secondly, the grafting density of these films can be tuned to optimize binding kinetics and capacity. Finally, being a bottom-up technique, SI-CRP can also be used to modify complex, patterned or structured biosensor substrates with a conformal DNA recognition and binding interface. This article provides an overview of the state-of-the-art on the use of SI-CRP techniques to enhance or facilitate DNA biosensing. On the one hand, SI-CRP techniques have been used to generate high binding capacity surface coatings. On the other hand, these reactions have also been demonstrated to be powerful tools to amplify DNA recognition and binding and allow visual detection. The examples discussed in this article not only underline the potential of SI-CRP reactions to engineer the properties of biosensor interfaces, but also, together with future advances in these polymerization techniques, provide exciting opportunities to further enhance the performance of DNA microarrays and DNA biosensors.

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1. Introduction

DNA microarrays [1] and biosensors [2] are analytical devices that explore the unique sequence selectivity of the DNA hybridization process to detect target DNA sequences [3]. This is of great relevance for medical diagnosis [4,5], for forensic investigations [6] as well as for fundamental studies, such as e.g. gene expression analysis [7–10].

In essence, for both DNA microarrays and DNA biosensors, target detection is the outcome of a 3-step process that involves (i) immobilization of the probe DNA; (ii) hybridization of the probe DNA with the target sequence and (iii) detection; i.e. transduction of the hybridization event into a measurable signal [11,12]. In case of a DNA biosensor, probe immobilization takes place directly on the transducer surface [2]. The hybridization of the target sequence depends on the stability, accessibility and reactivity of the surface-bound DNA. As a consequence, the immobilization of the probe DNA and the surface chemistry at the DNA recognition and binding interface are critical aspects in the development of DNA biosensors [13].

A variety of strategies has been developed for the immobilization of probe DNA, which includes the use of electrostatic interactions (e.g. using positively charged surfaces), non-specific adsorption (e.g. on graphite surfaces), highly specific non-covalent interactions (e.g. using avidin/streptavidin–biotin binding) as well as covalent surface attachment [11]. The latter approach typically involves the use of surfaces that present aldehyde or epoxy groups, which can undergo reactions with amino-modified probe DNA, or explore the chemisorption of thiol modified probe DNA on gold substrates. In addition to immobilizing the pre-synthesized nucleotides, arrays of surface-attached probe DNA can also be prepared via *in-situ* synthesis from appropriately functionalized surfaces [12,14].

In addition to two-dimensional substrates such as e.g. glass slides [15–17] or carbon or gold electrode surfaces [18–22], there has also been an increased interest in the use of polymer-based DNA immobilization platforms for the development of DNA biosensors or microarrays. The main attractive feature of these polymer-based interfaces is that they provide a three-dimensional platform with a much higher probe binding capacity as compared to the typical two-dimensional substrates. Examples of such

polymer based three-dimensional substrates that have been used include nitrocellulose films [23,24], as well as various hydrogel based coatings [25–47] which can be prepared either *in situ* or by deposition of pre-synthesized polymers.

The aim of this article is to illustrate the opportunities that are provided by surface-initiated controlled radical polymerization (SI-CRP) techniques for the development of DNA biosensors or DNA microarrays. SI-CRP generates densely packed assemblies of polymer chains that are tethered to the surface with one chain end and which are commonly referred to as polymer brushes [48–51]. SI-CRP techniques possess a number of unique characteristics that make them ideally suited for the development of three-dimensional polymer-based DNA biosensor and microarray interfaces. First of all, the controlled/“living” nature of the SI-CRP process allows to precisely control the thickness of the polymer interface, which can be advantageous e.g. for waveguide-based sensors [52]. Secondly, a variety of strategies is available that can be used to tune the grafting density of polymer brush thin films, which allows to engineer the accessibility and probe binding capacity of the interfaces. Finally, being a “bottom-up” methodology, SI-CRP can also be used to generate well-defined and conformal biosensor and microarray interfaces on geometrically complex substrates, such as e.g. (nano)porous membranes [53,54]. In addition to “bottom-up” synthesis via surface-initiated controlled radical polymerization, polymer brushes can also be prepared via the so-called “grafting-onto” strategy, which involves coupling pre-synthesized polymers to an appropriately functionalized surface. This strategy has also been successfully used to prepare DNA binding and detection interfaces [55–59]. As compared to surface-initiated polymerization strategies, the grafting-onto approach generally leads to polymer brush films with lower grafting densities and is restricted to relatively thin polymer brush films. This article exclusively concentrates on polymer brushes obtained via the “grafting from” strategy using SI-CRP methods.

The remainder of this article is organized in three sections, each of which highlights one specific class of polymer brush based DNA biosensing or microarray platforms. First, the use of SI-CRP to generate polymer brush interfaces that can covalently bind probe DNA will be discussed (Fig. 1). The second class of polymer brush

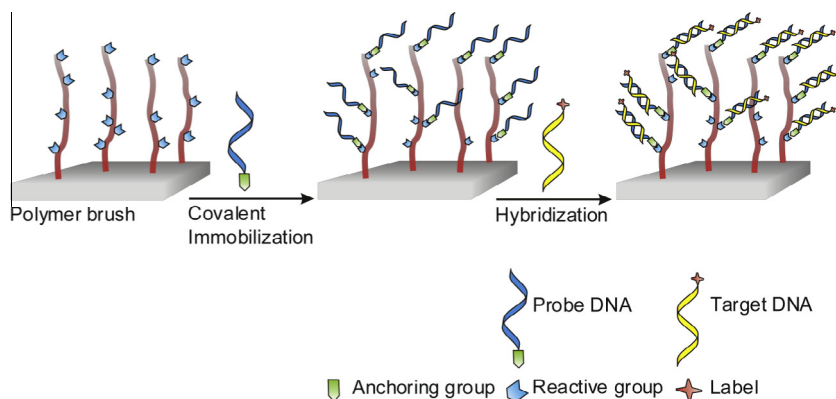


Fig. 1. Covalent immobilization of probe DNA on a polymer brush.

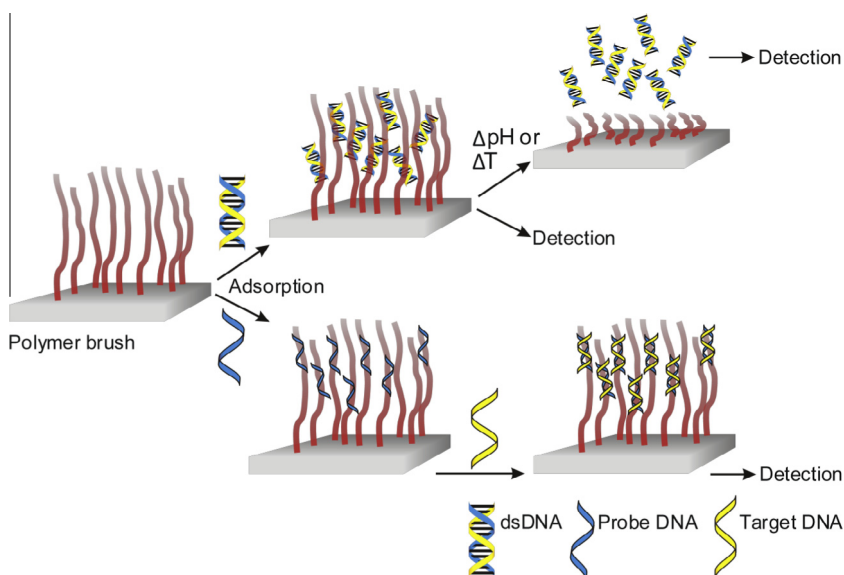


Fig. 2. DNA detection using polymer brush interfaces that non-covalently capture DNA.

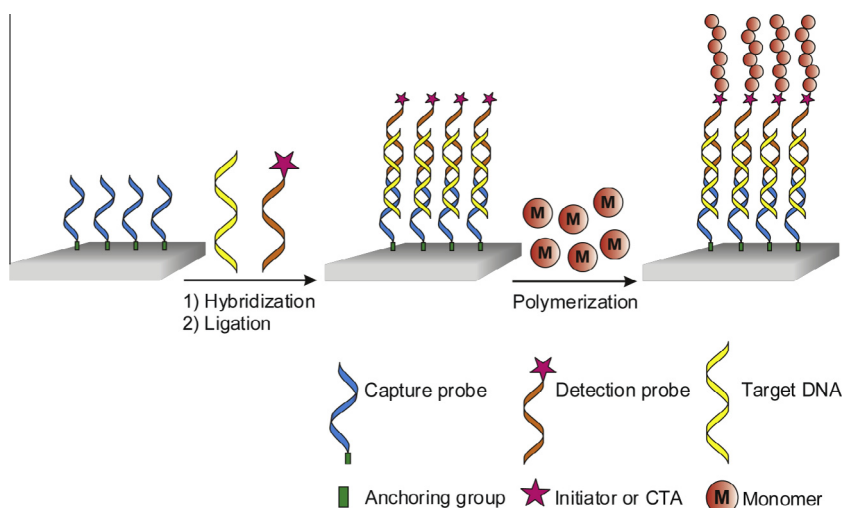


Fig. 3. SI-CRP mediated amplification of target DNA hybridization using a three-strand oligonucleotide system.

based interfaces that will be presented are those that are designed to capture DNA via non-covalent interactions (Fig. 2). The final section will highlight the use of SI-CRP as an amplification method that allows direct visualization of target DNA hybridization (Fig. 3).

2. DNA binding and detection with polymer brushes

2.1. Covalent DNA binding polymer brush platforms

Table 1 provides an overview of the different polymer brush based platforms, which have been prepared so far for the covalent immobilization of DNA. The first five examples (rows 1–4) are (co)polymer brushes with side chain functional groups that are able to covalently bind appropriately modified oligonucleotides [60–64], the

following three examples (row 5–7) [67–69] are homopolymer brushes incorporating functional groups that allow to covalently attach appropriately modified oligonucleotides and the last two examples (row 8) explore the incorporation of the oligonucleotide sequences into the polymer brush layer by direct (co)polymerization of acrylamide modified oligonucleotides [70,71].

Pirri et al. reported the use of poly(*N,N*-dimethylacrylamide-*b*-glycidyl methacrylate) (poly(DMA-*b*-GMA)) block copolymer brushes, which were prepared via surface-initiated reversible addition–fragmentation chain transfer (SI-RAFT) polymerization, to bind 23 base-pair amine-modified probe DNAs via the reaction with the oxirane functional groups in the polymer brush. By fluorescence measurements, it was shown that the fluorescence intensity reached a plateau when the concentration of probe DNAs

Table 1
Overview of polymer brush platforms that have been used to covalently immobilize DNA.

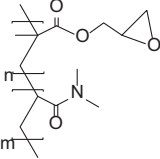
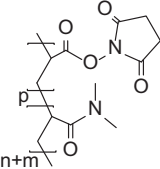
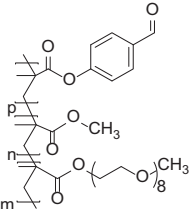
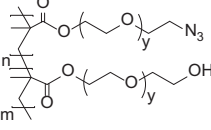

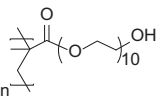
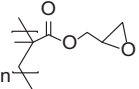
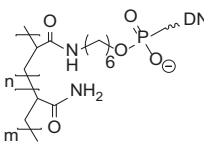
Polymer brush	Chemical structure	Substrate	SI-CRP method	M_n (g/mol)	M_w/M_n	Thickness (nm)	Remarks
P(DMA-b-GMA) [60]		Glass	RAFT	15,620 ^{a,b}	2.61 ^{a,b}	–	The RAFT process was used to grow a PDMA-PGMA block copolymer from thiol functionalized surfaces in the presence of initiator and CTA. The block copolymer coating presented higher fluorescence intensity upon hybridization with complementary target oligonucleotide demonstrating the higher binding of probe oligonucleotides compared to organosilanized surface.
P[DMA-b-(DMA-co-NAS)] [61]		Glass	RAFT	14,180 ^{a,c}	3.20 ^{a,c}	–	Diblock polymer coating bearing functional groups in the external block was demonstrated to bind probe oligonucleotides at a distance of 30 nm from the surface based on self spectral interference fluorescence microscopy (SSFM).
P(PEGMA-FPMA-MMA) [62]		Glass	ATRP	7000 ^a	1.25 ^a	–	(Co)polymer brushes bearing reactive aldehyde groups and non-fouling units were prepared. Increasing the temperature at which the ATRP initiator modified trimethoxysilane was cured was found to result in an increase in the number of the bound probe oligonucleotides.
P(OEGMA-N ₃ -co-POEGMA-OH) [63,64]		Silica-coated magnetic NPs	SET-LRP	12,100 ^a	1.35 ^a	–	Azide groups incorporated into the (co)polymer brush layer allowed to bind alkyne functionalized capture probe DNA via CuAAC. OEGMA was incorporated in the brush to suppress the non-specific adsorption. The hybridization of target DNA was evaluated in a sandwich assay using fluorescent reporter probe modified particles giving a limit of detection of 60 pM in 50% fetal bovine serum [63], which could be further improved to 0.5 pM in 100% serum upon assay and reagent optimization [64].
PAA [67]		Porous silicon (pSi)	ATRP	–	–	180 ^d	RCA primers were bound to PAA brushes via EDC/NHS coupling. Concatemeric DNA was produced via RCA presenting multiple copies of RCA template. Hybridization with the complementary sequence to two different RCA templates allowed multiplex scanning.
PPEGMA [68]		Plasma sputtered nylon	ATRP	–	–	30 ^e	SPR chips modified with non-fouling polymer brushes were used to monitor the post-polymerization modification with streptavidin and as well as the binding of biotinylated probe oligonucleotides to streptavidin and subsequent hybridization with a complementary oligonucleotide sequence.
PGMA [69]		Au	ATRP	–	–	30–60 ^f	Polymer brushes were grown from initiator modified substrates, which were prepared via polymer pen lithography. The density of these brushes could be tuned by feature size. Oxirane functional groups could bind fluorescent labelled probe oligonucleotides and the subsequent hybridization with the fluorescent labelled target sequence was monitored via FRET.

Table 1 (continued)

Polymer brush	Chemical structure	Substrate	SI-CRP method	M_n (g/mol)	M_w/M_n	Thickness (nm)	Remarks
P(acrylamide-co-DNA) [70,71]		Au	ATRP	–	–	17.9 ^f	Acrylamide modified oligonucleotides were copolymerized with acrylamide. SPR chips modified with these (co)polymer brushes were used to monitor the hybridization with the complementary sequence; a limit of detection of 22 pM was reported [70], whereas a limit of detection of 2.67 nM was reported on (co)polymer brush modified Au substrates based on electrochemical assay [71].

^a Number average molecular weight (M_n) and polydispersity (M_w/M_n) determined by gel permeation chromatography (GPC).

^b M_n and M_w/M_n of P(GMA) block.

^c M_n and M_w/M_n of P(DMA-co-NAS) block.

^d Thickness determined by cross-section imaging of scanning electron microscopy.

^e Thickness determined by ellipsometry.

^f Thickness determined by atomic force microscopy.

spotted onto the copolymer brush surface exceeded $10 \mu\text{M}$. The number of accessible probe DNAs attached on the copolymer brush surface was calculated as 0.3×10^{13} molecules/ cm^2 from subsequent hybridization experiments with fluorescent labelled target DNAs. The grafting density of PGMA chains was given as 1.4×10^{11} molecules/ cm^2 , which corresponds to ~ 20 probe DNA molecules per chain on the copolymer brush that could hybridize with the target DNA sequence. Hybridization experiments on the poly(DMA-b-GMA) coated substrates resulted in much higher fluorescence intensities as compared to a control experiment that was carried out with a (3-glycidyloxypropyl) trimethoxysilane modified glass slide, which reflects the higher probe binding capacity of the three-dimensional polymer brush interface [60]. In a subsequent publication, Di Carlo et al. reported qualitatively similar findings using a related RAFT-synthesized block copolymer brush platform in which poly(N-acryloyloxysuccinimide) (PNAS) instead of PGMA was used as the upper, DNA binding block [61].

Bifunctional copolymer brushes composed of poly(ethylene glycol) methyl ether methacrylate (PEGMA),

formylphenyl methacrylate (FPMA) and methyl methacrylate (MMA) (poly(PEGMA-FPMA-MMA)) were prepared by Lee et al. via surface-initiated atom transfer radical polymerization (SI-ATRP) [62]. These copolymer brushes contained reactive aldehyde groups to immobilize 30 base-pair long amine-modified DNA probes and non-fouling PEGMA units to suppress non-specific adsorption. A series of experiments was carried out in which DNA hybridization on polymer films generated from ATRP initiator functionalized substrates, which were prepared at different curing temperatures, was compared. Interestingly, the authors observed higher fluorescence intensities, indicative of higher hybridization efficiencies, when high temperatures were used during the curing of the ATRP initiator modified surfaces.

Thomson et al. used single electron transfer living radical polymerization (SET-LRP) to graft copolymer brushes of ω -hydroxy terminated oligoethylene glycol methacrylate (OEGMA-OH) and its azide modified form (OEGMA-N₃) [poly((OEGMA-N₃)-co-(OEGMA-OH))] from the surface of 500 nm diameter silica coated magnetic nanoparticles (Fig. 4) [63]. Whereas the azide groups allow to bind

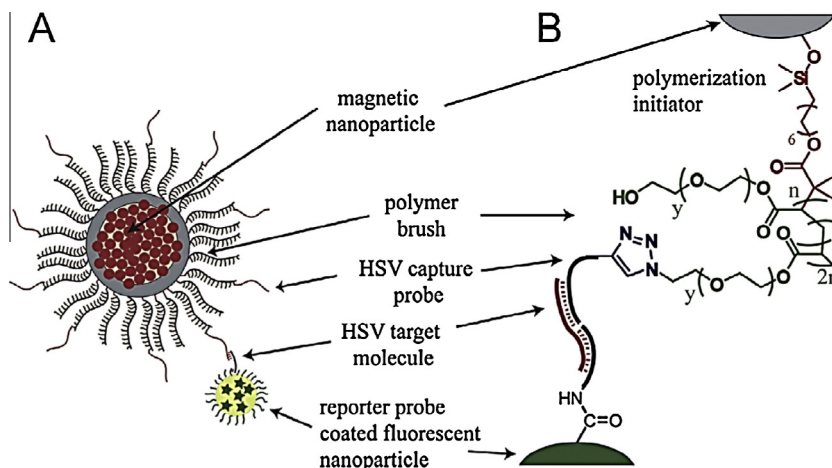


Fig. 4. (A) Schematic illustration of a sandwich type DNA detection assay that uses polymer brushes grafted from magnetic nanoparticles to covalently immobilize capture probes; (B) chemical structure of the polymer brush coating [64]. Reprinted from Biosensors and Bioelectronics, 50, D.A.C. Thomson, M.A. Cooper, A paramagnetic-reporter two particle system for amplification-free detection of DNA in serum, 499–501. Copyright 2013, with permission from Elsevier.

alkyne-functionalized capture probe DNA via copper catalysed azide-alkyne cyclo-addition (CuAAC), the OEGMA units provide a non-fouling background. Non-specific adsorption was further suppressed by quenching unreactive azide functionalities on the copolymer brushes with an alkyne substituted poly(ethylene glycol) derivative. The performance of the copolymer brush coated magnetic particles was evaluated in a sandwich assay using fluorescent reporter probe modified particles. The authors reported 6.6×10^{13} probes/cm² as the optimum probe density and limits of detection of 6, respectively, 60 pM in buffer and 50% fetal bovine serum. Assay and reagent optimization allowed to further improve the detection limit of target DNAs down to the 0.5 pM (25 amol) level [64].

In a recent study, Wang et al. used poly(acrylic acid) (PAA) brushes produced by SI-ATRP to immobilize long, single strand DNA sequences that consisted of many successive copies of the probe DNA sequence. These long “multi-probe” sequences were produced via rolling circle amplification (RCA) [65,66] from a short DNA primer that was attached to the PAA brush via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) mediated coupling chemistry (Fig. 5). The use of these long single strand DNA sequences that present a repetitive series of copies of the probe sequence is attractive since it provides access to surfaces that present very high probe surface concentrations. This array format was shown to allow multiplexing and was reported to have a dynamic concentration range of 0.1–100 nM and a limit of detection of 0.1 nM [67].

Plasma sputtered nylon films deposited on surface plasmon resonance (SPR) chips were modified with poly(polyethylene glycol methacrylate) (PPEGMA) brushes.

Activation of the side chain hydroxyl groups with N,N'-disuccinimidyl carbonate (DSC) and subsequent covalent attachment of streptavidin allowed to immobilize biotinylated DNA molecules. Both the streptavidin coupling as well as the binding of the biotinylated DNA and the subsequent hybridization with a complementary oligonucleotide sequence could be monitored by SPR [68].

Xie et al. have used polymer pen lithography to prepare gradient type surfaces that present micropatterns of PGMA brushes covering a range of brush thicknesses and feature sizes. These PGMA brush patterns were used to immobilize 3'-tetrachlorofluorescein (TET) labelled oligonucleotides, which were subsequently exposed to a tetramethylrhodamine (TAMRA) end-modified complementary DNA strand. The use of the TET/TAMRA label pair allowed to use FRET to monitor the hybridization of the nucleotides. Analysis of the fluorescence intensities revealed that both the amount of TET-labelled oligonucleotides as well as the quantity of hybridized double stranded DNA increased with increasing brush thickness [69].

In addition to covalently attaching probe DNA on a suitable reactive polymer brush platform, another strategy towards nucleotide functionalized polymer brushes involves direct surface-initiated controlled radical polymerization of an appropriate oligonucleotide functionalized monomer. This approach has been utilized by Henry et al. who prepared brushes of 17.9 nm thickness by direct surface-initiated atom transfer radical copolymerization of acrylamide and an acrylamide modified oligonucleotide sequence that is complementary to the Exon 16 sequence of the breast cancer related gene BRCA1. SPR analysis revealed that these surfaces present $\sim 1.1 \times 10^{12}$ probe nucleotides and have a limit of detection towards the target sequence of 22×10^{-12} M. Interestingly, this limit of

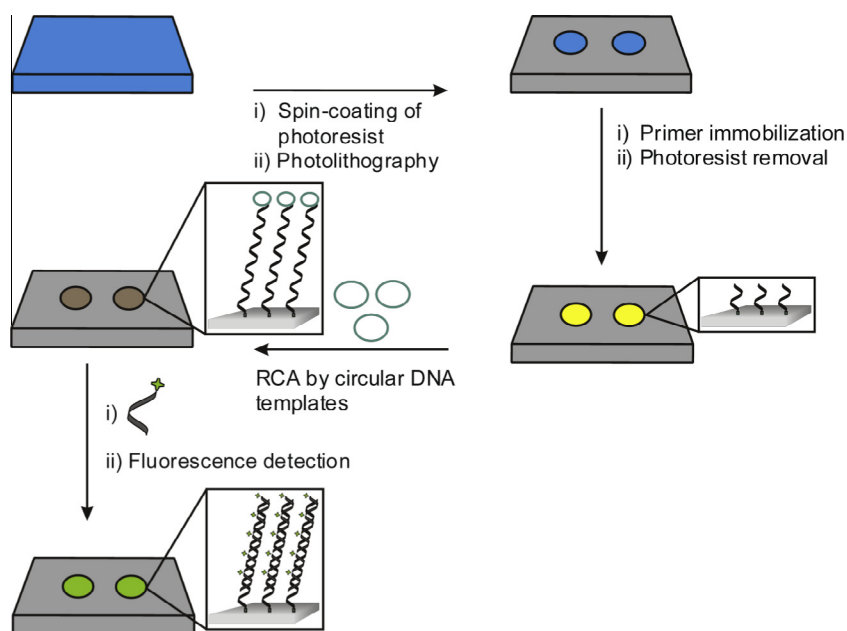


Fig. 5. Preparation of poly(acrylic acid) brush patterns using photolithography followed by the immobilization of primers on the polymer brush surface and subsequent DNA microarray fabrication by RCA of circular DNA templates. (Re-drawn from [67].)

detection is much lower than that of an SPR chip modified with a thiolated monolayer of the corresponding probe DNA, even though the probe surface density in the latter case is about 4 times high as compared to the DNA functionalized brushes. The authors attributed this to a cooperative refractive index change upon the binding of the target DNAs to the surface and the conformational changes of the DNA-polymer brush upon hybridization [70]. The authors also used the same copolymer brush platform to develop a sandwich type electrochemical sensor for the detection of the Exon 16 marker. Using a horseradish peroxidase (HRP) modified reporter probe, a limit of detection of 2.67 nM could be achieved [71].

2.2. Non-covalent DNA binding polymer brush platforms

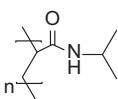
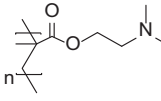
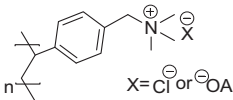
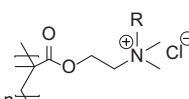
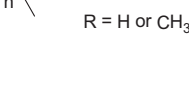
Table 2 provides an overview of the polymer brush platforms that have been used to non-covalently capture DNA. The examples in this table can be divided into two main categories. The first entry (row 1) presents three examples that use the temperature-induced stretching and collapse of surface tethered, thermosensitive poly(N-isopropylacrylamide) (PNIPAAm) chains to release captured DNA [72–74]. The other examples explore electrostatic interactions to capture DNA and can be subdivided in two groups. The poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA) platform used in the second row is a pH sensitive platform that allows to capture negatively charged DNA below the pK_a and release DNA above the pK_a [75,76]. The other 3 polymer brush platforms (rows 3–4) are representing permanently positively charged films that allow to entrap negatively charged DNA [77–79].

In a series of papers, Chen and Li have presented the development of several prototypical fluidic and lab-on-a-chip devices that use the thermoresponsive properties of a surface-grafted poly(N-isopropylacrylamide) (PNIPAAm) brush to release captured DNA [72,73]. The devices are first exposed to the sample at a temperature $<40^\circ\text{C}$, which is below the lower critical solution temperature (LCST) of the PNIPAAm brush, to capture the DNA. Upon raising the temperature to 60°C (above the LCST of the PNIPAAm brush), the surface tethered polymer chains collapse, which results in release of the entrapped DNA. The released DNA is amplified using polymerase chain reaction (PCR) and analyzed with electrophoresis. Using this method, the authors were able to detect DNA present at sample concentrations down to $0.5\text{ ng}/\mu\text{L}$ [74] and were able to resolve mixtures of two DNA strands containing 528 and 584 base pairs, respectively. Chen and collaborators have extended their DNA capture-release-amplification strategy also to the use of pH-sensitive poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA) brushes. The PDMAEMA brushes were used to capture DNA at pH 6 and release at pH 8, concomitant with a change in the surface charges of the polymer brush. Using these pH responsive polymer brush interfaces the authors could detect DNA that was present at sample concentrations as low as $0.1\text{ ng}/\mu\text{L}$ [75,76].

Demirci and Caykara prepared cationic poly[(vinylbenzyl)trimethylammonium chloride] (PVBTAAC) brushes via RAFT polymerization. These brushes were evaluated for their DNA adsorption capacity using ellipsometry. From the ellipsometric thickness and refractive index values and using Langmuir equation, the maximum DNA

Table 2

Overview of polymer brushes that have been used for the non-covalent immobilization of DNA.

Polymer brush	Chemical structure	Substrate	SI-CRP method	Thickness (nm)	Remarks
PNIPAAm [72–74]		Si	ATRP	73–75 ^a	Due to the hydrophilic character of polymer brush below the LCST, DNA molecules can diffuse into the water boundary on the polymer brush and be captured within the polymer brush layer. Heating above the LCST results in release of DNA.
PDMAEMA [75,76]		Si	ATRP	129, 179, 285, 363 ^a [75] 363 ^a [76]	pH-sensitive polymer brushes at thicknesses above 179 nm could capture DNA molecules at pH values below pK_a . DNA molecules could be released at pH values above pK_a [75]. The brushes were used to develop a microfluidic device for the detection of breast cancer recurrence DNA [76].
PVBTAAC [77]		Si	RAFT	51.9 ^a	Cationic polymer brushes were shown to capture negatively charged DNA molecules with a maximum adsorption capacity of $0.791\text{ mg}/\text{cm}^2$ calculated based on ellipsometric thickness and refractive index values using Langmuir equation.
PDMAEMA [78]		Cellulose paper	ATRP	–	Protonated polymer brushes bearing positive surface charges could bind negatively charged probe DNA. Subsequent hybridization with the complementary target DNA could be monitored by addition of PicoGreen [78].
QPMAEMA [79]		Filter paper	ARGET ATRP	–	Quaternized cationic polymer brushes could bind negatively charged DNA molecules to be analyzed and the hybridization of the complementary target sequence could be followed by a Dot blot analysis using biotinylated PNA probe and an enzyme mediated calorimetric assay [79].

^a Thickness determined by ellipsometry.

adsorption capacity at equilibrium was calculated as 0.791 mg/cm² [77].

In another example, linear, PDMAEMA brushes grown from cellulose paper were used to immobilize and detect DNA. The polymer brush coated cellulose paper was used in combination with PicoGreen (PG), which is relatively non-fluorescent when unbound, but lightens up upon intercalating with the surface bound double stranded DNA, i.e. upon hybridization of probe and target DNA. First, probe DNA was captured by the cationic polymer brush. Subsequent addition of complementary target DNAs results in successful hybridization, which could be visualized and quantitatively evaluated by addition of PicoGreen. The authors reported a limit of detection as low as 0.3 nM in homogenous solution and of ~1000 nM in biological serum [78].

Hoven et al. have grown quaternized PDMAEMA brushes from filter paper by activator regenerated by electron transfer atom transfer radical polymerization (ARGET ATRP) of DMAEMA and subsequent quaternization with methyl iodide. The brush modified filter paper was used for DNA detection using a Dot blot format. DNA detection involves first electrostatic immobilization of the sample DNA followed by exposure to and hybridization of a biotinylated peptide nucleic acid (b-PNA) probe. PNA was used as a probe as it mimics the selective binding properties of oligonucleotides, but has a non-charged backbone, thus avoiding non-specific electrostatic adsorption onto the polymer brush. The b-PNA could bind streptavidin modified-HRP, allowing an enzyme mediated calorimetric assay. The limit of detection was given as 10 fmol to determine

single mismatch discrimination due to the low non-specific interactions of the polymer brush modified filter paper and due to the high specificity of PNA probe to be hybridized to the sample DNA [79].

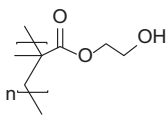
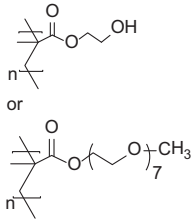
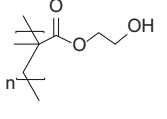
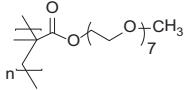
2.3. Surface-initiated controlled radical polymerization assisted DNA detection

In addition to utilizing surface-initiated controlled radical polymerization to generate polymer brush films that can covalently or non-covalently bind DNA, surface-initiated controlled radical polymerization has also been explored as a tool to allow direct visualization of DNA detection [80–82]. Table 3 provides a summary of the different surface-initiated controlled radical polymerization strategies that have been developed to amplify and facilitate DNA detection. The first two rows [83–87] use a three-strand oligonucleotide system and ATRP to amplify hybridization of the target DNA both from planar gold substrates as well as gold nanoparticle surfaces (Fig. 3). The entries in the third row of Table 3 utilize AGET ATRP for signal amplification [88–90], which is an attractive further development as it avoids the need for inert conditions. The last two examples use RAFT polymerization on gold substrate and porous polyacrylamide gel for the amplification [91,92].

Fig. 3 illustrates SI-CRP assisted DNA detection, which is also referred as amplification-by-polymerization, based on a three-strand oligonucleotide-system [83–85]. First, DNA capture probes of complementary (C) and non-complementary (NC) sequences are immobilized on a gold substrate.

Table 3

Overview of different surface-initiated controlled radical polymerization strategies that have been used to amplify and facilitate DNA detection.

Polymer brush	Chemical structure	Substrate	SI-CRP method	Remarks
PHEMA [83–85]		Au	ATRP	Surface-initiated ATRP from initiator modified target probes allows visual detection of DNA hybridization. The further functionalization of the hydroxyl side chain functional groups of polymer brush with an ATRP initiator resulted in the formation of branched polymer brushes which allowed to detect one and three-base mismatches [83]. Non-specific adsorption could be further eliminated by passivating the surface with a non-fouling ethylene glycol monolayer [84]. The addition of Cu(0) to the polymerization system could eliminate the need for inert conditions required for the polymerization [85].
PHEMA or POEGMA [86,87]		Au NPs	ATRP	Immobilization of capture probe DNA on the surface of gold nanoparticles allowed the calorimetric detection of DNA hybridization. Hybridization of the target DNA resulted in the formation of polymer brushes which could provide both the particle stability and the calorimetric DNA detection [86,87].
PHEMA [88–90]		Au	AGET ATRP	Air tolerant AGET ATRP used to amplify DNA hybridization in different detection schemes using PNA based probe surfaces [88], electrochemical detection schemes [89] or polylysine-based macroinitiators for signal amplification [90].
POEGMA [91,92]		Au Porous polyacrylamide gel	RAFT	Signal amplification by RAFT polymerization using CTA-modified detection probes. This strategy was used for signal amplification both on planar gold substrates [91] as well as porous polyacrylamide hydrogels [92].

After that, the capture probe modified surfaces are exposed to the target DNA sequence (C'D') and finally an ATRP initiator modified detection probe (D). Subsequently, immersion of the substrates into an ATRP reaction solution containing the catalyst and HEMA as monomer results in SI-ATRP at those parts of the surface that present the complementary capture probe. The SI-ATRP process changes the surface reflectivity and opacity, thus allowing direct visualisation of the hybridization with the naked eye. In contrast, areas that did not present the complementary capture probe did not reveal any visible changes in the surface properties. By performing a single step SI-ATRP of HEMA resulting in a linear PHEMA brush, this approach allowed to visualize detection of target DNA at concentrations down to 0.1 μM [83]. This limit of detection could be decreased to 1 nM by applying a two-stage amplification strategy, in which the hydroxyl side chain functional groups of the initially grown PHEMA polymer chains are modified with 2-bromoisobutryl bromide and then used to initiate a second ATRP reaction, leading to a branched-type PHEMA brush. This SI-ATRP enhanced detection scheme was also successfully used to detect one and three-base mismatches [83]. By first passivating the gold substrate with a thiol modified oligo(ethylene glycol) monolayer, non-specific adsorption can be reduced, resulting in an decrease in background noise [84]. By utilizing gold nanoparticles instead of planar gold substrates, this strategy allowed calorimetric DNA detection. Whereas binding to target DNA resulted in the formation of a stabilizing POEGMA shell, solutions with particles that did not bind a complementary DNA strand underwent a colour change from red to blue [86,87]. Whereas the original reaction conditions required the use of an oxygen-free environment for the SI-ATRP step, the need to operate under inert conditions could be eliminated by using Cu(0) as a reducing agent [85]. Under these conditions, single stranded target DNA at concentrations of 1 fM could be detected.

Another attractive strategy to SI-ATRP enhanced DNA biosensing that obviates the need for inert reaction conditions is the use of activators generated by electron transfer for atom transfer radical polymerization (AGET ATRP). This technique was successfully used to amplify hybridization

of target DNA on gold surfaces that presented the complementary probe PNA with a limit of detection of ~ 200 fmol (Fig. 6) [88]. AGET ATRP was also used to extend the concept of polymerization enhanced DNA biosensing based on the three-strand oligonucleotide system to an electrochemical detection format [89]. To this end, the side chain hydroxyl groups of the PHEMA chains were modified with aminoferrocene using 1,1'-carbonyldiimidazole (CDI) as the coupling agent. Using this electrochemical detection scheme, a limit of detection of ~ 15 pM towards target DNAs could be achieved [89].

Fig. 7 outlines an interesting further development of the SI-ATRP enhanced DNA detection method. This strategy uses surface attached PNA probes to selectively capture the target DNA. Successful binding of target DNA to the surface attached PNA is subsequently amplified by electrostatic adsorption of ATRP initiator modified polylysine (PLL). The use of the PLL macroinitiator has two distinct advantages; (i) as binding to the PNA/DNA duplex involves electrostatic interactions, there is no need to chemically modify each individual probe DNA sequence; (ii) as the PLL contains multiple ATRP initiator sites, it will generate a branched PHEMA brush, which facilitates detection. In order to avoid non-specific binding of the PLL macroinitiators to the surface attached probes, this strategy uses neutral PNA instead of negatively charge DNA probes. This approach provides a limit of detection of 1 nM corresponding to 3 fmol of target DNA detected. With the naked eye, this detection system allows the detection of target DNA at concentrations of ~ 10 nM [90].

In addition to ATRP, RAFT polymerization has also been successfully used for the amplification-by-polymerization detection of target DNA. Using OEGMA as the monomer this enabled the visual detection of target DNA at concentration down to 1 fM based on a three-strand oligonucleotide system [91]. The RAFT mediated chemical amplification strategy was also successfully used to visualize detection of target DNA that was entrapped in a porous polyacrylamide hydrogel with the aid of chain transfer agent (CTA)-coupled detection probes that were partially complementary to the target DNA and could result in

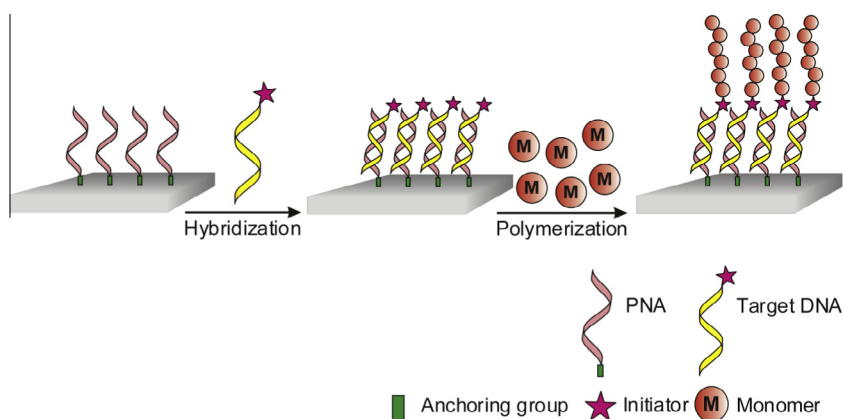


Fig. 6. SI-CRP mediated amplification of target DNA hybridization using surface immobilized PNA probes [88].

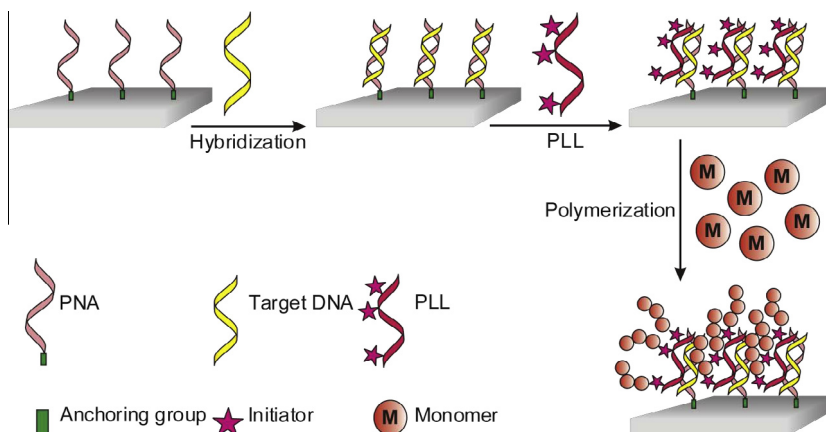


Fig. 7. Detection of target DNA hybridization using surface-immobilized probe PNA and a polymer-based macroinitiator for signal amplification [90].

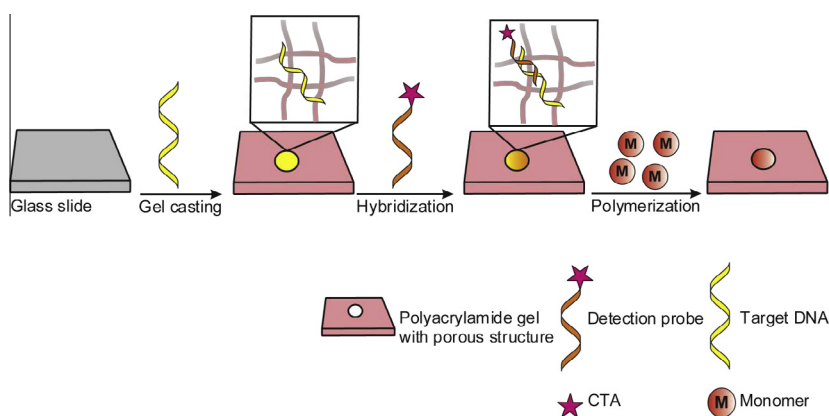


Fig. 8. Amplification of target DNA hybridization using RAFT chain transfer agent modified detection probes [92].

polymer growth when hybridization took place (Fig. 8). The calculated limit of detection for this in-gel DNA detection system was 2.8 pM [92].

Krull and co-workers investigated DNA hybridization on surfaces that presented both the probe DNA as well as PHEMA brush chains grown via surface-initiated atom transfer radical polymerization [93]. The co-presentation of the surface-grafted PHEMA chains was explored to suppress oligonucleotide-to-oligonucleotide and/or oligonucleotide-to-surface interactions. The presence of the PHEMA grafts reduced non-specific adsorption, sharpened melting curves and resulted in increased resolution. In a following study, the selectivity of these mixed films was evaluated towards determining single nucleotide polymorphisms (SNP) based on distinctive sharpness of the melt curves and melting temperature differences compared to the fully complementary target DNA [94].

3. Conclusions and outlook

Surface-initiated controlled radical polymerizations (SI-CRP) represent a powerful toolbox that allow to facilitate

or enhance DNA biosensing. On the one hand, SI-CRP enables to produce high binding capacity polymer coatings that can entrap DNA either covalently or non-covalently. On the other hand, SI-CRP also provides interesting and manifold opportunities as an amplification method that allows visual detection of DNA hybridization. This article has presented various examples in which SI-CRP has been successfully used for one of these applications. Nevertheless, there also seems to be room for further advancement. The grafting density of the surface-tethered brushes, for example, is a parameter that has only received very limited attention. Variation of grafting density, and in particular, the investigation of less densely grafted brushes may provide opportunities to further increase binding capacity. Other opportunities to generate DNA biosensor surfaces with high binding capacities exist in the use of complex, three-dimensional nano/microstructured sensor surfaces. SI-CRP is also ideally suited to conformally coat these complex substrates with a high binding capacity polymer coating. These opportunities, together with further advances in SI-CRP, offer exciting prospects to engineer the structure and properties of DNA recognition and binding interfaces and to improve the performance of DNA biosensors.

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