P-042	**Novel colorimetric detection of a serum biomarker with polydiacetylene-based nanoparticles**	

Chan T. [1,2] and Gu F. X. [1,2*#]
[1] 200 University Ave. W., Waterloo, Canada N2L3G1 [2] University of Waterloo - Waterloo, Canada [*] Supervisor [#] frank.gu@uwaterloo.ca

INTRODUCTION AND OBJECTIVES

Over the past decade, there has been a growing interest in point-of-care testing (POCT) devices for diagnosing various diseases. POCT devices offer several advantages over traditional diagnostic methods, which are elucidated in-depth elsewhere (Yager 2008). Briefly, POCT devices provide faster diagnostic results than microbial cultures, by detecting and quantifying biomarkers – molecules present in the body at specific concentrations that are indicative of the presence and/or severity of a disease. Compared to other biomarker-based methods, such as ELISA or FISH, POCT devices: are extremely portable and self-contained; require no specialized training to use; provide easily interpretable diagnostic results; and require little sample preparation.

For the best patient outcomes, most diseases, including sepsis, require early treatment, and thus early diagnosis (Pierrakos 2010). A possible POCT strategy for diagnosing sepsis is detecting the serum concentrations of human C-reactive protein (CRP). CRP is a general acute-phase reactant protein that rises in concentration up to 1000-fold in the blood in response to inflammation and infection (Steel 1994). CRP concentrations greater than 10 mg/L are indicative of infection (Chiriboga 2009), while other biomarkers are present at much lower concentrations. Consequently, the primary challenge during the development of a POCT device is the amplification of the detection signal for low concentration biomarkers.

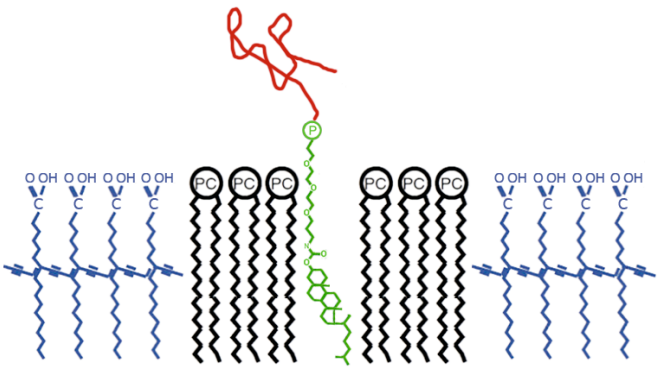

Figure 1 : Schematic diagram of the aptamer-functionalized nanoparticle's surface.

In this study, we have developed a polydiacetylene (PDA)/phospholipid nanoparticle (NP) that generates a visible colour change upon detection of CRP. Polydiacetylene is ideal for POCT applications due to the dramatic visible colour change it displays when the polymer is subjected to external stimuli, such as pH change or mechanical stress (Lee 2010). While other groups have developed polydiacetylene/phospholipid particles to detect antibodies (Kolusheva 2001) and thrombin protein (Kim 2011), the target receptors used lack the specificity offered by antibodies and aptamers, or require several modifications to the NP components. In comparison, our NPs are synthesized with minimal modifications to the polydiacetylene monomer, phospholipid, and target receptor. The target receptors tested include an ssRNA aptamer and anti-CRP antibody (Figure 1). The detection efficiency and stability of the NPs are assessed and compared *in vitro* in near physiological conditions.

MATERIALS AND METHODS

Materials

10,12-tricosadiynoic acid (TRCDA) was purchased from GFS Chemicals (Ohio, USA). 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from Avanti Polar Lipids (Alabama, USA). Human C-reactive protein was purchased from RayBiotech (Georgia, USA). Anti-CRP IgG produced in rabbit was purchased from Sigma (Missouri, USA). N-[ß-Maleimidopropionic acid] hydrazide, trifluoroacetic acid salt (BMPH) and 2-mercaptoethanolamine·HCl (2-MEA) were purchased from Thermo Fisher Scientific (Illinois, USA). Single-stranded RNA aptamer with a 3'-cholesteryl-TEG modification was purchased from Integrated DNA Technologies (Iowa, USA), with the following sequence: 5'-GCC UGUAAGGUGGUCGGUGUGGCGAGUGUGUUAGA GAGAUUGC-3'.

Nanoparticle synthesis

The nanoparticles were prepared using a modified previously described method (Kolusheva 2001). Briefly, TRCDA was dissolved in chloroform, then filtered with Pall 0.45 µm PTFE membrane (New York, USA). After the solvent was purge with N_2 and dried *in vacuo* overnight, the filtered TRCDA was dissolved in a chloroform:ethanol solution to 60 mM. DMPC was dissolved in the same solution at 40 mM. TRCDA and DMPC were subsequently mixed together, dried *in vacuo* for 6 hours, then rehydrated with nuclease-free water containing the aptamer. The aqueous solution was heated to 70 °C, then pulse sonicated for 3 minutes using a Branson S-450D sonicator (Connecticut, USA). After cooling, then storing at 4 °C overnight, the aqueous solution was irradiated at 254 nm for 30 seconds using a

UVP CL-1000 UV crosslinker (California, USA), resulting in a blue colour solution.

The same procedure was slightly modified to form antibody-functionalized NPs. Briefly, the dried lipid mixture was rehydrated without the presence of aptamers. After the NPs were formed, they were crosslinked with BMPH, then subsequently crosslinked with the antibody. The antibody was previously selectively reduced at the hinge region with 2-MEA according to manufacturer's instructions. The formulated NPs were subsequently characterized using transmission electron microscopy to determine the mean diameter of the nanoparticles and verify the geometrical structure.

Target detection assay

To replicate physiological conditions, the CRP suspended in sterilized phosphate buffered saline (PBS) pH 7.4 at a various concentrations, including 10 mg/L. The NPs were purified and kept in aqueous solution. Varying ratios of NP to CRP were added to a 96-well microplate. After a few minutes of incubation at room temperature, the absorbance measurements were recorded at 550 nm and 650 nm to determine the degree of colour change after target detection. Absorbance readings at 260 nm and 280 nm were also recorded to determine aptamer and protein concentrations, respectively.

RESULTS AND DISCUSSION

To assess the detection efficiency of the NP, it was mixed at varying ratios with a 10 mg/L CRP solution. The addition of CRP resulted in a colour change from blue to light purple (Figure 2), indicating the NPs detected the CRP in solution. The NP was also tested with ethanol to compare against the NPs mixed with CRP, as well as verify the successful formation of the nanoparticle. Since the addition of ethanol causes a large pH change in solution, successfully formed NPs would undergo a visibly noticeable colour change from blue to pink after addition of ethanol (Figure 2).

Figure 2: Image from the target detection assay. Left to right: NP only, NP:CRP (1:1), NP:CRP (2:1), NP:EtOH (1:1).

The absorbance spectrums of the varying ratio mixes were recorded to quantify the colour change (Figure 3). The NP exhibited a maximum peak at 650 nm. The appearance of a significant peak at 550 nm was indicative of a colour change, as demonstrated by the addition of ethanol. Mixtures of NP:CRP showed minor peaks at 550 nm, which were not evident for the sample of only NPs. However, maximum peaks still occurred at 650 nm.

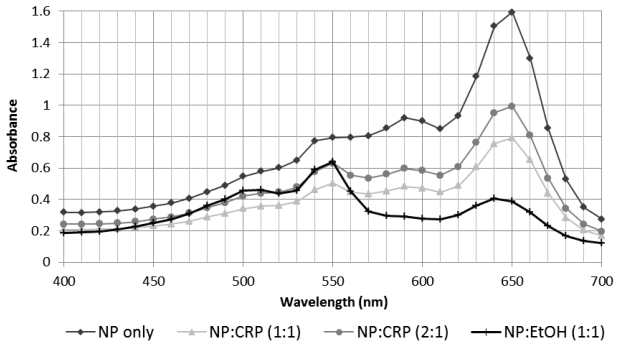

Figure 3: Absorbance spectrums from the target detection assay.

Based on the observed data, the absorbance spectrums corresponded with the observed visible colour changes. For instance, the retention of a maximum peak at 650 nm corresponded with the light intensity of blue still present in the NP:CRP samples. However, further studies are required to optimize the amount of target receptor used during synthesis to achieve more dramatic visible colour changes.

CONCLUSION

A novel strategy for incorporating target receptors onto polydiacetylene-based NPs was developed. The addition of the biomarker CRP resulted in the NPs turning blue to light purple, indicating the biomarker detected by the NPs. Future studies are needed optimize the synthesis process to generate a colour change similar to that observed when mixing the NPs with ethanol. Furthermore, detection of lower concentration biomarkers should be analyzed in the future.

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