Lab-on-a-Syringe Diagnosis of Kaposi's Sarcoma in the Developing World

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ABSTRACT

Kaposi's sarcoma (KS) is the leading cancer in untreated Human immunodeficiency virus (HIV) infected individuals, effecting 1 in 20 patients. Two challenges present themselves in determining the presence of Kaposi's sarcoma associated herpes virus (KSHV), the cause of KS: 1. The presence of similarly presenting diseases, such as Bacillary angiomatosis (BA) and 2. The detection of KSHV in biopsy samples. Here we show work on creating a multiplexed colorimetric detection scheme for KS and BA based on gold and silver nanoparticles as well as work on integrating this scheme with technology to lyse biopsy samples.

KEYWORDS

Biosensors, Gold Nanoparticles, Kaposi's sarcoma

INTRODUCTION

When the acquired immunodeficiency syndrome (AIDS) epidemic began in the early 1980s, often the first sign patients were infected was the appearance of red cancerous skin lesions, symptoms of an opportunistic disease known as Kaposi's sarcoma (KS).[1] In 1983 Human immunodeficiency virus (HIV) was discovered as the cause of AIDS,[2-3] and a little over ten years later the cause of KS was connected to a second virus, Kaposi's sarcoma associated herpesvirus (KSHV).[4] Kaposi's sarcoma is still the leading cancer in untreated HIV infected individuals, and still plays a role in the developed world even after highly anti-retroviral therapy (HART) has made a tremendous impact.[5] While KSHV transmission is still being studied,[6] it is known that in some populations the virus hastily spreads, and in some places effects over 40% of the population.[7] While the seroprevalance of KSHV is this high, the disease only causes cancer in select individuals, and the clinically relevant question becomes detecting KSHV in cancer biopsy samples, and not simply antibodies in patient blood serum. In addition to the difficulty associated with diagnosing KS based on biopsy samples, additional considerations arise because of the existence of a number of similarly presenting diseases, such as Bacillary angiomatosis (BA). BA is a bacterial infection, unlike the viral disease KS, and presents with similar red-purple nodules. Further, both KS and BA can often look the same on hematoxylin and eosin (H&E) stained histology slides, requiring more advanced immunohistochemistry to distinguish. Ultimately, KS detection involves two unique challenges: 1.The need for biopsy based diagnostics, and 2.The presence of diseases that appear the same as KS when viewed using histology.

To date, a considerable amount of work has been conducted on creating diagnostics for the developing world. A tremendous amount of biosensors have been constructed using various mechanical, electrical, and optical components, and have achieved all sorts of milestones in terms of measurements like sensitivity, specificity, and cost.[8] Of the numerous biosensors created, some of the most interesting involve those that use metal nanoparticles and the bright colors associated with their localized surface plasmon resonance to couple detection reactions to colorimetric changes.[9] However, for all of the accomplishments in creating point-of-care diagnostics based on nanoparticle aggregation reactions, limitations still exist in the form of sample preprocessing, the requirement to work in various buffers, and limitations in detecting multiple targets.

In this work we demonstrate how gold and silver nanoparticle aggregations can be combined into one multiplexed solution capable of detecting both KSHV and BA oligonucleotide sequences. This multiplexed detection in a single solution is particularly important because it enables more complicated microfluidic systems to later be integrated for biopsy lysis. We show results of this multiplexed detection reaction and demonstrate the limit of detection to be approximately 1nM. Further, we show preliminary work at creating an integrated device for biopsy based diagnosis (Figure 1).

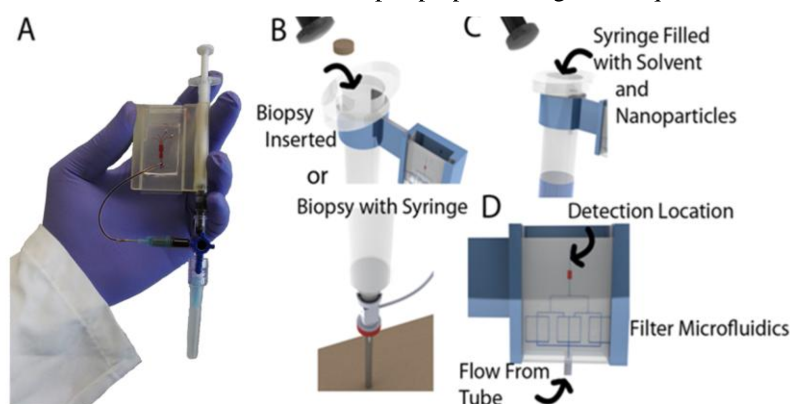

Figure 1: A prototype Lab-on-a-Syringe Diagnostic is shown (a). Samples can be collected using an attached biopsy needle (b), mixed in the syringe (c), and injected into an attached microfluidic chip for readout (d).

EXPERIMENT

Sequences were chosen for KSHV and the two bacteria species that cause BA, *Bartonella quintana* and *Bartonella henselae,* using BLAST Primer Design. Oligonucleotides were attached to gold nanoparticles based on thiol-gold chemistry. Gold nanoparticles and silver nanoparticles were mixed at concentrations of 1.5nM and 325 pM for use in the two-color multiplexed experiments. Due to silver's higher absorption cross section, a lower concentration was used. In the sensitivity experiments various different concentrations of DNA were added to 40uL of both silver and gold independently to measure the sensitivity of each channel. In both two-color and sensitivity reactions solutions were given 2h at 65˚C to react, and their near UV and visible spectrums were collected. Scanning electron micrographs (SEMs) were collected of nanoparticles spotted onto a silicon wafer to further illustrate aggregation.

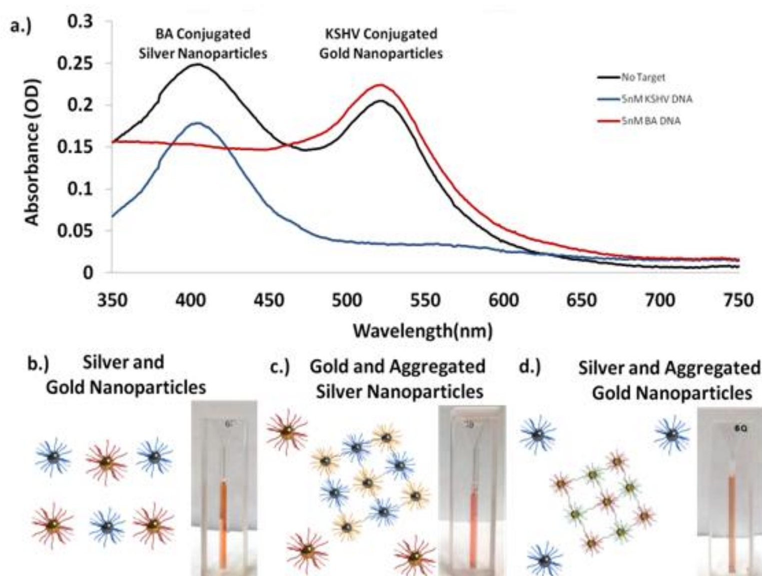

Figure 2: (a) An optical spectrum illustrates the change in the nanoparticles resonance as they aggregate. (b) A solution of conjugated gold and silver nanoparticles before the addition of target is shown. (c) Upon introduction of KSHV DNA the gold particles aggregate and the solution turns darker orange. (d) Upon introduction of BA DNA the silver nanoparticles aggregate, and the solution becomes bright pink. All DNA solutions added were 5nM.

RESULTS AND DISCUSSION

Attachment of oligonucleotides to gold and silver nanoparticles yielded solutions stable in high salt concentrations (300mM NaCl) for durations on the order of 1 month. As observed in the literature, the resonant peak of the nanoparticles shifted slightly (1-3nm) with the attachment of oligonucleotides. The results of a melting temperature analysis indicate that aggregates of gold nanoparticle-conjugates functionalized for KSHV, and silver nanoparticle conjugates functionalized for BA would disassociate between 70 ^{o}C and 80 ^{o}C, and 65 ^{o}C was used in all further experiments to prevent non-specific aggregation.

In multiplexed detection reactions using both gold and silver nanoparticle conjugates we were able to observe both sets of nanoparticles independently aggregating (Figure 2). When BA DNA was introduced into the system the silver nanoparticle conjugates would aggregate, and their absorbance peak would almost completely disappear. Similarly, upon introduction of KSHV DNA the gold nanoparticles would aggregate and their characteristic resonance peak would disappear. In the case of silver nanoparticles aggregating the solution changed from bright orange to a bright red, similar to the gold particles alone; in the case of gold nanoparticles aggregating, the solution would change to a murky orange. Further, SEMs also reveal that the particles were indeed aggregating in the presence of target DNA (Figure 3).

The limit of detection of both detection reactions were characterized separately (Figure 4). Our results indicate that our non-optimized system had colorimetric detection limits of approximately 1nM for the silver conjugates and 2nM for the gold conjugates. Further, both detection reactions could be resolved to lower limits (<1nM) using a spectrophotometer. We suspect the limit of the silver nanoparticles was higher because of the lower concentration of nanoparticles needed to achieve a similar absorbance of gold. This lower concentration leads to a higher DNA/nanoparticle ratio, a major

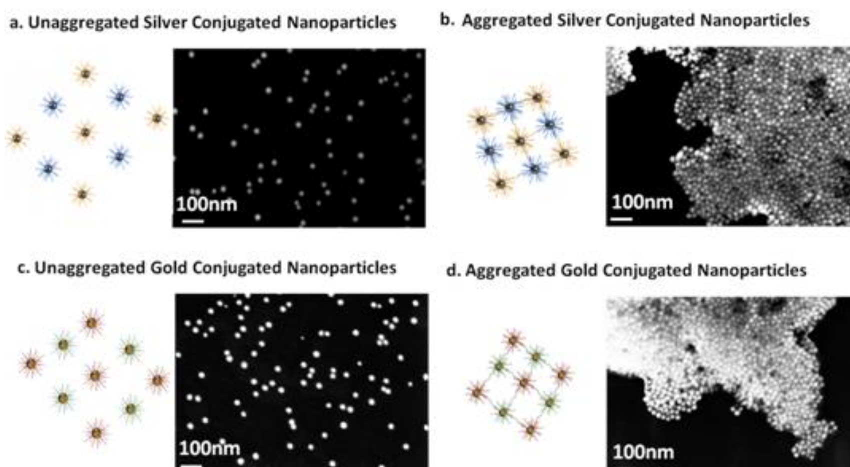

Figure 3: Scanning electron micrographs of unaggregated oligonucleotide conjugated silver and gold nanoparticles on a silicon wafer. After the addition of target DNA the silver and gold nanoparticles form aggregates.

factor in the size and number of aggregates formed. These results line up well with similar reported numbers for both gold and silver nanoparticles, with detection limits around 100pM for slightly larger silver particles and 1nM for similar sized gold.[9]

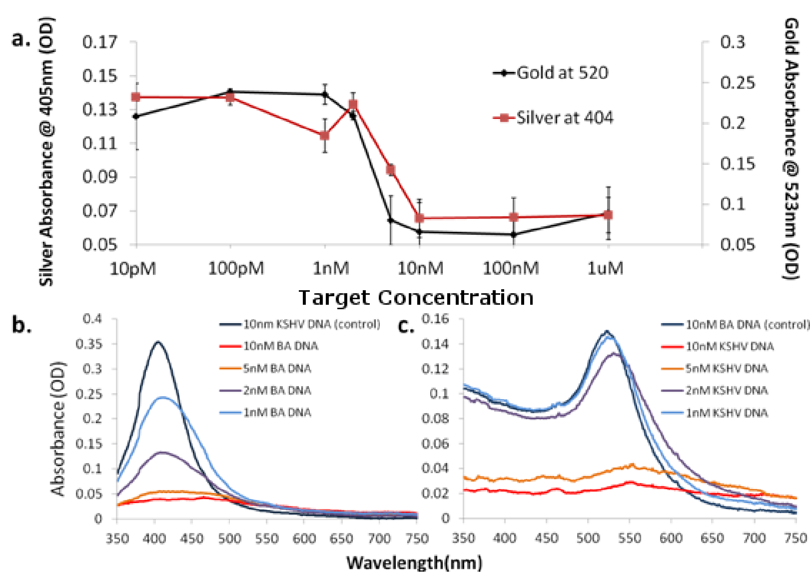

Figure 4: Sensitivity of both gold and silver nanoparticles conjugates. A color change was observed for silver solutions around 1nM DNA, and for gold nanoparticles around 2nM. (b) and (c) show the absorbance of the two systems between 1nM and10nM, in the systems transition region.

CONCLUSIONS

In this work we have demonstrated a one-solution multiplexed colorimetric detection of KSHV and BA DNA sequences using gold and silver nanoparticles. In order to use this technique on unamplified DNA sequences improvements in sensitivity are still required, however, because of its simple one solution nature, the techniques here can easily be integrated with microfluidic technology forming diagnostic devices capable of solving both major challenges associated with KS diagnosis; multiplexed detection and biopsy lysis. Further, a number of possible methods exist for increasing the sensitivity of colorimetric nanoparticle-based detection, some as simple as using larger nanoparticles.

Since the development of therapeutics capable of controlling HIV have been created the incidence rates of KS in the developed world has decreased. However, in regions where HART is prohibitively expensive, KSHV is still a major cause of cancer. Better diagnostics, built utilizing the system described above, could be useful in diagnosing KS and helping determine subsequent treatment. In future work we hope to integrate the reaction with biopsy-based detection, and solve both challenges involved in KS diagnosis.

ACKNOWLEDGEMENTS

This work was funded in part by the Clinical and Translational Science Center under NIH grant UL1 RR 024996. M. Mancuso would like to acknowledge a National Science Foundation Graduate Research Fellowship under Grant No. DGE-0707428. This work was performed in part at the Cornell NanoScale Facility, a member of the National Nanotechnology Infrastructure Network, which is supported by the National Science Foundation (Grant ECS-0335765) and at the Nanobiotechnology Center (NBTC), an STC Program of the National Science Foundation under Agreement no. ECS-9876771.

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