3D multiplex, paper-based rapid diagnostic tests using plasmonic gold nanoprobes

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Abstract

Plasmonic properties of gold nanoparticles are a promising tool to develop sensing alternatives for the traditional, but disadvantageous, enzyme catalyzed reactions and other biological recognition elements in biosensors. The need for sensing alternatives, especially in under developed areas of the world, has given rise to the application of non-enzymatic sensing and quantification approaches to biochemical analysis. Because of environmental and cost disadvantages of enzymatic reagents, metallic nanoparticles, with higher focus on the gold ones, have been synthesized and modified with the purpose of achieving sensitive probes for relevant health biomarkers over the years. The integration of these new recognition elements into versatile platforms, such as paper, gives the opportunity for the development of new, easy-to-use, low-cost, sustainable diagnostic tools. More specifically, the pairing of paper µfluidics and gold nanoparticles is a promising approach for the development of point-of-care devices, which can be used together with information technologies, such as smartphone embedded cameras and cloud computing, to develop complete systems to be used in under developed areas, for various health applications. Here, we present three individual, low-step, wet-chemistry assays, for each of the target health markets (free cholesterol, glucose and uric acid), and the fabrication and calibration of a µfluidic, multiplex, paper-based device, to serve as a platform for said assays. We found that the optical properties of differently produced AuNPs are influenced by the presence of the target analytes, by changes in size, conformation and interparticle distance, both in colloidal gold mixtures and in AuNPs immobilized in a chromatography paper substrate. The direct influence of glucose, in different quantities, towards the reduction of a gold salt, in alkaline medium, is used as a sensing approach [1], while for uric acid and cholesterol sensing, the introduction of molecules with thiol moieties, for surface modification and functionalization, was the approach. The use of digitonin [2] and 2-thiouracil (2-TU) [3], which present affinity towards cholesterol and uric acid, respectively, allows us to tune optical properties of AuNPs, depending on analyte concentration. In the case of glucose and uric acid, the interaction between nanoparticles and increased concentrations of the analytes cause a blue-shift, related to diminishing sizes of AuNPs, resulting in optimal sensitivity ranges of measurement (1.25 – 50 mM for glucose, 0 - 5 mM for UA, both including the clinical measuring range), while for cholesterol, interactions produce an increase in interparticle distance and consequent decrease of optical density. UV-Vis spectrophotometry, dynamic light scattering (DLS) and transmission electron microscopy (TEM) were used to determine the influence of the biomolecules in the plasmonic properties of AuNPs and the changes in size and conformation, in addition to applying Raman spectroscopy and other characterization techniques to study AuNP properties.

The studied AuNPs were posteriorly translated to the chromatography paper substrate, by two different approaches, for the creation of lab-on-paper applications: (i) consecutive deposition of reagents for direct synthesis of AuNPs into the paper substrate (for glucose sensing); (ii) direct deposition of surface modified and functionalized AuNPs, to serve as gold-based probes (for free cholesterol and uric acid sensing). We found that for the sensing of glucose, direct synthesis of AuNPs result in increasing color intensity of pink/red AunPs, because different amounts of particle are produced. This color intensity can be correlated to glucose concentrations, using digital, colorimetric calibration employing different color spaces, mainly RGB. For uric acid sensing, 2-TU functionalized AuNPs deposited into the paper substrate are influenced by the introduction of UA by color change indicative of anti-aggregation effects (from blue/purple to red), resulting in digital color calibration with a linear correlation and a sensitivity range between 71/87.5 µmol/L (depending on the color space used for calibration) and 1 mmol/L, which includes the clinical range of measurement. For cholesterol sensing, digitonin functionalized AuNPs (DAuNPs) interact with cholesterol in the paper substrate, to cause a decrease in color intensity, correlated to cholesterol concentrations using the HSV color space.

With the purpose of developing a platform to host the paper-based, colorimetric assays, a µfluidic, multiplex device is presented, for simultaneous, multiparametric sensing of the target health markers, resulting in a low-cost, low-complexity tool for diagnostics in low-resource areas of the world.

References

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[2] Raj, V., Johnson, T., & Joseph, K. *Biosensors and Bioelectronics*, *60* (2014) 191-194.UA

[3] Bera, R. K., Anoop, A., & Raj, C. R. *Chemical Communications*, *47*(41), (2011) 11498-11500

Figures

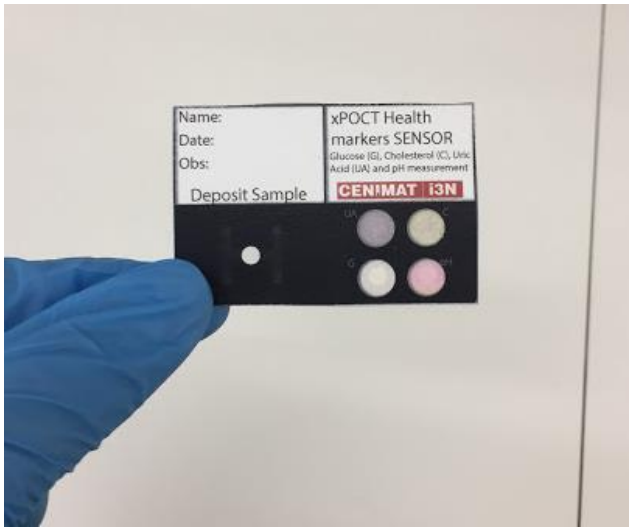

Figure 1. Real-size image of paper-based, 3D multiplex platform for screening of glucose, cholesterol and uric acid.

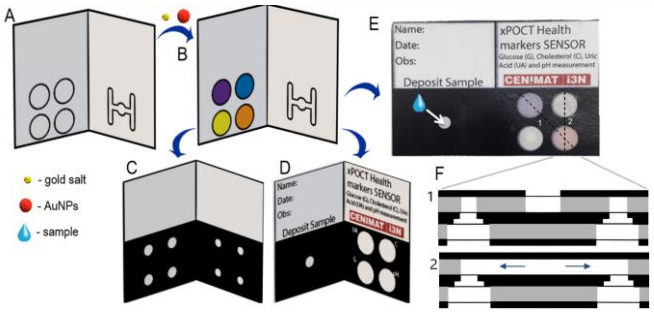

Figure 2. Schematic Illustration for assembly and operation of 3D µPAD.

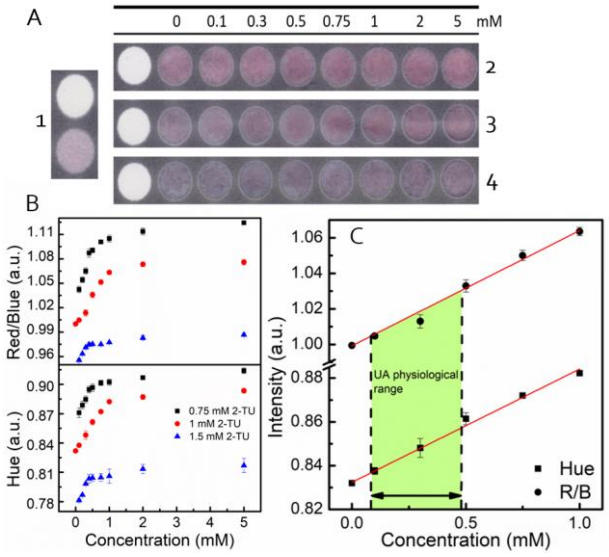

Figure 3. Paper-based assay of uric acid colorimetric behavior and color calibration.