Nanospring™-enhanced Enzyme-linked Immunosorbent Assay for Increased Sensitivity and Early Detection

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ABSTRACT

GoNano Technologies' Nanospring platform provides a unique approach towards increasing the sensitivity of current commercial ELISA test kits while minimizing the procedural requirements for the production and use of such test kits. The high surface area and hydroxyl rich surface of the silica Nanosprings provides an ideal substrate for antigen or other biomolecule attachment. The ability to control the surface chemistry of the Nanosprings could minimize functional surface area loss from non-functionalized surface sites or enable greater levels of multifunctional engineering.

Keywords: ELISA, nanomaterials, nanosprings, glass, silica.

1 INTRODUCTION

Enzyme-linked immunosorbent assay (ELISA) is a well known method for detecting the presence of antibodies or antigens with current applications in disease and pregnancy testing, and drug screening [e.g., 1-6]. While ELISA has a high detection sensitivity, further increase in the sensitivity of ELISA test kits would provide significant advantages in earlier detection of diseases when concentrations of antibodies are below the detection limits of present methods. For example, cancer – the second most common cause of death in the US – is more easily treated during the early stages of the disease and early detection can significantly increase patient survival rate. For this reason screening programs such as the Center for Disease Control's National Breast and Cervical Cancer Detection Program have been established to detect cancer before noticeable symptoms become manifest. While most of these screening programs consist of more traditional detection methods such as mammograms, pap smears, and prostate exams, many companies are producing ELISA test kits to complement such screenings. An example is Cervatec™ ELISA test kits for cervical cancer screening produced by mtm laboratories AG.

For simple sandwich ELISA methods (illustrated in Figure 1), an antibody is attached to a solid surface support following which a test sample with or without a target analyte protein is washed over the surface of the antigen such that any target protein present binds to antibodies. A secondary antibody linked to an enzyme is then introduced, binding to any target protein. If the test sample is void of target protein, then all the enzyme-linked secondary antibody is removed with washing and no enzyme catalyzed signal results. If the test sample contains the target protein, then the enzyme-linked secondary antibody is retained on the surface resulting in a positive signal. Most
ELISA test kits use 96-well plates with well diameters of ~5 mm. The interior surface of these wells serve as the binding surface for the test.

While there are many variations of the ELISA method, such as indirect ELISA, sandwich ELISA, competitive ELISA, and reverse ELISA, these techniques are all dependent on attachment to a solid surface. One method for increasing the sensitivity of ELISA is to increase the available surface for binding, which results in more test molecule attachment and subsequently larger concentrations of enzyme. Increased sensitivity of ELISA has already been demonstrated using high surface area gold nanoparticles [7]. However, in this study, gold nanoparticles were used with magnetic microparticles and an external magnet in order to retain the nanoparticles during washings. The target protein in this study (p53) when present acted as a bridge between p53 antibodies immobilized on the magnetic microparticles and p53 antibodies immobilized on the gold nanoparticles. By utilizing the high surface area nanoparticles, this study reported an increase in sensitivity of 25 times that of the standard test kit.

GoNano Technologies’ Nanospring™ technology represents an attractive approach to providing a high surface area that will lead to increased sensitivity [e.g., 8,9]. Nanospring mats are immobilized on the surface on which they are grown and therefore eliminate the need for magnetic methods to retain the nanomaterial throughout the ELISA process. X-ray photoelectron spectroscopic analysis of one-dimensional silica nanostructures has been undertaken to characterize their surface chemistry.

## 2 RESULTS AND DISCUSSION

Figure 2 is a scanning electron microscope (SEM) image showing part of a silica Nanospring mat. The loosely intertwined Nanosprings create an inverse porous material with 100% open porosity. At higher resolution, each individual Nanospring is comprised of multiple silica (SiO$_2$) nanowires ~5 nm in diameter wrapped around each other in a helical configuration. If a 5 mm diameter well in a 96-well plate, with a well footprint of approximately 20 mm$^2$, was coated with Nanosprings the available surface area becomes an astonishing 30,000 mm$^2$ or 300 cm$^2$: an increase of over three orders of magnitude.

In addition to increased sensitivity of ELISA techniques, the ability to coat Nanosprings with metal or metal oxide nanoparticles, as demonstrated in Figure 3, enables the creation of multifunctional ELISA test kits that could identify the presence of multiple biomolecules simultaneously. For example, coating of the surface with gold nanoparticles enables the use of thiol chemistry methods for attaching functional groups to the gold nanoparticles while silane chemistry methods can be used for attaching functional groups to the silica Nanosprings, as illustrated in Figure 4. Such a multifunctional test kit would be a significant advance in disease and drug screening technology allowing for the testing of multiple diseases or drugs with a single test. Also, multifunctional ELISA test kit would be better able to compete commercially with protein arrays.

![Figure 2: SEM image of a silica Nanospring mat.](image)

![Figure 3: Transmission electron microscope image of a single silica Nanospring decorated with gold nanoparticles.](image)
surface area to volume ratio of an oxide material increases, so should the quantity of hydroxyl-terminated silicon sites.

Figure 4: Illustration of how gold nanoparticle decorated Nanosprings could be used to achieve multifunctionality.

Figure 5: XPS hydroxyl (-OH) to oxide (-O-) signal ratios of silica nanowire mats and silica wafers with native oxides before and after storage in various media.

The presence of hydroxyls on the surface is significant in terms of functionalizing the surface. Hydroxyls are often necessary for the chemical attachment of biomolecules onto the silica surface, and therefore by maximizing the hydroxyl concentration, we can maximize the antigen or antibody attachment, thus minimizing functional surface area loss, and maximizing the sensitivity of the ELISA test.

Furthermore, it has been shown that the surface chemistry can be tuned to enable variable attachment efficiencies of specific antigens by altering the quantity of hydroxyls on the surface (Figure 6). Therefore it is possible to limit the amount of blocking coating used by maximizing the attachment efficiency of antigens or antibodies, and thereby maximize the usage of available binding sites as explained. Alternatively, the surface chemistry can be tuned to be less efficient for antigen or antibody attachment. Thus, as an alternative to, or in tandem with, nanoparticle coatings for multifunctionality, subsequent low efficient coatings of different antigens or antibodies could utilize only the silica Nanosprings for multifunctional attachment. Thus, in combination with the high surface area, standard test quantities of multiple antigens or antibodies can be utilized in a single test well.

Figure 6: Plot of the OH : SiO$_2$ signal intensity ratios versus pH of storage media. ■ Wafer ➤ NW

GoNano Technologies Inc. proposes the use of Nanospring inserts that could be used within present commercial test kits with little to no change in the production and testing procedures for these test kits. Because Nanospring mats are attached to the surface on which they are grown, no steps are necessary to prevent the material from being removed with washing, as is the case with suspended particles [7]. The low temperature (~300°C) atmospheric pressure method used to manufacture the Nanospring mats enables them to be formed on a wide range of substrates including Pyrex®, polyimides, aluminum, and silica glass. From these materials, Nanospring coated inserts can be produced for mulitwell plates currently used in commercial ELISA test kits.

3 CONCLUSIONS

One dimensional silica nanostructures have been shown to exhibit large hydroxyl concentrations on their surface. Furthermore, the ability to tune this hydroxyl concentration has been demonstrated, suggesting that sensitive control over biomolecule attachment can be achieved. For ELISA applications, the results of this study suggest that the Nanospring material can be used as a platform for the
engineering of test kits that exhibit multifunctionality and increased control over biomolecule attachment, while eliminating the need for magnetic methods of nanomaterial retention.

REFERENCES


