

Nanospring[™] Enhanced ELISA for Increased Sensitivity and Early Detection

GoNano Technologies' Nanospring material is proposed as an enhancement of enzyme-linked immunosorbent assay (ELISA) to increase detection sensitivity. The high surface area and hydroxyl rich surface chemistry provides greater attachment capability and decreased functional surface loss to antigen uncoated sites. Such applications of the Nanospring material could have implications for early disease and pregnancy detection, and enhanced drug screening capabilities.

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. While ELISA is known for having high detection sensitivity, further increase in the sensitivity of ELISA test kits would provide great advantages in early detection of diseases when concentrations of antibodies are too low to detect with present commercial test kits.

Cancer for example is the second most common cause of death in the US, responsible for approximately 25% of all deaths according to the National Center for Health Statistics. It is well known that cancer is more easily treated in the early stages and cases of early detection have a much higher survival rate than cases of later detection. For this reason screening programs such as the CDC's National Breast and Cervical Cancer Detection Program (NBCCEDP) have been established to detect cancer before noticeable symptoms manifest. While most of these screening programs consist of more traditional detection methods such as mammograms, pap smears, prostate exams, etc., many companies are producing ELISA test kits to complement such screenings. An example would be Cervatec[™] ELISA test kits for cervical cancer screening produced by mtm laboratories.

For simple ELISA methods, an antigen is attached to a solid surface support following which a test sample with or without a specific primary antibody is washed over the surface of the antigen such that any antibody present binds to antigen. A secondary antibody linked to an enzyme is then introduced, binding to any primary antibody. If the test sample is void of primary antibody, then all the enzyme-linked secondary antibody is removed with washing and no enzyme catalyzed signal results. If the test sample contains the target primary antibody, then the enzyme-linked secondary antibody is retained on the surface resulting in a positive signal.

While there are many variations of this simple ELISA method such as sandwich ELISA, antigen antibody role reversal, etc. these ELISA 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. This in turn results in more test molecule attachment and larger concentrations of enzyme for catalyzed signal. The problem to be solved then is how to increase the available surface. Most ELISA test kits use 96-well plates with well diameters of approximately 5 mm. The interior surface of these wells serve as the binding surface for the test.

When searching for higher surface area materials, researchers often look to porous material. While such a material does increase the available surface area, it also adds the complication of diffusion requirements. Because the surface resides inside a pore, any molecule that would attach to this surface would need to pass through the opening of the pore, resulting in a bottleneck the size of the cross-sectional area of the pore. The result is an increased diffusion time, sometimes referred to as charging. Such a material would not be conducive to ELISA in which the efficiency of the test is dependent upon surface accessibility. An alternative to porous materials would be inverse porous materials in which the solid and void are reversed to create long one dimensional structures, shown in figure 1. Such materials are said to have 100% open porosity because all of the surface is immediately available to liquids or gasses flowing through it. One dimensional structures in which two of the three dimensions are on the order of 1 nm to 100 nm are often called nanowires, nanorods, or nanotubes.

GoNano Technologies' Nanospring material is an inverse porous material with 100% open porosity in which the Nanospring (NS) material is composed of nanoscale coiled structures. Each coil is composed of multiple silica nanowires of approximately 5 nm in diameter wrapped around each other in a helical configuration. Figure 2 shows a TEM image of a silica NS that has been coated with silver resulting in a silver coil nucleation within the NS. The ability to coat or decorate the NS material with other materials can enable multifunctional capabilities. NS can be coated on the surface of any material that can withstand the deposition temperature of 350°C. With a 270 m²/g surface area to mass ratio, coating a surface with NS mats can increase the surface area by approximately 1500 times. Thus given a 5 mm diameter well in a 96-well plate, each

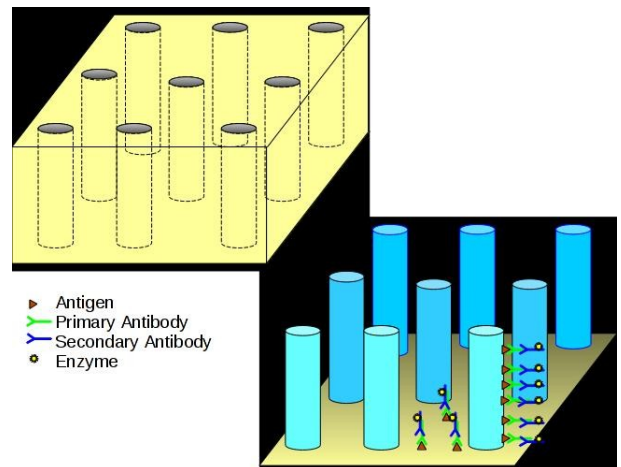


Figure 1: Porous vs inverse porous materials

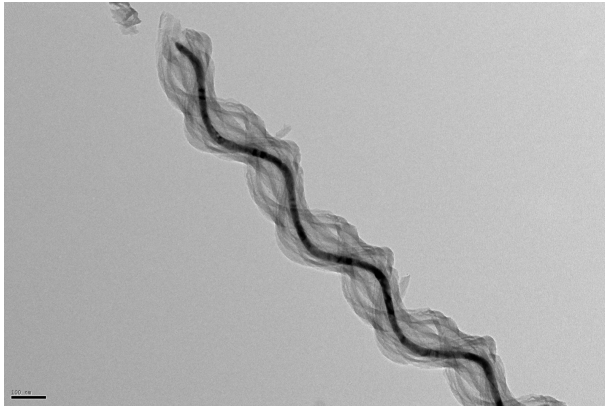


Figure 2: TEM image of Ag coated Nanosprings. Scale bar 100nm

well has a footprint of approximately 20 mm². With the addition of a NS mat at the bottom of this well, the available surface area becomes approximately 30,000 mm² or 300 cm².

The use of high surface area nanomaterials for the purpose of increasing the sensitivity of ELISA techniques has been proven by Jia *et. al.* (Biosensors and Bioelectronics 2009;24:2836) in which gold nanoparticles and magnetic microparticles were used to increase the sensitivity of an ELISA test kit by 25 times. This method required the suspension of particles. One disadvantage to using zero dimensional nanoparticles is their tendency to agglomerate which reduces the available surface area. While the problem of agglomeration is not insurmountable with the addition of surface surfactants to prevent agglomeration, it does add extra steps in the preparation of the material and the use of the test kits.

GoNano Technologies Inc. proposes the use of Nanospring inserts that could be used within present commercial test kits with little if any change in the production and testing procedures for the test kits. Because NS 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. The reason for using NS coated inserts as opposed to directly coating the multiwell plates is that most all plates used for commercial ELISA test kits cannot withstand the deposition temperatures of the NS coatings. Rather, GoNano Technologies has shown that NS mats can be deposited on a low cost fiberglass weave out of which holes can be punched to produce the inserts to go into commercial test kits. The use of a fiberglass weave being a non-planar substrate further increases the resulting surface area.

Another advantage to using the NS mats for ELISA is the control over the surface chemistry. In most ELISA preparation methods, the initial antigen coating is followed by a blocking coating to eliminate activity of uncoated surfaces. Any of these blocked surfaces do not participate in the ELISA and therefore result in decreased sensitivity. Preliminary results of the surface stoichiometry of one dimensional nanostructures suggests a very rich hydroxyl concentration can be achieved on the surface. Figure 3 shows the ratio of intensities of hydroxyl signal to oxide signal taken from X-ray Photoelectron Spectroscopy of silica nanowires versus planar native oxides on a silica surface. In all cases, the hydroxyl to oxide signal ratio for the native oxide is less than 2, whereas the same ratio for the silica nanowires can be more than 11. The higher intensities of the hydroxyl signal for the nanowire mats can be explained in terms of the size of the nanowires. At the nano-scale, the surface area to volume ratio is orders of magnitude greater than for bulk material, which means more of the silicon sites reside at the surface and are susceptible to hydroxyl termination. Thus we would expect that as the surface area to volume ratio of an oxide material increases, so should the hydroxyl to oxide signal ratio of the O 1s core level electrons.

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

GoNano Technologies' Nanospring material provides a unique solution to increase the sensitivity 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 material provides an ideal substrate for antigen or other biomolecule attachment. The ability to control the surface chemistry of the material could eliminate functional surface area loss from antigen non-coated sites.

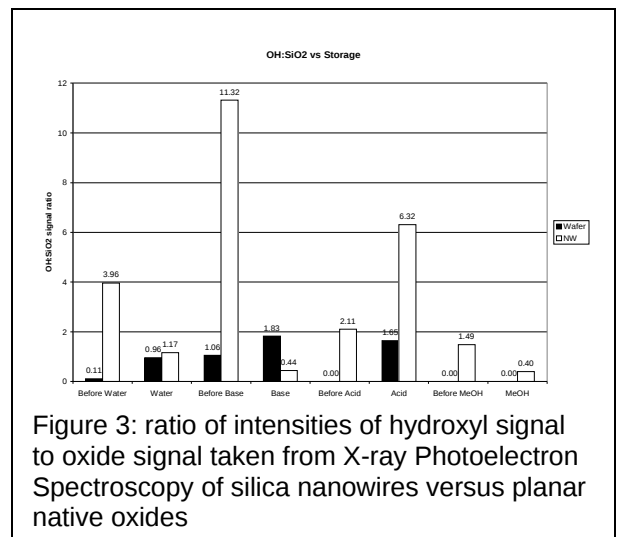


Figure 3: ratio of intensities of hydroxyl signal to oxide signal taken from X-ray Photoelectron Spectroscopy of silica nanowires versus planar native oxides