The Self-assembly, Characterization of Hepatocytes on Nano-sized Gold Colloid and Construction of Cellular Biosensor

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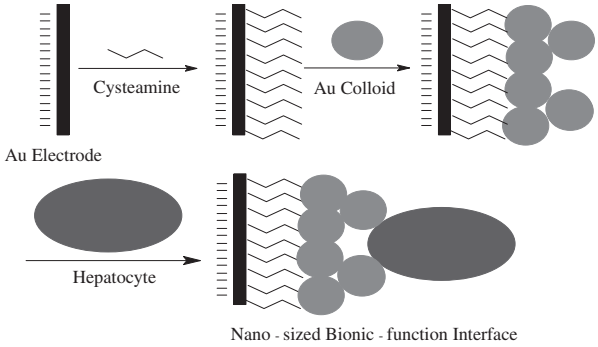
Hepatocytes were successfully assembled on nano-sized gold colloid associated with a cysteamine monolayer on a gold electrode surface to construct a novel cellular biosensor for determining lactate.

Nano-sized self-assembly has a wide application in chemistry, biochemistry, and many other fields. Willner and coworkers[1] reported on the organization of gold colloid films on indium tin oxide surface using aminopropyl siloxane or mercaptopropyl siloxane as base monolayers. The resulting gold colloid films exhibit high stability and allow further modification to construct various biosensors and bioelectronic devices by immobilizing enzymes on the monolayers of the gold nano-sized particles.[2] This self-assembled process could either keep the biological activity of enzymes and proteins or maintain the stability of gold colloid particle.[3] In our previous work, the adsorption characteristic of gold colloid and its use for immobilizing horseradish peroxidase (HRP), hemoglobin (Hb) on gold colloid surface to assemble successfully a spatial ordered three-dimensional film have been reported.[4] Therefore, it is possible that living cells are assembled on nano-sized gold colloid surface.

We report here a novel cellular biosensor based on the nano-sized bionic-function interface of hepatocyte/gold colloid which was characterized with atomic force microscopy (AFM), scanning electron microscopy (SEM) and electrochemistry.



Scheme 1. Preparation process of hepatocytes/gold colloid-cysteamine-modified gold electrode.

Scheme 1 outlines the preparation process of hepatocytes/gold colloid. The cleaned gold electrode (or film) was first immersed in 0.1 M deoxygenated cysteamine aqueous solution for 2 h at room temperature in darkness. The resulting monolayer-modified film was obtained and rinsed thoroughly with twice-distilled water and soaked in water for 12 h to remove the physically adsorbed cysteamine. Then, it was dipped into the gold colloid solution (24 nm) for 18 h at room temperature in darkness. Finally, the gold colloid/cysteamine modified gold film or electrode was dipped into the medium (pH 7.0) containing ca. 5×10^5 cells/mL hepatocytes at 37 °C for 24 h in a 5% CO_2 incubator.

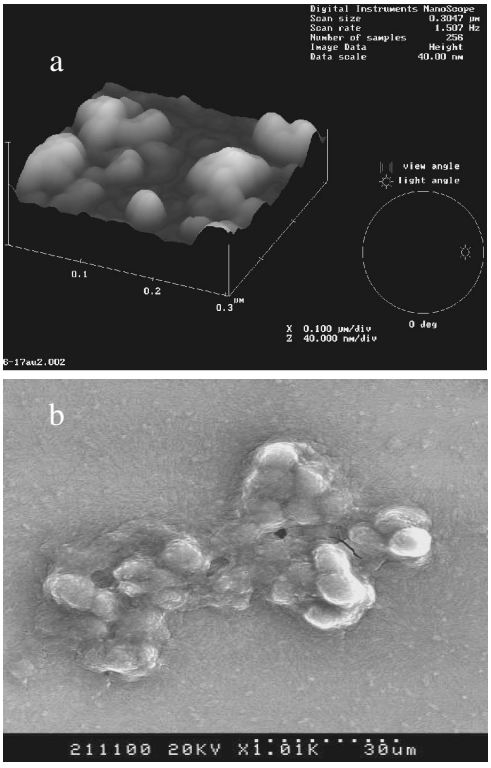


Figure 1. (a) AFM image of gold colloid particles immobilized cysteamine modified gold electrode, and (b) SEM image of aggregates of hepatocytes assembled on nano-sized gold colloid.

AFM and SEM were used to characterize the self-assembly process. Figure 1a shows the representative AFM image of gold colloid immobilized on the cysteamine modified gold electrode (or film). The nano-sized gold colloid particles or clusters are connected each other, and an almost continuous monolayer of gold colloid or clusters is formed. The diameter of the gold colloid particle is about 24 nm, while that of the cluster is about 100 nm. When hepatocytes were assembled on nano-sized gold colloid particles or cluster surfaces, the AFM image would be obviously changed, but the sections of an ellipsoid-like hepatocytes (13–15 μm) was only showed. Undoubtedly, hepatocytes were assembled on the nano-sized gold colloid or cluster sur-

faces. Figure 1b shows the SEM image of aggregates of hepatocytes assembled on nano-sized gold colloid surface. AFM and SEM images gave the direct visual evidence.

In such a way, the hepatocyte/gold colloid-cysteamine modified gold electrode, a nano-sized bionic-function interface was obtained. It was reported that hepatocyte contains the redox couple of NAD^+/NADH and lactate dehydrogenase (LDH).[5] Obviously, the catalysis system containing NAD^+/NADH and LDH provided by hepatocytes is naturally formed for determining lactate. When small amount of lactate molecules infiltrate inside of hepatocytes through cell membrane, the following reaction can be occurred:

$$\text{Lactate} + \text{NAD}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{Pyruvate} + \text{NADH} + \text{H}^+$$

Here, the redox couple of NAD^+/NADH plays a role of mediator. The electron transfer between lactate and gold colloid particles attached tightly to hepatocytes is realized through cell membrane.

It was reported[6] that the oxidation peak potential of NADH was about 0.80 V in PBS at the bare gold electrode. Various single crystal gold electrodes show the respective electro-oxidation to NADH, which could decrease its overpotential.[7] At the gold colloid-cysteamine modified gold electrode, it could be seen that the oxidation peak potential is about 0.25 V while adding a certain amount of NADH (in Figure 2a, inset). The anodic overpotential of NADH was reduced by 550 mV. The cellular biosensor based on this interface showed a good electrocatalytic activity to lactate without the help of promoters or mediators in solution.

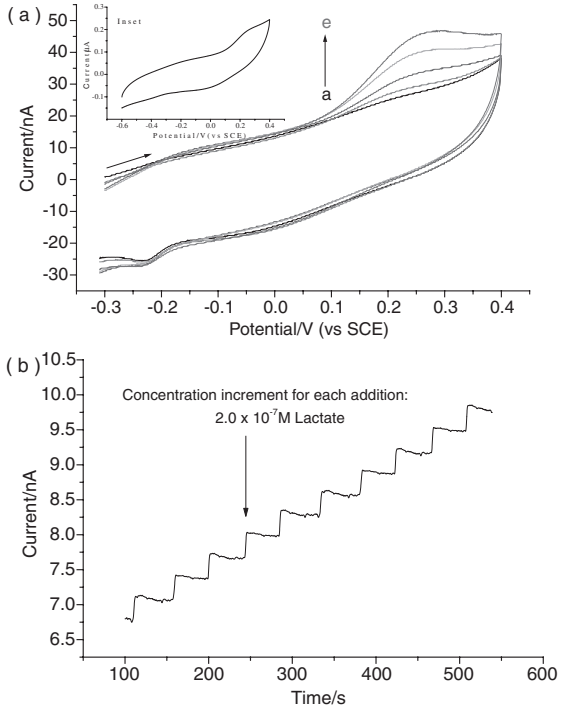


Figure 2. (a) Voltammograms of hepatocytes/gold colloid-cysteamine-modified gold electrode in pH 8.0 PBS at 100 mV/s, in the absence of lactate(a) and presence of 6.0×10^{-7}, 3.0×10^{-6}, 6.0×10^{-6}, 9.0×10^{-6} M lactate respectively (b,c,d,e); Inset: Voltammogram of 5.0×10^{-4} M NADH at the gold colloid-cysteamine modified gold electrode; (b) Amperogram of the cellular bisensor for lactate at operating potential of 250 mV.

The electrochemical responses of hepatocytes/gold colloid-cysteamine-modified gold electrode to lactate in pH 8.0 PBS were observed (Figure 2a), and an obvious increase in the anodic peak current was observed when the lactate was added. The oxidation peak of NADH which was produced in the enzymatic reaction, is broader, and it is relevant to the oxidation speed of NADH and regeneration velocity of NAD^+ on the resulting nano-sized bionic-function interface. It revealed that the resulting cellular biosensor possesses a biological and electrochemical activity as a reagentless biosensor.

The effect of operating voltage on the response of lactate was also studied. Amperometric current–time curves at different potential were researched. At the operating voltage from 0.1 to 0.35 V, the oxidation current increased with increasing of the potential. However, when operating voltage is more than 0.25 V, the noise increases. Therefore, 0.25 V was chosen as the operating voltage. Figure 2b shows the steady-state current response of the resulting cellular biosensor to lactate. This response is linear in the range from 1.0×10^{-7} to 1.0×10^{-5} M. The detection limit was down to 4.0×10^{-8} M (three times the ratio of signal to noise). The response time was about 3 s and the RSD was 3.5% for 8 successive determination at lactate concentration of 2.0×10^{-7} M.

In conclusion, SEM and AFM can be successfully used as powerful tools to characterize the assembly of hepatocytes on nano-sized gold colloid surface. The resulting cellular biosensor displays an excellent electrocatalytic performance to lactate with high sensitivity.

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