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功能化奈米粒子於生物檢測及分析上之應用

Applications of Functionalized Nanoparticles in Biological Diagnostics and Assays

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摘要

我們已成功的發展出將硒化鉻/硫化鋅奈米粒子置於核心且在表面提供三種功能性末端基的新穎成網合成技術。我們以化學反應構築成一個官能基網，而將奈米粒子定位於核心。我們也對此樹枝球體奈米元件做詳細的光物理性質與核磁共振光譜的探討。

我們運用化學輸送加強物質 oleic acid，來成功的證明了跨越皮膚螢光量子點的輸送。多光子螢光影像的分析證明了 oleic acid-量子點系統進入為透過皮膚表面細胞脂性結構所完成的。我們的工作顯示螢光量子點可被運用於非破壞性生物影像的應用。

關鍵詞：螢光奈米粒子、樹枝狀探針、成網合成，螢光、奈米粒子、跨越皮膚輸送、多光子影像

Abstract

A new netting process to efficiently fix CdSe/ZnS nano-particle (QD's) in central core and, in the meantime, to provide three functional ends in the peripheral regions has been developed. The photophysical and spectroscopic profiles of the resultant dendritic, flrorescent nano-devices were discussed.

We have demonstrated the transdermal delivery of fluorescent quantum dots using the chemical enhancer oleic acid. Multiphoton image analysis shows that oleic acid-QD system delivers the QD's through the lipid domains of the corneocytes near the surface stratum corneum. Our work demonstrates the feasibility of non-invasive delivery of fluorescent QD's for bioimaging applications.

Keyword: nanoparticle, dendritic antenna, fluorescent probe, methathesis, fluroescence, QD, transdermal delivery, multiphoton imaging

1、Introduction

Since the recent progress of solution-phase methods for the synthesis and stabilization of luminescent gold and semiconductor nanoparticles,[1] research in this filed has dramatically expanded and evolved into a multi-disciplinary science. Their applications have included biological markers,[2] DNA sensors,[3] molecular recognitions,[4] and nanoscale electronics.[5] The use of polymer[6] and dendrimer[7] systems interacting with colloidal metal nanoparticles has been explored as a handle for particle size control, organization, and stabilization. To date, the primary focus has been their optical properties. They are governed by strong quantum confinement effects and are size dependent. The absorption onset and fluorescent emission shift to lower energy (longer wavelength) with increasing size. Moreover, the emission pattern is narrower, symmetric, and excitation frequency independent. Therefore, nanoparticles of varying sizes can be excited simultaneously with a single excitation source, resulting in well-resolved colors of emission. The emission efficiency can be further improved significantly by passivation of the nanoparticle surface with a thin shell of organic material.[8] The

shell also imparts photochemical stability with reduction of photo-bleaching effect.

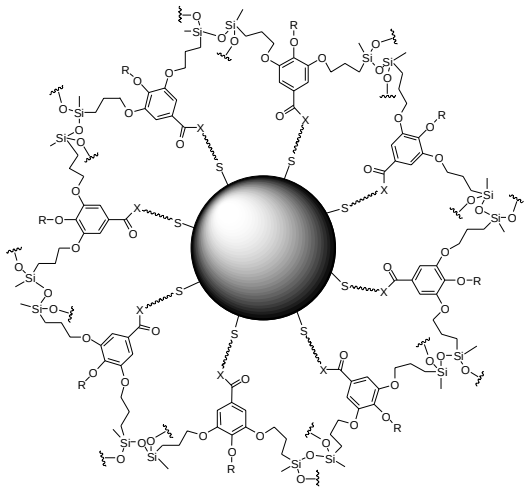
In recent years, the development of novel nanostructures has attracted considerable attention. In particular, it was recognized that the unique luminescent properties of semi- conducting quantum dots may be explored for bioimaging applications. In this area, two approaches have been used to deliver QD's into biological systems. One can either modify the QD surface chemical properties to target biological organelles or directly inject the QD's for in vivo applications.[9-12]

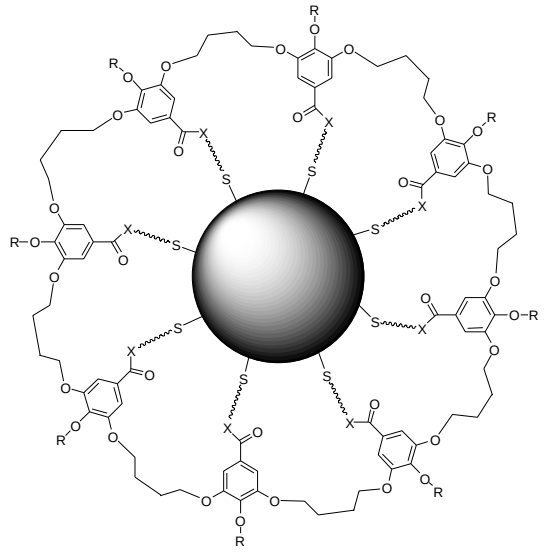
In this work, we feel that the non-invasive delivery of QD across biological barriers offers another exciting possibility for exploring QD interactions with biological systems. Specifically, we are examining the possibility for the delivery of QD's across the skin barrier. In addition to utilize the unique fluorescent properties of QD's in the imaging of physiological specimens, the nanometer size range of QD allows researchers to probe into the QD delivery process into biological systems. The latter point is especially interesting since QD's are comparable in size to many protein molecules and oligonucleotides. Therefore, properly modified QD's can act as probes clarifying the delivery of proteins, genes, or other biological nanomaterials for therapeutic applications.

2. Experiment

2.1 Nanoparticle-encapsulated, Functional Dendermers as Fluorescent Probes for Biochemical Applications

Several strategies have been explored to dissolve high quality hydrophobic nanoparticle-hybrid in aqueous solvents.[13] They mainly hinge on anchoring the nano-surface with a thiolated molecule bearing a carboxyl end group to secure water solubility. In that context, the adsorption onto the nano-surface is dynamic, resulting in a poor stability of the filmed-nanoparticles in water. Moreover, any chemical reactions may lead to slow dissolution of the naked particles and thus to the diffusion of heavy metals in to the solution. To retard this process and to secrete the toxicity of the nanoparticles for bio-organisms, a complete and engineered encapsulation of the nano-fluorophores is indispensable.[14] On the basis of these considerations, we thought to develop a conceptually novel approach by which the individual core/shell nanoparticle is embedded in a cross-linked 3,5-diallyloxy-4-hydroxybenzoate netting scaffold, Figure 1. One additional advantage is the peripheral 4-hroxy group can be readily functionalized with a carbohydrate, a peptide segment, or a DNA marker by diethylene glycol ether, 4-bromobenzoxy, and 2-nitrobenzoxy spacers.





R = $(CH_2)_2O(CH_2)_2OH$, X = NH, O
= $(CH_2)_2O(CH_2)_2O(t\text{-Boc})$
= $(CH_2)_2O(CH_2)_2OTBS$
= CH_2Ph
= $CH_2C_6H_4\text{-}Br$
= $CH_2C_6H_4\text{-}o\text{-}NO_2$

They are expected to retain the photophysical properties of the original nanoparticles and to exhibit a greater stability in biological buffers as compared to nanoparticles anchored with thiolated molecules like mercaptopropionic acid. We herein describe our preliminary results toward this end with detailed spectroscopic determination of the final dendritic nano-devices.

2.2 Examination of Transdermal Delivery Pathways of Fluorescent Nanocrystals by Multiphoton Fluorescence Microscopy

We have succeeded in using the chemical enhancer oleic acid to deliver fluorescent QD's, transdermally. Water-soluble QD's provided by Prof. C. J. Chen (Chemistry, NTNU) was mixed with a solution of PBS buffer and ethanol (1:1 ratio by volume) with 5% oleic acid. The QD solution is then placed in contact with human skin (provided by Dr. S. J. Lin, NTU hospital) for two days. The QD labeled skin is then placed under our home-built multiphoton fluorescence microscope for examination. In our multiphoton system, a femtosecond titanium-sapphire laser coupled with an upright microscope equipped with x-y-z scanning capabilities is used for non-linear excitation and detection of transdermally delivered fluorescent nanoparticles.

3、Result and discussion

3.1 Nanoparticle-encapsulated, Functional Dendermers as Fluorescent Probes for Biochemical Applications

There are several delicate approaches to encapsulate nano-size particles like Au and CdSe/ZnS in polymeric[15] and dendritic[16] molecules for the purposes of size control, stabilization, and organization. One major approach was to swell dendrimers in organic solvents in the process of forming nanoparticles. The encapsulated nanoparticles reside mainly inside the cavities among internal repeating units. Therefore, the cavity size governs the number and size of particles incorporated. Since the cavity sizes that are large enough to hold the nanoparticles in place are mainly located near the peripheral regions of the dendrimers, they tend to escape with time. Another means was to establish strong adsorption or binding directly to the nanoparticles and then to create a shell assembly to surround them via bond-forming cross-linking events.[17]

Retrosynthetic Analysis. As mentioned above, our goal was to utilize a netting process to efficiently lock CdSe/ZnS nanoparticle in central core and, in the meantime, to provide functional ends in the peripheral regions at will. As shown in Figure 2, 3,5-diallyloxy-4-hydroxy-benzoic acid (**1**) was devised as the netting unit.[18] The carboxyl group will be amidated by 2-mercapto-1-ethanamine. The resultant thiolate end then allows for priming to the nanoparticle. Finaly, the 4-hydroxyl group will be extended via a di-ethylene-glycol-ether tether with a primary alcohol end for installing functional probes Alternatively, it can be extended via 4-bromobenzoxy or 2-notrobenzoxy tether for further coupling and photo-initiated deprotection, Figure 2.

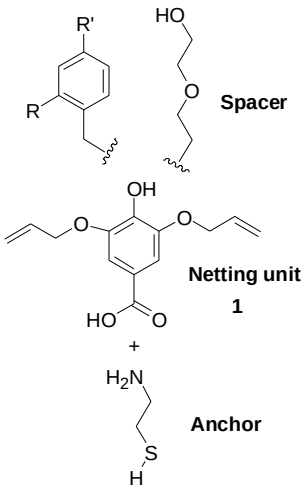
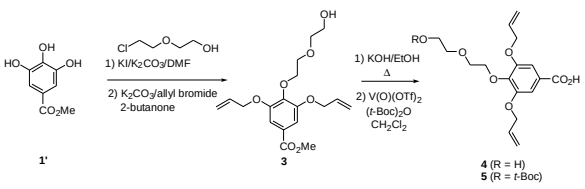


Figure 2. The targetd the spacers, netting unit, and the anchor to nanoparticles

The allyloxy units at both C3 and C5 positions are used for netting and cross-linking by ring-closing metathesis methodology developed by Grubbs.

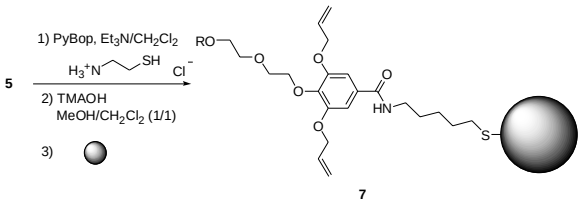
Synthesis of the dendrons. We used methyl gallate as the starting material. The di-ethylene glycol spacer at its C-4 position was first installed by treatment with 2-chloroethoxy-ethanol in the presence of KI and K_2CO_3 in refluxed DMF for 3 days,[19] Scheme 1. The reaction time may be reduced down to 20 min with similar chemical yield (68%) by microwave heating. Subsequent double allylations at C-3 and C-5 hydroxyl groups of **2** with allyl bromide were attained by refluxing in 2-butanone in the presence of K_2CO_3 leading to **3** in 90% yield.[20] The methyl ester-**3** may be saponified by KOH in hot EtOH for 4 hours to furnish the free acid-**4** in 68% yield. The peripheral hydroxyl group was protected as *t*-butoxycarbonate-**5** in 40% yield by using vanadyl triflate-catalyzed acylation developed in our laboratory.[21]

Scheme 1



The targeted 2-mercaptoethanamine anchor was attached to **5** by a common amidation procedure. Namely, treatment of **5** with 2-mercaptoethanamine hydrochloride in the presence of PyBop and Et_3N in CH_2Cl_2 at 0 ^oC for 12 h furnished amide-**6** in 50% yield , Scheme 2. Subsequent anchoring of the *thio end* group in **6** by tetramethyl-ammonium hydroxide in the presence of TOPO-stabilized CdSe/ZnS[22] in a solution of $CHCl_3$ and MeOH (1/1) under reflux afforded **7**, thus completing the targeted dendron-nano particle complex ready for the final intramolecular ring-closing metethesis to secure the orgaic net.

Scheme 2

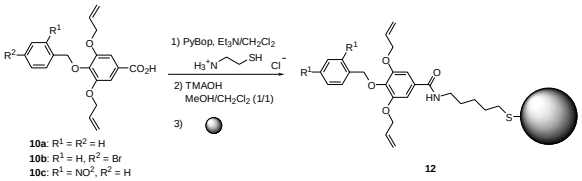


Type-II system was similarly prepared by starting with methyl gallate. The benzoxy, 4-bromobenzoxy, or 2-nitrobenzoxy spacer at its C-4 position was first installed by treatment with the respective bromide in the presence of K_2CO_3 in refluxed acetone for 10 h.[23] In all cases, the resultant benzyl ethers, **8a-c**, were provided in 55% yields. Subsequent double allylations at C-3 and C-5 hydroxyl groups of **8** with allyl bromide were attained by refluxing in 2-butanone in the presence of K_2CO_3 for 4 h leading to **9a-c** all in 70% yields.[24] The respective methyl esters may be saponified by KOH in hot EtOH for 2 hours to furnish the free acid-**10a-c** in 65% yields.

The targeted 2-mercaptoethanamine anchor was attached to **10a** by treatment with 2-mercaptoethanamine hydrochloride in the presence of PyBop and Et_3N in CH_2Cl_2 at 0 ^oC for 24 h furnished amide-**11a** in 35% yield, Scheme 3. Subsequent anchoring of the *thio end* group in **11a** by tetramethyl-ammonium hydroxide in the presence of TOPO-stabilized CdSe/ZnS[25] in a solution of $CHCl_3$ and MeOH (1/1) under reflux for 24 h afforded **12a**.

The identity of the dendritic nano-device **12a** was confirmed by proton NMR spectroscopy and UV-vis and emission spectra.

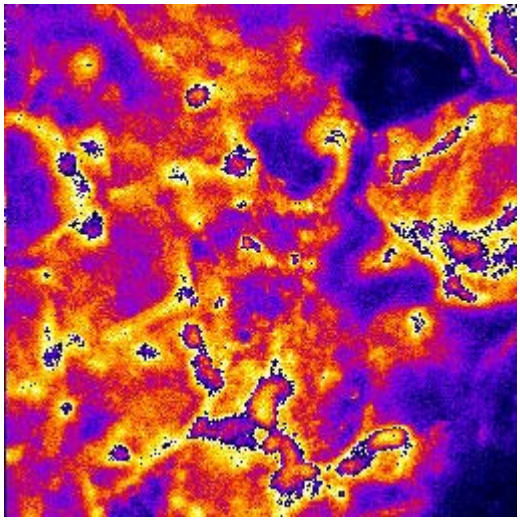
Scheme 3



The final cross-linking and netting step is currently under investigation and will be reported in due course.

3.2 Examination of Transdermal Delivery Pathways of Fluorescent Nanocrystals by Multiphoton Fluorescence Microscopy

Shown in Fig. 3 are two examples of the QD treated human skin images acquired with our multiphoton instrumentation. In both examples, it can be seen that the lipid region of the corneocytes near the surface stratum corneum is heavily labeled with the fluorescent QD's, indicating that the QD - oleic acid system results in the transdermal QD delivery through the lipid domains of the cells.



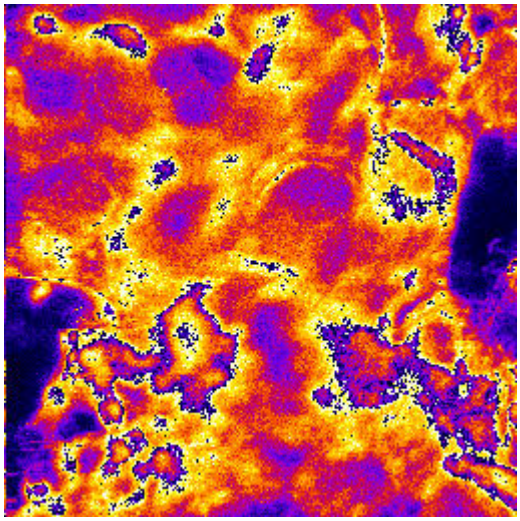


Figure 3. Multiphoton images of QD labeled human skin specimens.

We have successfully demonstrated the delivery of fluorescent QD's across the human skin barrier. Our results demonstrate the feasibility of using fluorescent QD's in bioimaging applications. The delivery process can also be used to investigate the interaction of nanometer biological materials in therapeutic applications. In the future, we plan to extend this approach in no-invasive QD delivery for in vivo applications.

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