Detection of Fluorescence from a Mixed Specimen Which Consists of Microparticles Attached Different Fluorescent Substances Using a Light Waveguide Incorporated Optical TAS

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We have already developed a transparent epoxy-resin-based optical TAS chip which has a specially prepared light waveguide structure of radially arranged configuration at an intersection portion with a microfluidic channel, in order to detect “directivity” of fluorescence from fluorescent substance attached micro particles [1]. According to further medical requirements, fluorescence analysis involving specimens incorporating individual fluorescent substances for each cell will also be required. In order to realize physiological information analysis based on the conventional method, it is necessary to equally divide the originally detected fluorescence and to introduce each power into highly sensitive photo detector by way of adequately selected interference filters. As a result, the powers of the fluorescence to be processed will drastically be decreased, and, finally, photo-electric conversion of extremely weak fluorescence may be difficult with a sufficient S/N ratio. In order to solve such problems, we divided the detected fluorescence “unequally” into two powers (for example, 10% and 90%) by a specially prepared beam splitter. Then, we utilized the weak fluorescent component for identification of the fluorescent substance processing by a lock-in amplifier. On the other hand, the remaining strong fluorescent component was used for directivity analysis based on the previously reported method (Fig.1) Even with the light power of approximately 10% of the originally detected fluorescence, it was possible to identify fluorescent substance (Fig.2). At the symposium, we will report the latest results using mixed specimens.

Figure 1. Schematic diagram of acquiring discrimination signals of particles contained in a mixed specimen.
Figure 2. Typical numerical results of discrimination signals.

References:
Involvement of nano efflux pump in organic solvent-tolerance in *Escherichia coli*

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Much attention has been directed to microbial production of a variety of chemicals including fine chemicals and biofuels. As some of these products are highly toxic to microorganisms, accumulation of these products in the bioproduction process often leads to low productivity. Efficient bioproduction process can be developed by understanding microbial tolerance mechanisms to various chemicals. Some of these target products are organic solvent-like compounds. Therefore, organic solvent-tolerant microorganisms have been intensively studied so far. *Pseudomonas putida* IH-2000, which can grow in the presence of 50\% (v/v) toluene, was reported as an organic solvent-tolerant bacterium for the first time (Nature, 388:264 (1989)). Since then, several physiological and biochemical approaches have been applied in an effort to clarify the mechanism of microbial tolerance to solvents (Extremophiles, 2:239 (1998)). In the case of *E. coli*, energy-dependent efflux systems, lipopolysaccharides, a composition of fatty acids, a maintenance of the proton motive force, and an alkyl hydroperoxide reductase have been reported to be involved in organic solvent-tolerance. Among these tolerance mechanisms, the AcrAB-TolC pump, a major efflux pump (bacterial nano pump) in *E. coli*, plays an important role in organic solvent-tolerance in *E. coli* (Fig. 1).

In this study, we focused on AcrAB-TolC pump and examined its involvement of organic solvent-tolerance.

![Figure 1. AcrAB-TolC pump in *Escherichia coli*](image-url)

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In this study, we focused on AcrAB-TolC pump and examined its involvement of organic solvent-tolerance.
Superparamagnetic microparticles can be dispersed in either aqueous or organic solvents. When an external magnetic field is applied to the solution, in which superparamagnetic microparticles are dispersed, a magnetic moment is induced in each superparamagnetic microparticle and the microparticles will eventually coagulate to form clusters due to magnetic interactions. Various structures are formed by superparamagnetic microparticles depending on the control parameters such as the volume fraction of particles, the thickness of the experimental cell and the strength of the external magnetic field [1-4]. In previous numerical studies [1,2], it was shown that superparamagnetic particles form wall structures in a direction parallel to a DC magnetic field. The structures formed by superparamagnetic particles have been utilized in a DNA electrophoresis [5] and a photonic device [6]. In this study, we observed the structures formed by superparamagnetic particles in a DC magnetic field and obtained the following results: (a) When the experimental cell is shallow and the volume fraction of superparamagnetic microparticles is low, individually dispersed straight chain structures are formed; (b) when the experimental cell is shallow and the volume fraction of superparamagnetic microparticles is high, wall or labyrinth structures are formed; (c) When the experimental cell is thick and the volume fraction of superparamagnetic microparticles is high, the thickness of wall or labyrinth structures increase and thick columns structures are formed.

References:
Control of the patterns formed by vertically aligned carbon nanotubes

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Synthetic methodologies of nano-structures are generally speaking classified into two; that is, bottom-up and top-down ones. The formation of nanostructures via a combination of both bottom-up and top-down processes is a recent trend; e.g., Nanomaterials can be self-assembled in nano/micro channels fabricated by top-down ultra-fine technique [1]. It is also well known that patterns can be formed by nanoparticles via convective self-assembly process such as the coffee ring phenomenon [2]. Carbon nanotubes (CNTs) are expected to be utilised in a variety of fields including electronics, optics and biomedicine. CNTs are generally synthesised by arc discharge, laser ablation and chemical vapour deposition (CVD) [3]. In the present study, we synthesised concentric patterns composed of fullerene nanofibres in cavities of various shapes utilising the coffee ring effect and clarified the effect of the temperature and concentration of C₆₀ molecules on the pattern formation. Concentric patterns were also formed by iron oxide nanoparticles by the same methodology, and then vertically aligned CNTs were synthesised using the iron oxide nanoparticles as a catalyst by plasma enhanced CVD (PECVD). The present result suggests that different patterns of CNTs can be grown as designed by controlling the self-assembly process of catalytic nanoparticles.

Chemical Synthesis and Characterisation of Neo-Glycolipids

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We synthesized neo-glycolipids (new types of glycolipids) composed of rare sugars, glycerol and lipids (RSGLs) using 6 different types of rare sugars by combination of the modified Fischer and enzyme reactions. Rare sugars are known useful and have quite a few unique functions such as antioxidant action, Induction of apoptosis of cancer cells, and reduction of blood sugar level. We characterized and analysed the production of RSGLs by thin layer chromatography (TLC), Fourier-transform infrared (FT-IR) spectroscopy and matrix assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF-MS). The results of TLC, FT-IR and MALDI-TOF-MS showed that we successfully synthesized RSGLs. We investigated the cytotoxicity of RSGLs by lactate dehydrogenase (LDH) and alamar blue assays. We can estimate cell membrane damage by LDH assay and cell metabolism by alamar blue assay. We clarified the effect of the concentration of RSGLs on cytotoxicity. We analysed the dependence of the surface tension of the media, critical micelle concentration and activity of housekeeping enzymes on the concentration of RSGLs to clarify the cytotoxicity mechanism of them.
By combining the electrophoresis and conventional Coulter methods, we previously proposed the electrophoretic Coulter method (ECM) technique that enables the simultaneous analysis of the size, number and zeta potential of individual specimens [1]. We also demonstrated that the ECM can identify whether the present cell state is alive or dead for solutions of human B lymphoblast (IM-9) cells, both before and after dosing of an apoptosis inducer [2].

We have demonstrated that the ECM can the separation of targeted components and the identification of living or dead cells in individual cells using differences in size and/or Zeta potential. In principle, the ECM can be applied to various biological cells or biomaterials provided their sizes and/or Zeta potential values change.

In this study, we investigate that the ECM can characterize whether analyzed cells are senescent cells or non-senescent cells, senescent cells which are present in premalignant lesions and sites of tissue damage and accumulate in tissues with age [3].

References:

Isolation of novel sulfate-reducing magnetotactic bacteria from freshwater sediments

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Magnetotactic bacteria (MTB) synthesize magnetosomes composed of membrane-enveloped magnetite (Fe₃O₄) and/or greigite (Fe₃S₄) nanoparticles in the cells. Mostly known MTB belonging to the Deltaproteobacteria are dissimilatory sulfate-reducing bacteria (SRB) that biomineralize unique bullet-shaped magnetite nanoparticles. SRB are generally strict anaerobes obtaining energy for growth by oxidation of a wide variety of organic compounds with reducing sulfate to hydrogen sulfide. Desulfovibrio magneticus strain RS-1, the first isolate of the Deltaproteobacteria, is a dissimilatory sulfate-reducing bacterium that has ability to synthesize bullet-shaped magnetite nanoparticles under anaerobic conditions. We report here sulfate-reducing MTB, strain FSS-1, FSO-1, FSO-2 and FSO-3, which were isolated from two freshwater sediments. Strain FSS-1 was grown in liquid medium containing casamino acids as electron donor and sulfate as electron acceptor, while strain FSO-1, FSO-2 and FSO-3 were grown in liquid medium containing formate as electron donor and sulfate as electron acceptor. On the basis of phylogenetic analysis, strain FSS-1, FSO-1 and FSO-2 appeared to be novel species of the genus Desulfovibrio. Strain FSO-3 also appeared to be novel strain of D. magneticus. Strain FSS-1 was able to produce approximately 9 bullet-shaped magnetite nanoparticles in each cell and response to an external magnetic field.
Convective self-assembly of iron oxide microparticles dispersed in water/ethanol solvent

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Self-assembly of nano/micro particles during the evaporation process of solvents has been recognized as a simple approach to attaining complex yet ordered nano/micro structures on a two-dimensional (2D) plane. In fact, the absolute comprehension of this phenomenon would lead to numerous industrial applications, e.g., Lithography, inkjet printing and micropatterning in electronics and photonics. Magnetite (Fe₃O₄) is one of the most intensively studied magnetic materials since it has shown considerable application potential in various fields [1]. In this study, we investigated the patterns formed by magnetite microparticles (MPs) after the evaporation of water/ethanol-based droplets, in which the particles were dispersed. Magnetite particles of 1.0 and 2.8 μm were separately dispersed in water/ethanol-based solvent, followed by dilution with either water or ethanol. The droplet was dropped either on the surface of a heated glass substrate or in a hole made by PDMS placed on a glass substrate at different temperatures and concentrations [2]. Note that the substrate was washed by APM before each experiment. Finally, the patterns formed on the substrate were observed with a metallurgical microscope after the solvent had completely evaporated (see Figure 1).

As the evaporation proceeds, magnetic MPs moved from the central part of a droplet towards the circumference. In the early stage of the evaporation process, some MPs moved outwards to the periphery and then they were transported towards the droplet centre forming ringlike clusters as shown in Figure 1 (b, c and d). At the end of the evaporation process, the ringlike structures accumulated at the centre while increasing the width of the whirl. We noticed that using an ethanol solvent led to the construction of ringlike structures, while the dilution with water led to a dendritic structures as shown in Figure 1 (a). The ringlike clusters made by 2.8 μm particles were different from those made by 1.0 μm particles. The circular patterns were more seriously deformed in the case of particles of 2.8 μm diameter than those in the case of 1 μm as shown in Figure 1 (c and d).

![Figure 1: Optical micro/milligraph images of some patterns formed by Fe₃O₄ MPs at 50 °C. The scale bars represent (a) 100 μm; (b) 1 mm; (c) 1 mm and (d) 1 mm. All the images were observed in cavities made by PDMS. The diameter of each particle is 1.0 μm (a,b,c) and 2.8 μm (d). The samples were diluted with water (a) and ethanol (b, c, d). The concentration of the MPs is (a) 0.1 mg/ml; (b) 0.04 mg/ml; (c) 0.01 mg/ml and (d) 0.12 mg/ml.](image)

References:
Early detection of malaria infection utilising the magnetic characteristics of hemozoin synthesised by malaria parasites

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Malaria is the largest parasitic protozoal infection in the world, noting that there are malarial patients in over 100 countries. According to WORLD MALARIA REPORT 2016, there are 216 million people annually infected and the number of deaths is 445,000 with malaria, which is a serious global problem. Currently, microscopic examination of Giemsa-stained blood is regarded as a standard method in malaria diagnosis. However, this process is time-consuming and requires well-trained operators, in particular to ensure detection with a small number of parasites. Thus, it is urgently necessary to develop a highly sensitive, simple diagnosis method.

In this study, the magnetic properties of hemozoin synthesised by malaria parasites were evaluated (see Figure 1), and the spectrum of hemozoin was measured by Flourier transform infrared spectroscopy (FT-IR) with the reflection mode and using surface enhanced infrared absorption spectroscopy (SEIRAS). It was found that the magnetisation of natural hemozoin was not high due to the attachment of biomolecules to the surface of hemozoin. The FT-IR results showed that the sensitivity of the spectrum of hemozoin obtained by SEIRAS was higher than that obtained by the ordinary FT-IR measurement. Therefore, it may well be possible to diagnose malaria in the early stage utilising the magnetic characteristics of hemozoin and measuring the SEIRAS of hemozoin.

Figure 1. Magnetisation-magnetic field curves measured by SQUIRD. (a), (c) Natural hemozoin; (b), (d) Synthesised hemozoin.
Synthesis of nonspherical magnetic nanoparticles in super-critical ethanol

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Functional nanomaterials are widely used in various fields. Among them, magnetic nanoparticles have attracted a lot of attention in a variety of fields in recent years. The dynamics and secondary structures of magnetic nanoparticles can be controlled by external magnetic fields and their unique characteristics are utilised particularly in the electronics, medical and biomedical fields; e.g., data storage devices, contrast agents for MRI, biosensing, carriers of drug delivery systems (DDSs) and hyperthermic treatment of cancer cells. Super-critical fluids possess both gas-like and liquid-like characteristics such as high diffusivity and solubility, thanks to which they are commonly utilised for various purposes including chromatography and biomass degradation [1]. In the present study, magnetic nanoparticles are synthesised dissolving ferrocene in super-critical ethanol [2,3]. The structures and magnetic properties are thoroughly characterised by scanning and transmission electron microscopy (SEM and TEM) and a superconducting quantum interference device (SQUID). The effect of the synthetic temperature on the shape, structure and magnetic properties of the magnetic nanoparticles is clarified. SEM images of magnetic nanoparticles synthesised in super-critical ethanol is shown in Figure 1. It is clearly shown that spherical particles were formed when the particles were synthesised at 250 °C, whereas dendritic tripod-like particles were formed at 300 °C. Four-leaf like particles were synthesised at 350 °C and particles composed of various shapes such as cubes, stars and spheres were formed at 400 °C.

Reference:
Chromatographic Behaviour of Magnetic Fullerenes

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Metallofullerenes have attracted a lot of attention from nanotechnology researchers in recent years thanks to their unique molecular properties, which change depending on encapsulated atom(s). Fullerenes, in which iron groups are encapsulated, have not yet been synthesised, the cause of which is still unclear. Under such circumstances, we initiated a new research project to investigate the magnetic properties of Fe-metallofullerenes based on theoretically predicted properties [1]. We also started study on metallofullerenes focusing on iron halide, which has also not yet been produced, as well as iron group elements.

Previously we reported the synthesis of FeCl-fullerene complexes such as FeClHC₆₀, which had been generated by arc discharge between pure carbon and FeCl₂-embedded carbon electrodes as described elsewhere [2]. In the study, we detected the mass to charge ratio (m/z) and ideal isotopic pattern which resembles the simulated one for FeCIHC₆₀. The isotopic pattern of FeCIHC₆₀ clearly indicated the presence of an Fe atom, noting that according to quantum calculations, FeCIHC₆₀ has a magnetic moment. It is theoretically predicted that endohedral FeCIHC₆₀ is more stable than exohedral one, and therefore can be the smallest magnetic nanoparticles of approximately 0.7 nm diameter. In the present study, we investigated the chromatographic behaviour of FeCIHC₆₀ to obtain the optimal conditions to isolate it for detailed analysis such as the molecular structure, optical spectrum, and magnetic properties. We investigated the chromatographic behaviour by high performance liquid chromatography (HPLC) changing columns and mobile phases. We used 5 columns and 4 types of mobile phase compositions; i.e., (toluene : n-hexane) = (1:0), (8:2), (7:3), and (6:4), to change the interactions among the stationary phases, mobile phases, and fullerenes. As a result, we found the optimal isolation conditions, under which the intensity ratio of FeClHC₆₀ to other components detected at the same retention time increased from 30.4 to 73.3%, and under all of the conditions, FeClHC₆₀ was eluted faster than an empty C₆₀. It is supposed that the difference in the retention time indicates the FeCl is attached to the surface of a fullerene cage or encapsulated FeCl is attracting π-electrons from the cage considering the interactions in the HPLC columns. I will discuss the predicted molecular structures from the view point of quantum calculations in more detail.

References:
Encouragement of protease reverse reaction utilising supercritical carbon dioxide

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Enzymes control multiple reactions simultaneously as biocatalysts in our body, showing their specificity and selectivity against substrates. Substances are effectively decomposed by enzymes without any structural change of those enzymes. Therefore, enzymes have been actively used in recent years in various fields such as medical and food sciences and industries. Supercritical fluids are located in the state, where the temperature and pressure are higher than those corresponding to their critical points. Supercritical fluids possess both gas-like and liquid-like properties; e.g., gas-like diffusivity and liquid-like solubility. It is known that the activity of lipase, which decomposes lipids, increases in supercritical carbon dioxide (scCO₂), although the activity of most enzymes decreases in scCO₂, whereas the activity of cyclodextrin glucanotransferase (CGTase) changes in the presence of organic solvent. Protease is an enzyme, which decomposes proteins via hydrolysis, but it is expected that hydrolytic enzymes may proceed the reverse reaction in scCO₂, noting that two reactions; that is, decomposition and synthesis, are simultaneously occurring, but decomposition reactions in most cases prevail over the synthetic ones (Figure 1). The objective of the present study is to synthesise dipeptide, increasing the protease reverse reaction in scCO₂.

We constructed a supercritical fluid cell so that carbon dioxide in a supercritical state can be contained and maintained in the cell. We used five amino acid residues as substrates for the reactions by protease. The products were detected by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), according to which protease reverse reaction could have been promoted in some cases. The details of the detection of the reverse reaction will be explained and the encouragement of the reverse reaction in scCO₂ will be discussed at the poster session.

Figure 1. Hydrolysis reaction. A hydrolase (E) is generally combined with a substrate (S) to form an enzyme/substrate complex (ES). Hydrolysis reaction proceeds to form enzyme/product complex (EP), and then a product (P) is produced.
Synthesis of hybrid nanoparticles composed of doxorubicin/PLGA/chitosan/graphene oxide

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In recent years, stimuli-responsive drug delivery systems have been actively studied to control drug release using near-infrared (NIR) light and ultrasound, and controlling the temperature and pH [1]. Among nanocarriers, it is well known that the biocompatibility of poly(lactic-co-glycolic acid) (PLGA) is high and that its absorbency and biodegradability by biological cells and tissues are also high, thanks to which PLGA-based nanoparticles (NPs) have been intensively studied for biomedical applications; e.g., as vehicles for drug delivery to target cells/tissues and as scaffolds for tissue engineering [2,3]. Chitosan-coated PLGA nanoparticles have been studied to increase the efficiency of gene delivery and protein expression both in vitro and in vivo [4]. It is also known that graphene oxide (GO) possesses high biocompatibility, low toxicity and high thermal conductivity. Therefore, GO has been paid a lot of attention to as a vehicle for drug delivery and a medium for photothermal therapy [5,6]. In the present study, doxorubicin (DOX)-loaded PLGA NPs, which are immobilized with chitosan and GO, (DOX-PLGA-GO NPs) are synthesized for a photo-responsive drag delivery system. The structures are characterized by scanning and transmission electron microscopy, Raman spectroscopy and Fourier transform infrared spectroscopy (FTIR). The surface charge and size distribution of the NPs dispersed in water are also measured by Zetasizer. Fig. 1 shows scanning electron microscopic images of PLGA/CS and PLGA/CS/GO NPs. Without the modification with graphene oxide, PLGA/CS NPs showed that the surface was quite smooth, whereas after the coating with graphene oxide, the surface of PLGA/CS/GO) became relatively rough.

References:
Encouragement of enzyme reactions using magnetic buckypaper in a rotational magnetic field

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It is known that the activity of enzymes immobilized on the surface of magnetic particles is increased in rotating magnetic fields up to a certain frequency and that it decreases once the frequency exceeds the critical value due to the break-up of clusters formed by magnetic particles \cite{1,2}. It is therefore supposed that the activity of enzymes immobilized on magnetic materials may be increased with an increase of the frequency of rotational magnetic fields if the clusters are not broken into smaller pieces. Buckypaper is a thin sheet made of carbon nanotubes (CNTs). It consists of entangled CNT fibres, thanks to which the surface area of buckypaper is large and buckypaper is mechanically strong.

Enzymes are catalysts for efficient biochemical reactions. The activity of enzymes is changed depending on the ambient conditions such as the temperature, pH, pressure and so on. It is supposed that the activity of enzymes may be increased by increasing the collision frequency of enzymes with substrates, noting that the activity has in fact been increased by rotating enzymes immobilized on magnetic particles by rotational magnetic fields.

In this study, $\alpha$-amylase was immobilized on the surface of CNTs and magnetic CNTs; i.e., CNTs, in which iron was intercalated, and magnetic buckypaper was synthesized, dispersing magnetic CNTs in phosphate-buffered saline (PBS) using a homogenizer and filtering the solution through a membrane filter. Finally, the magnetic buckypaper was sandwiched between two layers of buckypaper. The activity of $\alpha$-amylase was measured in the presence of a rotational magnetic field, using purified starch as a substrate and bovine serum albumin (BSA) as an inhibitor of enzyme reaction. The activity of the enzyme was estimated measuring the absorbance of photons of 700 nm wavelength. The effect of the frequency of a rotational magnetic field on the activity of $\alpha$-amylase immobilized on CNTs will be shown and discussed at the poster session.

References:
Curcumin Bioperine loaded PLGA Nanoparticles for Atherosclerotic activity

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Atherosclerosis characterized by the development of cholesterol-rich arterial plaques is a major cause of heart disease. The arterial plaques have highly upregulated inflammatory cytokines which are secreted by the macrophages that finally become cholesterol-laden foam cells. These foam cells gradually lead to atheroma. Current therapeutics aimed to treat atherosclerosis display side effects such as organ damage, diabetes, and often fail to fully reverse or repair the damaged arteries. Alternative options such as nutraceuticals might offer some edge over these synthetic substances in preventing the above-mentioned associated side effects. The inclusion of nanotechnology in delivering these natural substances further progresses the treatment procedures to achieve targeted delivery at the atherosclerosis plaque sites. An exciting strategy to treat atherosclerosis plaque would be to reprogram the M1 macrophages to M2 macrophages that block the formation of plaque cells that will reduce the inflammation induced by cytokines and elicit anti-atherosclerotic activity. In this present study, we have developed Curcumin Bioperine PLGA nanoparticles synthesized by the solvent evaporation technique to achieve the above-mentioned hypothesis. The dual drug NPs will be characterized for their size, shape morphology, chemical interaction and in vitro cell studies by various techniques to meet the objective of the proposed work.

Reference:
Electrospun cell free nanofibrous porous scaffold for two stage-controlled delivery of miRNA-1 targeting fibroblast for reprogramming of heart

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Cardiovascular disease is the predominant cause of death worldwide. Injury to heart is often due to death of cardiomyocytes due to restricted blood flow. In damaged heart, loss of cardiomyocytes is often replaced with cardiac fibroblast which is activated and converted into myofibroblast which helps to preserve the structural integrity. Nevertheless, Depletion of cardiomyocyte leads to reduced cardiac dysfunction such as pathological cardiac dilation and causes fibrosis. After Myocardial infraction, regeneration or repair of the myocardium will be the best possible therapy after end stage heart failure. The current therapies that can help to restore the loss of cardiomyocyte is limited to heart transplantation. Directly reprogramming of adult cardiac fibroblast with micro RNA holds great promise for restoring heart function. In the present work, we have developed polymeric nanocarrier encapsulated with miRNA polyplexes for attaining a controlled two stage delivery of miRNA and also to achieve a high transfection efficiency. The electrospun nanofibrous 3D Scaffold is used as an Extra cellular matrix to overcome off-target effect and to enhance the reprogramming of cardiac Fibroblast by controlling the release of miRNA. To overcome, daunting challenges in current technology, Nano mediated mircoRNA delivery targeting cardiac fibroblast serves as a non-viral carrier and also provides new insight to investigate the role microRNAs in controlling molecular network that regulates cardiac cell fate. It also serves as a new research model, as a novel cell free therapeutic strategy for repair and regeneration for end stage heart failure.

References:

Human Endothelial Cell Scaffolding Based on Functionalized Carbon Nanowalls

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Graphene¹ and Carbon Nanotubes² have been used in the past as scaffoldings for biological cells. The Carbon Nanowall (CNW) films were grown and N₂ gas plasma functionalized on silicon substrates by Surface-Wave Microwave Plasma-Enhanced Chemical Vapor Deposition (SWMWPECVD) technique. These substrates were subsequently characterized using Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM), Transmission Electron Microscope (TEM), Auger Electron Spectroscopy (AES), X-ray Photoelectron Spectroscopy (XPS), Raman Spectroscopy, Surface Zeta potential and Contact Angle measurement and. These functionalized CNW (CNW-N₂) were utilized in highlighting the potential of these CNW substrates as scaffolds for cellular proliferation facilitating Human endothelial cell attachment. It was found that the cell adhesion was stronger on CNW than on glass cover slips. The results from this study make functionalized CNWs an attractive candidate that could be applied in tissue engineering, regenerative medicine, Lab-on-chip devices and biosensors.

Figure 1. Graphical Abstract

References:
Morusin Loaded Niosomes for Anti-Cancer Nano Drug Delivery

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Cancer remains the principal cause of death worldwide. Generally in order to impede the growth of proliferating tumor cells cytotoxic drugs are given however significant toxicity is observed which is why extensive research is being carried out on using natural substances as potent inhibitors of cancer. Morusin, a water insoluble prenylated flavonoid is known for its numerous medicinal properties. It manifests its anticancer potential by suppression of genes involved in tumor progression. However, poor solubility of the drug results in low bioavailability and rapid degradation thus hindering its clinical utilization. In order to overcome this, we have synthesized a niosome system composed of non-ionic surfactant span 60 and cholesterol using thin-layer evaporation technique to improve the aqueous-phase solubility of the drug. Highly cytocompatible niosomes of 480 nm average size with smooth and uniform spherical morphology were synthesized in a facile manner. Unlike free morusin, nanomorusin was found to be freely dispersible in aqueous media. Having an extremely high drug entrapment efficiency (97%), controlled and sustained release of morusin resulting in enhanced therapeutic efficacy was observed in cancer cell lines of 4 different lineages. The results demonstrate that morusin-niosome system is a promising strategy for enhanced anti-cancer activity against multiple cancer types and could be an indispensable tool for future targeted chemotherapeutic strategies.

References:


Stimuli Responsive Nano-theranostics

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Stimuli-responsive nanomaterials are nanoscale agents that can undergo physicochemical and/or structural changes in response to specific stimuli. The stimuli can be internal, as in case of physiological or pathological variations at target site, or external, such as optical, magnetic, radiation etc. Current decade has seen spur in interest in these nanoscale materials in cancer theranostics due to their ability to control the release of cargo spatially as well temporally in response to the specific stimuli. Herein, this presentation deals with such stimuli responsive inorganic nanomaterials that were utilized for cancer theranostics and showed better outcome. In addition, these intelligent nanoprobes were biocompatible with extreme low to nil toxicity that seems more beneficial for clinical translation.

References:
Multifunctional Nanoparticles for Bioimaging

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Tracking and monitoring of various foreign bodies, including drugs, chemicals, bio/nano materials etc., in a living organism, including humans has always been an extremely challenging task. The actual importance of knowing where a certain entity is in the body, in real-time opens up a huge trove of information that can be used by the biomedical fraternity to further understand the fate and effects of that particular entity. Nanotechnology has had a great impact on the medical scenario since its advent, particularly concerning drug delivery. Yet, the issue of the fate of these drug delivery vehicles remains unresolved. Conventional dyes and contrast media which would emit signals when excited with light, fluorescence, electrons, ultrasound, X-ray, magnetic resonance, positrons etc. were employed for this purpose. The problem with these dyes and contrast media (some radioactive) is that they are usually toxic, have low signal-noise ratio, poor photostability, low quantum yield, insufficient in vitro and in vivo stability, etc. This has triggered a rapid interest in developing imaging moieties that can overcome these limitations.

Here, we present a range of highly biocompatible nanomaterials which can be utilized as imaging probes with various excitation sources as mentioned above. Additionally, most of the nanomaterials presented have multifunctional abilities such as a single probe can be imaged under different excitation sources depending on the purpose, apart from being able to induce cancer cell death on their own by photothermal/dynamic/magnetic hyperthermia etc.

References:

Inflammatory bowel diseases (IBD) are chronic, relapsing, remitting autoimmune diseases affecting the gastrointestinal (GI) tract. IBD has become a Global disease of the 21st century affecting nearly 5 million people worldwide, with almost $6 billion spent (2017) on treatments and occurs most often in patients aged 15 to 30 years. Two known forms of IBD are Crohn’s disease (CD) and Ulcerative colitis (UC). There is currently no cure for IBD, the therapeutic strategies are aimed at maintaining remission from inflammatory responses. Steroids are commonly prescribed for exacerbation of both CD and UC but prolonged use can lead to undesirable systemic side-effects. Presently, therapeutic approaches depend on conventional dosage forms such as delayed or controlled release methods by maneuvering the physiological conditions of the GI tract, especially the colon but are associated with inconsistent efficacy and inter-patient variability. Pharmaceutical strategies are currently implicating nano-delivery systems as carriers for active compounds as their reduced size leads to more effective targeting, increased bioavailability and reduced systemic adverse effects. This has shown promising results in addressing the physiological changes in IBD and are more effective than conventional method. Recently, polymeric nanoparticles have gained a lot of attention in the oral drug delivery system as they improve the bioavailability of the drug and do not produce any systemic toxicity. Another colon targeted nano-delivery system on the rise is nanoparticle-in-microparticle oral delivery system (NiMOS) and in addition silica nanoparticles have been modified to have selective drug delivery to the inflamed intestines in IBD. The understanding of nanoparticles in selective targeting and optimized release of the active compounds to the inflamed tissue can open additional therapeutic perspectives in the treatment of IBD.

References:

Co-delivery of Hedgehog Pathway Inhibitor and Epidermal Growth Factor Receptor Inhibitor via Polymer Nanoparticles Elicit Enhanced Therapeutic Efficacy in Breast Cancer

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Abstract
Breast cancer is the most common type of cancer that affects millions of women worldwide. It is a heterogeneous disease maintaining inter and intratumor heterogeneity. Single and combination chemotherapy has their own set of advantages depending on the stage of the disease though it does contribute to increased toxicity. Nanomedicine platform incorporating combination anti-cancer treatment might overcome these challenges and generate synergistic anti-cancer effects and also reduce drug toxicities. This study aims to develop polymeric nanoparticles (NPs) co-delivering a Hedgehog pathway inhibitor GANT61 and epidermal growth factor receptor (EGFR) inhibitor curcumin for the first time to provide enhanced anti-tumor activity in the heterogeneous breast tumor mass comprising of cancer stem cells (CSCs) and bulk tumor cells in a single lethal shot. The dual drug NPs synthesized using solvent evaporation technique revealed spherical, smooth surface morphology. Upon exposure to the dual drug NPs, breast cancer cells undergo cell death and cytomorphological changes along with a reduction in their target protein expression, CSC population, colony formation ability, and migration potential as revealed by their in vitro cell studies.

References:
Nanocellulose for Biomedical Applications

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Lately, a surging demand for green renewable materials have rekindled the interests of researchers for cellulose and its derivatives. Among the cellulose derivatives the sulfated cellulose promises several crucial bio-applications. In our earlier work, we functionalized the bacterial cellulose with sulfate groups via acetosulfation process get a highly transparent film with smooth surface, good integrity and mechanical properties through the so-called drop casting. Now, the sulfated polysaccharides are known to possess various bioactive properties like the antiviral, antitumor, antiangiogenic, anticoagulant, antioxidant, anti-inflammatory etc. This encouraged us to probe the bioactive properties of the bacterial cellulose sulfate in our following study. Here, the cell viability, antioxidant and hemocompatibility assays were verified to evaluate the bioactive properties of bacterial cellulose sulfate in both in vitro and ex vivo conditions. The results of the above evaluations verified that the bacterial cellulose sulfate possesses antioxidant properties, apart from being hemocompatible, cytocompatible and highly biocompatible. To explore its potential in tissue engineering and regenerative medicine, ultrafine nanoscale fibers were spun via electrospinning. Pure BCS failed to produce nanofibers due to poor spinability of its solution, hence novel BCS-PVA nanofibers were fabricated by simple electrospinning route to engineer ultrafine nanoscale fibers. The biological evaluation of the fabricated BCS/PVA nanofiber scaffolds was done using L929 mouse fibroblast cells, which confirmed that these nanofibers are excellent matrices for cell adhesion, proliferation and have remarkable properties suitable for various biomedical and tissue engineering applications.

Reference:

Cluster structures composed of $\alpha$-amylase and iron nanoparticles, and their enzymatic activity under an ac magnetic field

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We create cluster structures composed of $\alpha$-amylase molecules and iron nanoparticles. We modify the surface of iron particles, the average diameter of which is 40 nm, with 3,4-dihydroxyphenylacetic acid (DOPAC) and then activate carboxyl groups in DOPAC molecules on the particles using N-Hydroxysuccinimide (NHS) and 1-Ethyl-3(3-dimethyaminopropyl) carbodiimide, hydrochloride (EDC) in the presence of $\alpha$-amylase to covalently link the particles together thorough the enzyme molecules. Chain-like clusters are created when the particle-particle linking is performed under a dc magnetic field. We analyze the effect of the intensity of the dc magnetic field, the particle volume fraction and the enzyme concentration on the cluster structures. We next measure the activity of $\alpha$-amylase in the cluster structures and confirm that the enzyme molecules are still active. We find that the activity of $\alpha$-amylase in clusters under an ac magnetic field is increased by the heat generation of the particles. We also find that the activity increase of $\alpha$-amylase in chain-like clusters produced under a dc magnetic field is higher than that in the absence of a magnetic field.
Cluster formation of α-amylase/ferromagnetic particle hybrids under an ac/dc combined magnetic field and its effect on the enzymatic activity

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Ferromagnetic particles subjected to a radio frequency alternating magnetic field dissipate heat caused by magnetic hysteresis and eddy currents. We have demonstrated that an enzyme immobilized on ferromagnetic particles under an ac magnetic field is activated by heating [1,2]. In this study, we immobilize α-amylase on iron particles and investigate the cluster formation and the enzymatic activity of the α-amylase/iron particle hybrids under an ac/dc combined magnetic field. We find that the activity of α-amylase on the particles is raised by an ac/dc combined magnetic field compared to that under an ac magnetic field and the activity increase changes depending on the angle between the ac and dc magnetic fields. We analyze the effect of the amplitude of the ac magnetic field and the angle between the ac and dc magnetic fields on the enzymatic activity. We observe the cluster formation of α-amylase/ferromagnetic particle hybrids using an optical microscope and find that the average size of clusters formed under an ac/dc combined magnetic field is larger than that under an ac magnetic field. We make clear the relationship between the enzymatic activity and the cluster structure of the hybrids.

References:
PCR (polymerase chain reaction) is a method of amplifying specific DNA sequences and is widely used in biological and bio-medical studies. PCR is usually performed changing the temperature cyclically since the one cycle of PCR is composed of several processes with different optimal temperatures. In this study, we carry out PCR utilizing the photothermal effect of carbon-encapsulated iron (Fe@C) nanoparticles. We immobilize bobbin serum albumin on the surface of Fe@C nanoparticles, the average diameter of which is 25 nm, to prevent the attachment of DNA polymerase molecules on the particles. We then mix the surface-modified particles with a solution containing DNA polymerase, template DNA, primers and dNTPs. We carry out PCR by performing 25 cycles of temperature alternation between 65 and 95 °C utilizing the heat generation of the particles irradiated with light of 800 nm wavelength. The target DNA fragments are successfully amplified by the present method. Since the heating efficiency increases with an increase in the volume fraction of Fe@C nanoparticles, the reaction time is reduced by increasing the particle volume fraction. Furthermore, the amount of by-products per unit time also decreases as the volume fraction increases, which may be attributed to the rapid thermal cycling.
Up-motility phenotype of *Methylobacterium* sp. ME121 in co-cultivation with *Kaistia* sp. 32K

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It is thought that symbiotic events in nature are common, and this is so in the bacterial world. However, experiments to reproduce such events in the laboratory have not been aggressively pursued. It is expected that a bacterial property that is not observed in mono-culture may be observed when it is co-cultivated with another bacterium. Examples of past symbiosis experiments were mainly focused on growth and metabolic products.

Motile bacterium *Methylobacterium* sp. ME121 and non-motile bacterium *Kaistia* sp. 32K were isolated from the same soil sample [1]. Growth of these bacteria support each other when co-cultured in a minimal medium containing D-glucose, and furthermore, swimming velocity of ME121 was increased in the flask. Therefore, we aimed to elucidate an up-motile mechanism of *Methylobacterium* sp. ME121 in co-cultivation.

Co-cultivation of both ME121 and 32K was carried out in a Jar fermenter and growth rate, motility and medium pH were monitored. As a result, the swimming velocity of ME121 was also increased when mixed cultivate with 32K in a Jar fermenter. The co-cultivation medium pH was lowered to 3.5. This pH profile was only observed under co-cultivation conditions. This suggests that a motility-improving factor named the K factor was present in the 32K culture supernatant. This factor was purified and characterized as an extracellular polysaccharide of 5 to 10 kDa precipitated with 70% ethanol. The findings suggested that the mechanism by which the motility of the ME121 strain was improved was via an effect on the rotational force of the flagellar motor.

References:
Synthesis of gossypols having two glycosides and their anticancer effect

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Gossypol (Gos), a polyphenol isolated from cotton plants, shows antioxidative, antibacterial, and insecticidal activities and therefore, it is widely believed to play central roles in protection systems in these plants. In addition, Gos shows anticancer activity for human and contraceptive activity for male. However, its cytotoxicity and low water-solubility stemmed from its two aldehyde groups and naphthyl rings, respectively, strongly hinder its application as an anticancer drugs. Introduction of carbohydrate units onto these aldehyde groups would be the most promising strategy to simultaneously solve both these problems. In addition, the resultant Gos-based anticancer drugs should have potential cell/organ specificities originating from the carbohydrate units. In this study, βgalactoside/βlactoside/βglucoside/βmaltsoside having aminoxy groups (βGal/βLac/βGlc/βMal-ONH2, respectively) were synthesized through 4-steps synthetic scheme composed of acetylation of Gal/Lac/Glc/Mal, bromination of their anomeric positions, glycosylation with N-hydroxysuccinimide and deprotection of acetyl and N-hydroxysuccinimidyl groups by using hydrazine. We then, mixed these glycosides with Gos to give bis-glycosylated gossypols (βGosGal/βGosLac/βGosGlc/βGosMal, respectively) in which Gos and two βGal/βLac/βGlc/βMal units are tethered with enaminoxy linkages. Successful syntheses of these Gos-based glycoconjugates were confirmed through their ESI-TOF-MS and H NMR spectral analyses. We also carried out WST-8 assays to find that IC₅₀ of βGosGal, βGosLac, βGosGlc, and βGosMal for DLD-1 cells were 77.3, 46.4, 50.4 and 33.2 µM, and those for HepG2 cells were 59.0, 47.2, 77.5 and 42.0 µM, respectively.

\[
\begin{array}{c|c|c}
 & \text{R₁} & \text{R₂} \\
\hline
\beta\text{GosGal} & \text{OH} & \text{H} \\
\beta\text{GosLac} & \text{H} & \beta\text{Gal} \\
\beta\text{GosGlc} & \text{H} & \text{OH} \\
\beta\text{GosMal} & \text{H} & \alpha\text{Glc} \\
\end{array}
\]

\[\text{GosGal} \rightarrow \text{GosLac} \rightarrow \text{GosGlc} \rightarrow \text{GosMal}\]

\[\text{Imine form} \quad \leftrightarrow \quad \text{enamine form}\]

\[\text{Reaction condition}\]

(i) Ac₂O, Py, rt, over night, 85-90% (ii)30% HBr, Ac₂O, CH₂Cl₂, rt, 2h, (iii) NHS, TBAHS, CH₂Cl₂, (iv) 1M Na₂CO₃ aq., rt, over night, 35-56 % (v) hydrazine, dry MeOH, rt, 3h, 17-67% (v) dry MeOH, rt 3h, 31-99%
Glycosphingolipids (GSLs) on cell surfaces laterally aggregate into microdomains presenting densely packed carbohydrate clusters (glycoclusters), and the resultant glycoclusters play essential roles in various cellular recognition events. Especially, interfacial interactions between two microdomains on adjacent cells are now widely recognized as "carbohydrate-carbohydrate interactions (CCIs)". Such CCIs have attracted increasing research interest, since they initiate various cell-cell adhesions including cancer metastases. However, little information concerning CCIs has been obtained so far owing to fluidic and heterogeneous natures of cell surfaces. It is widely recognized that certain ions, Ca2+ in most cases, are essential to induce CCIs although the detailed mechanism is not clear.

In this work, we synthesized artificial glycoconjugates having tris-bipyridine ferrous complex cores as artificial models for mimicking native glycoclusters on the cell surfaces and monitor their spatial carbohydrate packing on additions of salts by using a circular dichroic (CD) spectrometer. Through these experiments, we found that not only cations but also anions bound to the metalloglycoclusters and dramatically changed the carbohydrate packings. We also found that the aqueous solution of the metalloglycocluster having 6 lactoside units (Lac6) showed enhanced viscosity in the presence of certain ion, suggesting ion-induced CCIs between the glycoclusters. These findings indicate that the ion-induced conformational changes of the glycoclusters are the very first step to induce CCIs. With the best of our knowledge, such ion-induced conformational change and the subsequent enhancement in the viscosity of their aqueous solutions have not been reported in literature.
Stabilization of gold and silver nanoparticles by thiols for LSPR applications to quality control of antibody drugs

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Localized surface plasmon resonance sensing, LSPR sensing, is a technique based on refractive index sensitivity of the extinction spectrum of nanoscopic gold and silver nanostructures. When the local refractive index varies due to a binding event of molecules or morphological change of adsorbed cells, the peak wavelength shifts. This can be evaluated out by a simple set-up consisting of a surface coated with noble metal nanostructures, a visible light source and a spectrometer in combination with a software capable of displaying the extinction peak as a function of time. It is best to fit the spectrum in real time for determining the peak shift.

We describe an LSPR sensor based on cap-shaped noble metal nanoparticles, prepared with a metal film on nanospheres (MFON) method. The nanosphere diameter is typically 100~150 nm with the top metal layer thickness in the range of 20 nm. These samples are characterized by peaks both in visible and near-IR regimes. The near-IR peak has refractive index dependence greater than 500 nm/RIU, some four times better than the visible peak.

We are interested in using silver due to its theoretically higher sensitivity, but it is hopeless useless in the presence of NaCl, which occurs quite often in biological measurements. We have solved this problem by coating nanoparticles with a layer of various thiol molecules. Those thiols were either sublimed or vaporized. Vaporized 1-butanethiol was found to be better at protecting the nanosphere layer. We also found that the same set of thiol treatments could stabilize Ag nanoparticles which are normally considered too reactive for use as a sensor material. We are currently trying to apply our technique to quality control of antibody drugs which are proving quite effective in combatting various diseases, but the use of partially denatured antibody not only makes it less effective but can also cause side effects. We have used thermally denatured antibody as a model for evaluating our LSPR sensors. Our preliminary results suggest that it is indeed capable to distinguishing intact and denatured antibodies readily. Further investigations are planned.
Butterfly wing scales as a model template for SERS applications

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As is well known, Raman spectroscopy is one of the few analytical techniques capable of giving information on chemical structures without the need to place the sample in vacuum, making it well suited for on-site inspection of chemical species as in environmental monitoring, forensic sciences and quality control. Its weakness of low sensitivity is being overcome by the technique of surface-enhanced Raman spectroscopy, which exploits strong near-fields associated with oscillations of free electrons inside noble metal nanostructures.

There are already a number of commercial vendors selling SERS substrates, but the price needs to be reduced significantly in order to make this technique widely available; a typical substrate costs on the order of 5,000 yen. Our group has been investigating (1) random-MFON structures whereby randomly adsorbed SiO$_2$ nanospheres are coated with a noble metal and (2) silver dendrites grown from surface-adsorbed base metal nanoparticles in a AgNO$_3$ solution.

Here, we report on yet another method based on exploitation of scales of butterfly wings$^1$. We found that coating of butterfly wing scales, characterized by intrinsic nanostructures, with silver gives rise to a surface capable of showing SERS effects. While effectiveness depends on the butterfly species, precise scales within a single wing, the amount of deposited silver etc., there is a surprising uniformity in SERS signal intensities when these parameters are selected appropriately. By exploiting naturally-existing nanostructures, we can minimize the number of manufacturing steps, thus reducing the overall cost. We can also obtain basic information on secret as to what makes a particular nanostructure work by selectively altering the underlying nanostructures. This would give us an option of artificially recreating the crucial nanostructures.

References:
Silver nanostructures formed from base metal nanoparticles: Optimization of the growth condition for SERS applications

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Recently, we have been examining new nanostructures for surface-enhanced Raman scattering. One such approach can be based on displacement reaction involving silver salt. Silver nanostructures, formed by displacement reaction of a base metal in silver nitrate, have been used for SERS before, but unlike most previous workers, we start with nano-sized base metal nanoparticles rather than a macroscopic chunk. We have found out that silver nanostructures with various morphologies can be prepared by changing the nanoparticle size, metal species, and reaction temperature. In particular we can prepare an unlimited variety of nano-sized base metal nanoparticles by simply depositing a metal onto a single layer of monodisperse silica nanoparticle adsorbed on a substrate surface [1], making this a powerful approach. Some of silver nanostructures we have obtained are dendrite, filament, and sheet [2], which are suitable for SERS measurements. For optimization of the SERS effect, we systematically formed many base metal nanoparticles with the following combinations of the parameters; nanosphere diameter (100, 400, and 700 nm), metal species (Cu and Al), and thickness (10, 30, 60 nm). These nanoparticles were immersed in 0.1 mM AgNO₃ aqueous solution for 15 min at different temperatures (4, 20, 50 °C) for the final displacement reaction.

The SEM images in Fig. 1 show representative silver nanostructures formed under different growth conditions. The figures in the parentheses correspond to the diameter of the silica nanosphere, base metal deposition thickness, and reaction temperature. They are all formed with Cu. Figure 2 shows an example of SERS measurement results. The intensity corresponds to the height of the 615 cm⁻¹ peak when 0.1 mM R6G was measured with different silver nanostructures. The sphere diameter is 700 nm and the irradiation wavelength is 633 nm. In our poster, we will show complete data covering the entire combination of the experimental parameters.

Fig. 1: Examples of silver nanostructures.

Fig. 2: SERS intensity of 0.1 mM R6G obtained with different nanostructures.

A plasmonic tool for evaluating enzymatic activity: monitoring acetylcholinesterase for environmental and medical applications

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Surface-enhanced Raman spectroscopy (SERS) is a powerful tool for identifying a minute amount of certain chemical species, particularly those Raman-active species with a thiol group that readily bind to a noble metal surface. We are interested in applying SERS to a study of acetylcholinesterase. This enzyme plays an important role in neurotransmission by converting acetylcholine into choline. Some poisons inhibit this process with the disastrous result, but regulating this process can potentially lead to a novel cure of various diseases. If acetylthiocholine is used as the substrate, the end-product of this process, thiocholine, is Raman-active and possess a thiol group so that it is a good target for SERS analysis. The traditional method is based on a colorimetric principle. Thiococholine is made to react with DTNB; the colored product is monitored at 570 nm with the optical path length of 1 cm, requiring 100 \textmu L or so of the sample. We intend to reduce the sample volume to less than a few \textmu L or so. For SERS measurements, we prepared our own SERS substrate with the following protocol. A glass substrate is treated with 1vol\% aminopropyl trimethoxysilane solution to promote binding of SiO\textsubscript{2} nanospheres in the subsequent step. Exposure of the substrate to nanosphere solution leads to formation of a silica nanosphere monolayer in less than one minute. Vacuum evaporation of Ag or Au results in what we call random MFON, metal film-on-nanosphere. The nanosphere diameter is normally 100 nm, and the deposition thickness is 100 nm. Figure 1 shows a portion of a glass slide with AgFON. Of the nine spots, with diameter of 3 mm, the four in the corners, whitish in appearance, are covered by AgFON. We are currently working on two aspects of this system. One is concerned with an assay protocol whereby the amount of thiocholine adsorbed on the sensor is to be optimized. The other aspect has to do with controlling the hydrophobicity of the sensor in such a way to concentrate the target molecule upon drying. As shown on the right-hand side in Fig. 2, by surrounding a SERS area with a superhydrophobic area, it is hoped that the sample can be concentrated significantly.

![Fig. 1: A photo of a SERS substrate](image1.png)

![Fig. 2: A schematic diagram for use of a superhydrophobic surface for concentration](image2.png)
Glycoside formation of d-type trichothecene at C-4 position by *Fusarium* app.

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[Introduction]

Trichothecenes are a group of mycotoxins produced as secondary metabolites when red mold fungi such as *Fusarium graminearum* infect important grain plants including wheat and corn (Figure 1). Ingestion of contaminated crops can cause toxic symptoms such as diarrhea and vomiting in humans and livestock. It has been reported that plants can conjugate glucose at the C-3 position of deoxynivalenol and T-2 toxin as a defense mechanism by reducing hydrophobicity of toxic compounds and resulting in their reduced toxicity. However, we recently found that by feeding trichodermol (TDmol) produced by *Spicellum roseum*, to *F. graminearum* and *F. sporotrichioides*, TDmol got conjugated to monosaccharide at C-4 position, maybe because TDmol has hydroxy group at C-4 position, but not C-3 position. Since glycoside conjugation of trichothecenes at C-4 position was never found before, we examined whether *Fusarium* fungi could also conjugate trichothecin (TCN), a TDmol analog, to monosaccharide at C-4 position.

[Methods]

*F. sporotrichioides* NBRC 9955 strain was inoculated into GYEP medium and the incubation was carried out 28°C with constant shaking for 2 days. Newly grown hyphae were harvested and washed with sterilized water on strainer with gauze. Hyphae were inoculated into GYEP medium and TCN was added to the medium to a final concentration of 40 µg/ml, and the incubation was carried out 28°C with constant shaking for 1-5 days. Each day 1 ml of the culture solution was harvested and extracted with an equal volume of ethyl acetate and applied to TLC, HPLC and LC-MS/MS.

[Results and Discussion]

Extraction of trichothecenes was performed every day from 1 day to 5 days, and TLC analysis revealed that the spot of TCN disappeared on the third day. It was confirmed that the peak of TCN also disappeared in HPLC at the same timing. LC-MS/MS analysis revealed that an unknown compound [M+NH₄]⁺ (m/z 444.2 Da) appeared on the same day (Figure 2). This result strongly suggested that TCN turned to be 8-keto-TDmol by losing butenyl group and afterwards got conjugated to a monosaccharide, assumingly glucose (Figure 3). Currently, in order to applying this compound to NMR for structural determination, we are in the process of purifying and collecting ~10 mg of it.
Production and structural analysis of unnatural type A trichothecenes using enzymatic reaction

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[Background]

Trichothecenes are a group of mycotoxins produced in important crops by fusaria as secondary metabolites. These toxins cause toxic symptoms when ingested by humans and livestock. Among them, type A trichothecenes including T-2 toxin, have structural characteristic that they have no keto group at C-8 position. It is also known that type A trichothecene producers have no gene to code the enzymes to hydroxylate at the C-7 position, thus, type A trichothecenes usually have no hydroxy group at C-7. However, recently, emergence of the newly evolved trichothecenes have been reported in nature, and some studies pointed out the occurrence of type A trichothecenes with hydroxy group at C-7. Hence in our laboratory, we have tried to produce type A trichothecenes with hydroxy group at C-7 to prepare the standard substances for detection, and succeeded in producing 7-hydroxy T-2 toxin (7-H T-2 toxin)(1). In this study, we use enzymatic reaction to produce other novel C-7 hydroxylated type A trichothecene using 7-H T-2 toxin as a substrate.

[Methods]

*F. graminearum* (Fg) ΔTri11 was inoculated in RF medium and incubation was carried out for 6 days to produce 7-hydroxyisotrichodermin (7-HIT). This intermediate trichothecene with 7-hydroxy group, was fed to a *F. sporotrichioides* (Fs), type A trichothecene producer, with gene disruption of Tri5, which code the first enzyme to produce trichothecene. The incubation was carried out at 28°C for 2 days, and 7-H T-2 toxin (1) could be produced.

Next, a crude enzyme was prepared from soil bacteria (No. 3743) having deacetylase activity of T-2 toxin at C-4 position. The enzyme deacetylated 1 to produce 7-hydroxy HT-2 toxin (2). Furthermore, recombinant TRI101, 3-O-acetyltransferase, was prepared and 1 and 2 were reacted with TRI101 and acetyl CoA to produce 3-acetyl-7-hydroxy T-2 toxin (3) and 3-acetyl-7-hydroxy HT-2 toxin (4), respectively. Structural analysis of these new trichothecenes were performed by MS/MS and NMR, and a calibration curve was prepared by HPLC based on the measurement result of qNMR.

[Results and Discussion]

qNMR analysis revealed that we succeeded to produce 1.30 mg of 1, by feeding 7-HIT to FsΔTri5. In HPLC analysis (at 195 nm), the slope of the calibration curve of 1 (peak area/µg) was 1.41x10⁶. Compound 2 (4.70 mg) was also successfully obtained by enzymatic deacetylation of 1, and the slope of the calibration curve was 1.22x10⁶. Enzymatic reaction of 1 and 2 with TRI101 resulted in the production of 3 (2.28 mg) and 4 (2.20 mg).

The slope of the calibration curve of 3 was 1.06x10⁶. MS/MS spectra of 1, 2, 3 and 4 were obtained and ready to be used as standard substances in detection for LC-MS/MS. NMR analysis confirmed the structure of 1, 2 and 3, but not 4 yet. We are in the middle of the process to prepare enough of compound 4 for NMR.
Characterization of germination and surface properties of *Paenibacillus chibensis* PB-434 spores

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Peracetic acid (hereinafter referred to as PAA) is one of bacterial agents, and it has bacterial sporicidal activity, is effective at a relatively low temperature, and the residue is safe. Therefore, it is used for sterilizing beverage, food and container. However, spores from *Paenibacillus chibensis* PB-434 (hereinafter referred to as PB-434) are highly resistant to PAA. If PB-434 spores survived in the sterilization treatment and grew through germination, they should cause food poisoning and food spoil. Since the sporicidal mechanism of PAA and the resistant mechanism of PB-434 spores have not been fully clarified, we focused on the characteristics of PB-434 spores.

In previous studies, it was suggested that PB-434 spores had different germination mechanism than *Bacillus subtilis* Marburg BS-168 (hereinafter referred to as BS-168) spores which are not resistant to PAA. Therefore, we investigated much more the details of germination mechanism of PB-434 spores. The results suggested that PB-434 spores required proteases for the germination regardless of presence or absence of PAA. We also investigate the aggregation mechanism of the spores, since we found that spores aggregated before spore purification but separated after purification. To clarify the aggregation mechanism, we used carbon nano materials with various functional groups and various metal ions. The results showed that some of carbon nanomaterials could make the spores aggregate (Figure 1). Then properties of the spore surface were also investigated by hydrophobicity test and zeta potential measurement. The results suggested that the surface of PB-434 spores is less charged (hydrophobic) compared to the surface of BS-168 spores. These properties of spores would be involved in the spore aggregation and the resistance to PAA. And the properties may be used to remove or reduce the spores from foods.

Figure 1. Spore aggregation with modified carbon nano tube.
Apoptotic cell death of skin cancer cells and leukemia cells induced by atmospheric pressure plasma irradiation

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Recently, atmospheric pressure plasma have been vigorously studied in various fields, for example surface modification, cancer treatment, water quality improvement, and sterilization [1], due to its simplicity of both equipment and handling. Some latest studies have shown that chemically active species or radicals generated in plasma induce selective killing or growth inhibiting only in cancer cells because attack by the radicals may induce apoptotic cell death more effectively on cancer cells than normal cells. In the early studies, the effect was investigated by irradiating plasma on the medium containing both cancer cells and culture solution. However, plasma irradiated culture solution has gotten to add to prepared cancer cells nowadays, since similar killing effect had been obtained by the method. [2] Such culture solution activated by plasma is called the plasma activated medium (PAM). In this study, we investigated the plasma irradiation time dependence of the PAM to evaluate of its effectiveness. Furthermore, we also conducted another experiment to measure the PAM's volumetric dependence of killing effect to confirm whether the plasma directly affects cancer cells or the radicals generated in the PAM attack to cancer cells.

Figure 1 shows plasma irradiation time dependence of relative number of living skin cancer cells (A375), when the PAM (culture solution: RPMI 1640) irradiated with atmospheric pressure helium plasma was added to the cells distributed to a 96 well plate. Almost all cancer cells died over 180 s irradiation. Figure 2 shows delay time dependence of relative number of living cells in the PAM, when the time adding the PAM to the cells after plasma irradiation was changed. Whereas almost all the cells died until 10 min, the number of living cells returned to 50% of that of control at 2 h (= 120 min), and finally it returned to 70% of that of control at 24 h (= 1440 min). Thus, it was found that the PAM irradiated with He atmospheric plasma for 180 s sustained the effect to kill skin cancer cells till 10 minutes thereafter.

References:


Figure 1. Plasma irradiation time dependence of relative number of living skin cancer cells in the PAM.
Figure 2. Delay time dependence of relative number of living skin cancer cells in the PAM.
Detection of Bisphenol A (BPA) in Tomato can using Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry

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BPA is one kind of environment hormone and is usually used as the material for plastic, which can be applied to metal products as antioxidant. However, when contacted with acidic liquids or liquid at high temperature, BPA can dissolve from these plastic into the liquid. Content of tomato can is acidic and during the production of tomato can, high temperature treatment is necessary, which indicates that elution of BPA is unavoidable. BPA may cause endocrine abnormalities in human body, including genital abnormalities and precocious maturity, suggesting that the detection of BPA in tomato can is important as a part the food safety.

In order to avoid the drawback that traditional matrices used in MALDI provides lots of fragments in the low mass region, new detection methods inorganic/organic compounds as matrices were developed. In this study, three kinds of compounds, anthracene (ANT), FeO nanoparticle (FeO) and active carbon nanoparticle (AC) were used as matrix. Concentrations of matrix solutions were 4 mg/ml, and BPA was dissolved in acetonitrile/H2O 7:3 with a concentration of 1 mg/ml. 1 µl of matrix solution and analyte solution was mixed on the sample plate and proceeded to the detection. From Figure 1 we can see that both ANT and AC can ionize BPA. However, compared with ANT, fragment level in the low mass region when using AC was much higher (data not shown). As a result, ANT was selected as the matrix for further study.

To perform the quantitative analysis, Bisphenol B (BPB) which has similar structure with BPA and considered to have the same ionization efficiency was selected as the inner standard compound. A series of mixture of BPA and BPB with different weight ratios were prepared and measured in order to acquire the calibration curve (Figure 2). 1 µl of tomato can content was mixed with 1 µl of BPB solution (1 µg/ml) and then mixed with 1 µl of matrix solution on the plate. After the measurements, with the peak intensity ratio between BPA and BPB, and with the calibration curve, the concentration of BPA was calculated as 1.16 µg/ml, while according to the standard provided by The Can Manufacturers Institute of Japan, the concentration of BPA should not achieve 0.01 µg/ml.

References:
Matrix-assisted laser desorption ionization (MALDI) is one of the soft ionization methods that does not decompose analyte during the ionization process. MALDI is often combined with a time-of-flight mass spectrometer (MS), and MALDI MS permits the detection of nonvolatile compounds such as biomolecules, which are difficult to detect by other methods. Since analyte is usually ionized as protonated species, organic acids such as α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), 2,5-dihydroxybenzoic acid (DHB), and 2,4,6-trihydroxyacetophenone (THAP) are often used as matrices. For compounds that are difficult to ionize by proton adduction, the addition of monovalent metal salts has been considered to be a promising method. Such compounds can be ionized as a metal-ion-adducted species, although the ionization of analyte is sometimes disturbed by counter ions. Transition metal ions used as ionization reagents were also investigated because of their affinity to some functional groups on peptides and proteins. Efficient production of Cu⁺, which can compete with other ionization probes (e.g., H⁺, Na⁺, K⁺), from the matrix is important, even though Cu⁺ adduction to analyte is carried out in the plume (gas phase) after photoexcitation or in the solid phase prior to photoexcitation.

In this study, we developed other MALDI matrices for Cu⁺ production and adduction. One is formed of nanometer-sized CuO particles loaded on a zeolite surface. The other is zeolite whose active sites have been substituted by copper ions. Zeolites are crystalline aluminosilicates with nanosized cages, and usually act as solid acid catalysts for many industrial processes. They have high catalytic activity owing to the charge imbalance at Si-O-Al bridging sites, which are compensated by cations. For the developed matrices, possible ionization and desorption mechanisms were investigated by several spectroscopic methods. These two matrices were applied to the desorption ionization of small molecules (arginine, tyrosine, heptacosan, melamine and creatinine). It was found that CuO/HM20 ionized analyte efficiently and CuM20 had selectivity for Cu⁺ adduction ionization [1].

References: