Particle Manipulation and Biosensor Applications using Optofluidic Ring Resonators

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Abstract—In recent years, much advancement has been made on optofluidics field which integrates optical elements in the form of lenses, lasers, waveguides and sensors with microfluidic devices. Here, for the first time, we present the potential usage of an optofluidic ring resonator in big particle trapping and manipulation ranging in size from 10-25 μm. When light at the resonant wavelength is coupled into the bus-waveguide, optical field confined within the ring resonator becomes amplified. The resulting high optical intensities in the evanescent field of the ring enable trapping of particles on the resonator. We are also trying to investigate a novel type of biosensor that could measure the mass of a single cell by observing resonant frequency shift of the resonator. This approach could potentially provide a new optofluidic based method for mass measurements of a single adherent cell in its physiological condition in a non-invasive manner.

Many research fields benefit from the ability to manipulate particles in the micro- and nanoscale regimes. This manipulation can be achieved with a variety of forces, including mechanical, magnetic, fluidic, electrokinetic and optical forces. Optical forces which provide dynamic and flexible manipulation can be used for micro- and nano particle manipulation either through a radiation pressure or by the force exerted by the gradient of the optical field of a highly focused laser beam, as in the optical tweezers which have been proven to be a useful tool for deflecting, sorting and transporting microparticles. Recently, researchers have developed methods such as slotted waveguides, photonic crystal resonators and plasmonic structures to generate high-intensity field gradients for the trapping of nanoparticles and biomolecules. Although these systems are highly advantageous for nanoscale particle trapping, they are limited for micro scale particle trapping because nanophotonic trapping length scale is much smaller than the actual particle size.

Figure 1: a) SEM image of a designed ring resonator being used for microparticle trapping and biosensing. b) The electric field profile for the fundamental TE mode propagating through an air-clad Si waveguide on SiO2.

Ring resonator is a kind of planar waveguiding device that can achieve high optical intensities. It consists of a ring waveguide adjacent to a bus waveguide. Light from a laser goes through the bus waveguide and evanescently couples into the ring resonator. When the optical path length around the ring is equal to an exact multiple of the wavelength of the excitation light, ring is ON resonance. Under this condition, the intensity of the light builds up and causes a dramatic increase in the optical field confined within the ring. This highly intensive evanescent field on the resonator is being used for trapping and sensing purposes. By using tunable laser source, these resonance conditions can be detected by a corresponding drop in the power output of the bus waveguide.

Figure 2: Optical transmission spectra of a designed ring resonator with radius 20 μm. At the resonance conditions, light builds up in the ring and output power of the bus waveguide decreases dramatically as sharp dips. When binding occurs along the surface of the ring resonator, resonant wavelength shifts which helps biosensing analyses. Output powers were plotted using a logarithmic scale.

To be able to make resonator single mode, silicon (Si) waveguide was designed to be 450 nm wide and 250 nm tall (Fig1). Low index silicon dioxide layer (SiO2) which lies beneath the high index Si waveguide helps to confine the light within the waveguide core. Silicon-on-insulator wafers were patterned using electron-beam (e-beam) lithography and etched using an inductively coupled plasma etching system. 1.8 μm of SiO2 was evaporated onto the chip using e-beam evaporation. Lift off processing was used to mask each resonator on the chip while SiO2 evaporation. Each ring resonator has different radius and spacing to the bus waveguide. Since the resonant wavelength is dependent on these properties, each resonator has a unique resonant wavelength associated with it.

In summary, we have demonstrated an optofluidic ring resonator device that can be used for micro scale particle trapping and manipulation. We present the potential usage of these devices for trapping of big planar particles ranging in size from 10-25 μm. We are also investigating a novel type of optofluidic biosensor that could measure the mass of a single cell by observing resonant frequency shift of the resonator. We are also planning to work on developing label-free biosensing applications by combining this optically driven ring resonator device with polymer brushes.

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Bioactivation of Titanium Based Orthopedic Implants via Titania Nanotubes

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Abstract—In this study, we present that medical grade titanium based plaques were oxidized with anodic oxidation in electrolyte solution. The resulting oxide layer is tube patterned with approximately 50 nm in diameter. Anodized titanium plaques were immersed into vancomycin containing simulated body fluid in order to load with antibiotics. The cell culture and related in vitro tests were carried out. Titania nanotube formation affected positively in osteoblast proliferation and bacterial inhibition.

Orthopedic implants have been largely used in people in both developed and developing countries with the prolonged lifetime. Complications such as bacterial infections and insufficient osseointegration are the primary disadvantages of post implantation. If not prevented, bacterial infection can result in serious and life threatening conditions such as osteomyelitis [1]. Although titanium and titanium alloys are all cytocompatible and show proper mechanical strength, in order to reduce chances of such serious complication, patients are often subjected to antibiotic drug therapy for 6–8 weeks after initial surgery. The antibiotics are systemically delivered either intravenously, intramuscularly or topically. Systemic antibiotic delivery entails certain drawbacks such as systemic toxicity. The local presence of antibiotics prevents the body from the disadvantages mentioned above [2].

If we think bone as a nanocomposite which consists of organic and inorganic constituents (such as collagen nanofibers and nano sized hydroxyapatite crystals) we can conclude that the best integration can be achieved with nanostructured biomaterial surfaces. The titania nanotube pattern showed better integration in previous studies [3].

The titanium alloy plaque samples were oxidized with size controlled titania layer by anodization method. In a typical procedure titanium plaques with the diameters of 10 mm are used as anode and platinum mesh structure was used as cathode. The electric potential was kept constant and the procedure lasted for 10 min. Vancomycin hydrochloride was chosen as antibacterial agent and was incorporated into apatite crystals. The plaques were then immersed in vancomycin hydrochloride containing 1.5X simulated body fluid (SBF) solution for 3 days [4]. The characterization of anodization and apatite formation procedures were carried out by AFM and SEM techniques. The release of drug from apatite crystals was determined spectrophotometrically.

Human osteosarcoma Saos-2 /An1 cell lines was used cell culture experiments. Cell cultures were conducted in sterile 24-well TCPS dishes in stationary conditions. Modified and antibiotic loaded titanium implants, having 10 mm diameter and 5μm thickness, were placed in 24-well TCPS dishes, fifty microliters of cell suspension (2x10⁶ cell/well) was pipetted onto the each implant then incubated in a humidified incubator. Adhesion and proliferation of Saos-2/An1 on the titanium implants were analyzed by crystal violet staining assay, after different culture times 2, 5, 7 and 10 days. The absorbance at 570 nm was measured. Osteoblast adhesion and proliferation studies results showed that titania nanotube modification did increase osteoblast attachment and proliferation (p<0,05).

The amount of total protein levels were measured 3, 5, 7 and 10 days of cultured cells onto the modified and non-modified titanium implants by Lowry’s method. Alkaline phosphatase activity (ALP) was measured to the cells cultured onto the implants for 5, 7 and 10 days. The cells present modified implant surfaces showed higher ALP levels compare to on the non modified titanium surfaces [5]. (p<0.05).

To evaluate the effect of surface modification and vancomycin on the matrix mineralization of Saos-2/An1, Alizarin Red staining of cell cultured on the implants was carried out, than photographed by the light microscopy. Growth of microorganisms in direct contact with the biomaterial surface plays a pivotal role in biofilm formation. Thus, in this work we have investigated bacterial adhesion on nanotubes loaded with vancomycin. A bacterial cell line Staphylococcus aureus ATCC 25923 was used in the experiments.

Data are expressed as means ± standard deviations of a representative of three similar experiments carried out in triplicate. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 11.5 software. Statistical comparisons were made by analysis of variance (ANOVA). In all statistical evaluations, p<0.05 was considered as statistically significant.

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Electrochemical Detection of Influenza A Virus based on DNA Hybridization by using Voltammetric and Impedance Biosensors

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Abstract-The aim of this study was to develop a novel electrochemical genosensor for the detection of DNA sequences related to the Influenza A virus genotype based on the intrinsic guanine oxidation signal. Easy wet-adsorption method was used to provide immobilisation of label-free probe DNA related to the Influenza A virus onto disposable carbon graphite electrode (GCE) surface without using any additional chemical agent for the immobilisation. The probe-modified sensor was able to clearly distinguish perfect match from non-complementary DNA in only 15 min. detection time. The voltammetric data were also confirmed and compared with electrochemical impedance spectroscopy (EIS). Several factors affecting on the hybridization and the rate of fullmatch/non-complementary discrimination are studied to maximize sensitivity and selectivity.

It has been known that a significant public problem of Influenza viruses cause three to five million severe cases in each year and between 250,000 and 800,000 deaths according to WHO data [1] These viruses may bring about such important diseases such as pneumonia, which can be fatal both adults and children and they can also cause economic loss[2]. The rate of influenza infections and excess deaths increased in each year. For all these important reasons, detection of the Influenza viruses is of importance in the medical and social area.

Several techniques have been reported for the detection of Influenza as viral culture, immunofluorescence staining (IFA), enzyme immunoassays (EIA), RT-PCR, DNA microarrays, and Taqman based real time PCR. The classical viral culture method is slow and it takes from 2 to 14 days and others have such disadvantages that immunofluorescence staining technique requires intact cells, highly skilled research man and specialised equipment, enzyme immunoassay is high cost and it is not a virus recoverable method. Although, PCR method have still been used for the sensitive and molecular detection of many viruses, after the amplification of selected gene region, toxic chemical as ethidium bromide is used in agarose gel electrophoresis step.

There are also some rapid tests used for the detection of influenza viruses and the rapid detection of Influenza viruses are important for taking preventative measures, changing the anti-viral therapy. However, the reliable discrimination of the influenza virus type is not possible with them because these tests either detect and distinguish between influenza A and B or detect influenza A only.

The alternative biological material detectors “biosensors” have been developed during the past two decade to fill a need for simple, rapid, inexpensive and portable testing devices for identifying biomolecular interactions. In these field, many DNA biosensors have been reported for the detection of DNA hybridization, DNA mutations, drug-DNA interactions by using optical, piezoelectric and electrochemical transducers recently [3,4].

The electrochemical detection of DNA hybridization events[5,6] can be monitored based on the intrinsic guanine signal or by mostly DNA hybridization indicators as antibiotics or some metal complexes [7-9] or by using some nano-sized materials[10].

Here, we described a simple, label-free and inexpensive voltammetric and impedimetric DNA biosensors for the detection of Influenza A viruses as an alternative to classical viral culture, immunofluorescence staining technique requires intact cells, highly skilled research man and specialised equipment, enzyme immunoassay is high cost and it is not a virus recoverable method. Although, PCR method have still been used for the sensitive and molecular detection of many viruses, after the amplification of selected gene region, toxic chemical as ethidium bromide is used in agarose gel electrophoresis step.

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Emerging Nanotechnology tools for drug delivery: Carbon nanotubes
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Abstract- Nanotechnology is rapidly developing field in especially engineering and medical sciences. Carbon nanotubes are one of the most studied nanomaterials in material sciences and physics but not in medical sciences and pharmacy. The adsorption and desorption properties of carbon nanotubes were investigated for ibuprofen, naproxen, oxaliplatin and paclitaxel. Multiwalled carbon nanotubes were also PEGylated and the effect of PEGylation was investigated.

Carbon nanotubes are first proposed to the market in 1991 and appeared to be a new and emerging nanotechnology tool for drug delivery in nanomedicine. Some of nanotechnology applications can be tailored early diagnosis and treatment of many diseases including some skin disorders and may be cancers. If nanoparticles are able to penetrate through skin layers or if they can be able to provide drug molecules on to the skin surface they can be used to deliver active substances for therapeutic purposes but exploration of their pharmaceutical applications remains at a very early stage. Recently it has been noticed that single-walled carbon nanotubes (SWNTs) and multi walled carbon nanotubes (MWNts) can be internalized by living cells and can pass across the biological membranes in cell culture studies. The internalization of carbon nanotubes by corneocytes has been shown in the literature. Figure 1 shows subsequent desorption of NAP and IBU after adsorption.

Skin penetration experiments using CNTs and drug complexes were also done and higher penetrations were observed (Figure 2). Higher penetrations were observed when drugs were provided with CNTs. The physical appearance of MWCNTs were determined using AFM (Figure 3). The tubular shapes of CNTs were clearly seen. On the figure and branches of CNTs and carbon particles can be observed. This gives information mainly about the length of nanotubes and approximate valuation of the bundles diameter.

It was found not to be very accurate to determine exact value of diameter of CNTs as it was reported in the literature because some nanotube does not lie directly on a mica and oscillated under the daze.

As a conclusion to prepare CN Ts-drug mixture is possible and it is resulted in drug adsorption. CNTs can adsorp drug molecule on their surface and it can deliver the drug and even they can release the drug by subsequent desorption. CNTs were found to be suitable material for drug transport having higher surface area and being an adsorptive material. PEGylation process was found to be effective and useful technique for further studies.

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Figure 1. Desorption of NAP and IBU from CNTs after adsorption (24h).

Figure 2. Penetrated amount of drugs through full thickness rat skin.

Figure 2. AFM images of DWCNTs and MWCNTs.

Figure 3. AFM images of PEGylated MWCNTs.
Interferometric Reflectance Imaging: Nanoscale Biological Imaging, Label-free Protein and Single Pathogen Detection

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Abstract — Utilizing the sensitivity provided by optical interference, we have demonstrated nanometer scale measurement capability in fluorescence microscopy studying conformation of DNA protein complexes. We have also developed a novel platform based on Interferometric Reflectance Imaging Sensing (IRIS) capable of high-throughput label-free biodetection in a micro-array format. The IRIS technique relies on a layered surface and imaging the reflectance when the sensor surface is illuminated, for example by a tunable laser, at different wavelengths. We have demonstrated dynamic multiplexed detection of antigens and antibodies in solution using corresponding probes on the IRIS surface as well as label-free measurements of DNA hybridization kinetics and single pathogen detection capability.

Optical interference is one of the key properties of light enabling important devices such as interference filters and lasers. We have utilized basic principles of optical interference in biological applications demonstrating nanometer scale measurement capability in fluorescence microscopy and label-free sensing of protein binding in a high-throughput micro-array format.

A novel application of interference to fluorescence microscopy promises nanometer resolution in biological imaging. We have developed a technique – spectral self-interference fluorescent microscopy (SSFM) – that transforms the variation in the intensity for different wavelengths in emission of fluorophores on a layered surface to nanoscale height information. Using SSFM, we have estimated the shape of coiled single-stranded DNA, the average tilt of double-stranded DNA of different lengths, and the amount of hybridization [1]. The determination of DNA conformations on surfaces and hybridization behavior provide information required to move DNA interfacial applications forward and thus impact emerging clinical and biotechnological fields. Recently, we have also applied SSFM to study the conformational changes of polymers [2] and DNA-protein complexes. [3] We combined SSFM with a complementary technique invented by Prof. Rant at Technical University of Munich (TUM) in which surface electric field provides highly-ordered DNA arrays [5] and SSFM can quantify conformational changes when DNA-protein complexes form.

Direct monitoring of primary molecular binding interactions without the need for secondary reactants would markedly simplify and expand applications of high-throughput label-free detection methods. We developed Interferometric Reflectance Imaging Sensing (IRIS) that monitors the optical phase difference resulting from accumulated biomolecular mass and demonstrated simultaneous detection of antigens and antibodies. [6] Dynamic measurements were made at ~10 pg/mm² sensitivity. We have also demonstrated label-free measurements of DNA hybridization kinetics. [6] Recently, we have developed a multi-LED discrete wavelength illumination system that allows for high spatial resolution imaging in IRIS modality. We have used this system to detect nanoscale particles and characterize their reflection signature thus measuring the size of the particle [7]. We have successfully detected 100 nm and 70 nm particles.

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Ecotoxic Effects of nanoTiO₂ on bacteria and algal cell population and lipid peroxidation

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Abstract—The ecotoxic effects of photocatalytic TiO₂ of E. coli and P. subcapitata, under different physico-chemical parameters, was examined. Nano-TiO₂ caused lipid peroxidation through generation of reactive oxygen species and was maximized at nanoparticle size of 18 nm and 42 nm for E. coli and P. subcapitata, respectively.

Nanotechnology has the potential to alter many aspects of our lives, including health care, consumer products, transportation, and energy. Manufactured nanomaterials have numerous industrial applications including electronics, optics, and textiles, as well as applications in medical devices, biosensors, and in environmental remediation [1-3]. Nanotechnology promises a wealth of benefits to society; however, the nanomaterials can impact both human and environmental health. Unfortunately, there is only limited study on the potential adverse effects of nanomaterials [4].

TiO₂ is a well-known antimicrobial material. It has been studied extensively over the last 25 years as photocatalyst for various purposes including the removal of organic and inorganic compounds from and the inactivation of harmful microorganism in water and air. It is the material reacts with cell membranes upon irradiation with a photon at ≤385 nm wavelength generating reactive oxygen spesies (ROS) and hydroxyl radicals (OH•), which subsequently leads to the inactivation of bacteria [5]. However, there is limited information on the ecotoxic effects of nano-TiO₂. When the materials are prepared in the form of very small particles, they are expected to show better antimicrobial characteristics because of larger specific surface area.

A wealth of information exists on the toxicity of TiO₂ towards bacteria and most of them are on the photocatalytic inactivation of the bacteria using different levels of UV radiation [6-8]. Blake et al. [6] reviewed the literature on the inactivation of cancer cells using TiO₂. They also provided background on photocatalytic chemistry, fundamental characteristics of target organisms, potential applications, and the toxicology of TiO₂. The results of our preliminary short-term batch and in vitro studies showed that there was also a short-term inactivation of bacteria in the absence of light [9]. Little published literature has confirmed this finding which indicates that there are additional mechanisms responsible for the toxicity effects. Adams et al [10] showed E. coli inactivation by TiO₂ in dark. Gurr et al. [11] reported from mammalian cytotoxicity studies that TiO₂ exerted oxidative stress to cells in dark under non-photocatalytic conditions. Warheit et al. [12] measured growth inhibition of P. subcapitata and reported a 72 hour EC50 of 87 and 61 mg/L for nanoparticle size of 38.5 and 100 nm, respectively. These researchers indicated that there is a clear dose-response relationship between survival and nanoparticle concentration however more studies are needed as to understand the mechanisms of microbial responses to nanoparticles.

In this work, the efficiency of photocatalytic inactivation of E. coli and P. subcapitata, under different physico-chemical parameters, was examined. Photocatalytic inactivation rates of the cells were dependent on several parameters such as physiological state of the cells, nanoparticle concentration and size, and the intensity of the light source. To test the effect of nanoparticle size, 11 sizes of TiO₂ were examined. All bacteria, algal cells and nanoparticle suspension were prepared in USEPA algal growth media. Following USEPA guidelines, the test duration was 4 days. Measurements included pH, conductivity, cell counts, and lipid peroxidation. The EC20 was calculated using TRAP ver 1.0. The most novel nanoparticle sizes of TiO₂ were 18 nm with an EC20 of 9.6 mg/L for E. coli cells, and 42 nm with an EC20 of 5.2 mg/L for algal cells. Toxicity was correlated to the surface charge of the material. TiO₂ caused lipid peroxidation through generation of reactive oxygen species and was maximized at nanoparticle size of 18 nm and 42 nm for E. coli and P. subcapitata, respectively. Direct contact between TiO₂ and cells is an important factor for lipid peroxidation.

In summary, the factors which did influence the bacterial and algal responses to nanoparticles include light availability, flocculation, particle size, and photoactivity. These factors are interrelated. When TiO₂ and organisms are introduced into the suspension, flocculation takes place right away. Flocculation between organisms and TiO₂ was observed and was consistent with Lin [13] which reported absorption of TiO₂ to P. subcapitata. However, it is believed that mainly membrane deformations causing the inactivation of the organisms. During the contact between nano-TiO₂ particles and the organisms, the generated ROS and OH• decomposing the cell membrane and causing the death or major inactivation of the organisms.

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Electrochemical monitoring of DNA hybridization by magnetic particles based sensor technology

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Abstract-An indicator-based and indicator-free magnetic assays connected with a disposable pencil graphite electrode (PGE) were successfully developed, and also compared for the electrochemical detection of DNA hybridization. The oxidation signals of echinomycin (ECHI) and electroactive DNA bases, guanine and adenine, respectively were monitored in the presence of DNA hybridization by using differential pulse voltammetry (DPV) technique. The detection limits (S/N = 3) of the magnetic assays based on indicator or indicator-free were found in nM concentration level of target using disposable sensor technology with good reproducibility.

The development of advanced biological sensor systems could impact significantly the areas of genomics, proteomics, biomedical diagnostics and drug discovery [1,2]. Recent advances in biosensors based on nucleic acid have led to the development of genosensor technology for gene sequence analysis and for nucleic acid ligand binding studies [3-5].

The electrochemical nucleic acid sensor system based on magnetic particles [6-11], labeling with an enzyme [8], using label-free system [7,9,11] or combining with metal nanoparticles [6,10], enables the sequence specific detection of DNA hybridization observed in low detection limits resulting from efficient magnetic separation.

A biomagnetic assay of DNA sequences related to breast-cancer gene (BRCA1) was reported based on label-free detection [7]. Coupling the label-free guanine detection with the efficient magnetic isolation of the hybrid as using biotinylated inosine-substituted probes, streptavidin-coated magnetic beads with potentiometric stripping analysis (PSA) measurements at a renewable graphite pencil electrode.

In this study, an indicator-based and indicator-free magnetic assays connected with a disposable graphite pencil sensor (pencil graphite electrode, PGE) were successfully developed, and also compared for the electrochemical detection of DNA hybridization. The changes at the oxidation signals of echinomycin (ECHI) and electroactive DNA bases, guanine and adenine, respectively were monitored in the presence of DNA hybridization by using differential pulse voltammetry (DPV) technique. The selectivity of these magnetic assays for DNA hybridization was also checked in the presence of single base mismatch and noncomplementary DNA sequences. There have not been yet any reports about both indicator-based and indicator-free magnetic assays connected with disposable sensor system by measuring the oxidation signals of ECHI, guanine and adenine in the same measurement scale.

The reported magnetic assays based on indicator-based and indicator-free methods connected with a disposable graphite sensor (PGE) were successfully developed for the electrochemical detection of DNA hybridization. There have not yet been any reports about both indicator-based and indicator-free magnetic assays connected with a disposable sensor system through measurement of the oxidation signal of indicator ECHI with the signals of DNA bases, guanine and adenine, in the same measurement scale. Easier, quicker and more sensitive detection scheme for DNA hybridization based on magnetic assay was explored herein in comparison to the traditional techniques reported earlier in the literature [5,12], in which several external indicators [Co(phen)3]3+, methylene blue, meldola’s blue have been applied by using advanced surface modification or regeneration schemes. The use of PGE brings the other advantage to our assay with a better reproducibility; such as, being cheaper, easy to use (single-use) and portable, which are crucial properties of devices for DNA chip technology contrary to other transducers; such as, gold electrode and hanging mercury drop electrode. A.E. acknowledges the financial support from TUBITAK (Project no.106S181). H.K. acknowledge a scholarship from PhD students obtained from TUBITAK.

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Direct Electrical Detection of E.Coli Based on Different DNA Modified Genosensors
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Abstract—Herein, we describe an impedimetric and voltammetric genosensor for rapid and direct electrochemical detection of E.Coli PCR (Polymerase Chain Reaction) products by using different modification techniques. Graphite and gold sensor surfaces were modified with probe sequences via different modification techniques. The aim of the study is to develop nanosensors for direct and rapid detection of microorganisms.

Recent advances in biosensors based on nucleic acid hybridization recognition have led to the development of genosensor technology for DNA sequence analysis.[1-3] Specifically, electrochemical hybridization biosensors demonstrate great promise for pathogen identification, mutation detection, and genomic sequencing.[4-5]. These novel, sequence-specific hybridization processes either involve monitoring the oxidation signal of the electroactive bases of DNA [6-7] or employing an electroactive hybridization indicator [8-9] that emits different signals to discriminate between single-stranded and double-strand DNA. Successful attempts to exploit the electrochemical detection of hybridization events and base pair mismatches using sample amplicons in order to obtain reliable, clinically-relevant measurements have already been reported.

Genosensors clarify the array technologies which based on immobilized 15–25 mer single strand DNA called as capture probe molecules [10]. These probe molecules are the complementary sequences of the specific part of the amplicon related to a virus, bacteria or a specific gene sequences. The specificity and the selectivity of the hybridization detection by using long amplicons are dependent on the conditions at the electrode surface such as; density, extent, and relative position of probe sequence.

In this work effect of surface chemistry onto hybridization and detection of microbiological disease was performed. E. coli probe and PCR products were used as a model case. For this purpose, disposable graphite and gold surfaces were modified by using a variety of different methods such as adsorption, covalent agent, poly-D-lysine, trimethoxysilane, carbon nanotubes (CNT), gold nanoparticles (AuNP) and streptavidine/biotin. The aim of this study is to select the most appropriate surface modified electrode for hybridization and clinical detections of microorganisms.

Inosine substitute probe sequence representing E.Coli bacteria was immobilized on the modified electrodes and the hybridization process occurred. Namely, DNA probe underwent hybridization to its target, mismatch and non-complementary oligonucleotides. Hyridization and mismatch detection was performed by electrochemical impedance spectroscopy and g differential pulse voltammetric signals of guanine.

There was also an enhancement in the hybridization signals due to the increased amount of immobilized oligonucleotides on the electrodes when it was compared to hybridization signal of unmodified electrodes

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Detection of Mycobacterium tuberculosis complex and Mycobacterium gordonae on the same portable surface plasmon resonance sensor

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Abstract-In the present study, we have developed specific detection system for M. tuberculosis complex and M. gordonae by using a commercially available SPR based portable-multichannel sensor system. The results showed that sensor platform can be used to detect DNA hybridization effectively down to a concentration of 0.05 μM. The sensor platform can be regenerated with 2.5 mM HCl quite effectively and reused several times without losing the signal intensity. The SPR sensors carrying the probe-ssODNs were kept in vacuum at room temperature in the dark for about 12 weeks, and used effectively.

Tuberculosis (TB) is a common and deadly infectious disease caused by several mycobacteria especially Mycobacterium tuberculosis complex (MTB) consisting of M. tuberculosis, M. bovis, M. africanum, M. microti. According to World Health Organization, [1] globally, there were about 1.7 million deaths from TB in 2007 occurring world-wide. On the other hand, Mycobacterium gordonae, typically regarded as a colonizing organism, is a nontuberculous mycobacterium (NTM) which are found primarily in natural and tap water, but are also found in soil, dust, animals, and food. Moreover, the presence of M. gordonae in hospital water system and other environments causes contamination and detected as a false positive result in many conventional TB test [2]. Therefore, distinguish of M. gordonae among MTB complex species is essential.

Surface Plasmon Resonance (SPR) is a rather new optical technique which allows following the interactions between biomolecules without using any label. It has been applied to the measurements of ligand-receptor interactions, drug screening, DNA-hybridization [3], enzyme-substrate interactions, polyclonal antibody characterization, epitope mapping and label free immunoassays.

In the present work, we developed a three channel mini (portable) SPR system for label free detection of M. tuberculosis complex and M. gordonae. A thiol-derivatized single-strand oligodeoxynucleotides (ssODN), which are complementary of the target characteristic sequence of MTB complex and M. gordonae were used as the “probes” and immobilized different channel of SPR chip. A probe-immobilization protocol was developed and the sensor carrying the probes as nano-overlays was used for the detection of the both “target” ssODNs and PCR products of M. tuberculosis complex and M. gordonae. A non complementary ssODNs was also used as control to exhibit the selectivity of the sensor.

The prepared sensor performances and calibration curves were examined for detection of both M. tuberculosis complex and M. gordonae and a steady increase in the SPR signal was observed when the target ssODNs concentration increased up 1 μM and the maximum relative refractive index was measured 82 ± 8.2 × 10⁻⁶ RIU and 146 ± 13 × 10⁻⁶ RIU, respectively. Note that the change of the relative refractive indices with the M. tuberculosis complex target concentration was linear only up to 0.5 μM with a linear regression coefficient of 0.962. A calibration curve of M. gordonae was also performed when the target ODNs concentration in 0-2 μM and linear relationship can be observed up to 1 μM with a regression coefficient of 0.989.

Cross-selectivity of the SPR sensors was tested to determine the MTB complex in mixed sample solutions that contained both MTB target and non-complementary sequences. Figure 1 presents changes in the refractive index responses in all 3 channels of SPR sensor chips when they were incubated with mixed sample solution. As expected, we just observed increase in relative refractive index in channel A which was carrying only the MTB-probe-ODNs.

The results showed that sensor platform can be used to detect DNA hybridization effectively down to a concentration of 0.05 μM and can also be used quite specifically in aqueous solutions at room temperature and by circulation the test solution for about 20 min at a flow rate of 5 μl min⁻¹ to effect complete hybridization. It was also demonstrated that the sensor platform can be regenerated with 2.5 mM HCl quite effectively and reused several times without losing the signal intensity. The shelf-life can be considered acceptable when the platforms carrying the probe-ssODNs are kept in vacuum at room temperature in the dark for about 12 weeks.

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![Changes in the refractive index responses](image-url)
Nano Alterations of Macro Systems in Biomedicine
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Abstract— Polymers are commonly used in medial area in the production of implants and prostheses where the biodegradable and biocompatible natural originated polymers are the desired ones for the preparation of drug carrier devices and tissue engineering scaffolds. Micro and nano modifications of the bulk or the surface of the prepared systems may be needed to enhance their biocompatibility. This presentation will focus on the types of nano modifications of the medical systems.

Polymers are the most preferable materials in medical applications because of their versatility and ease of synthesis. They are used in the production of blood bags, artificial veins, dental fillings, contact lenses, coatings for metallic implants, etc. Biodegradable and biocompatible natural originated polymers are the ones used for the preparation of scaffolds for tissue engineering, drug carriers as micro and nano capsules, hydrogels as filling materials. Scaffolds with fibrous or porous structures have walls with different chemistry, physical shape, crystallinity, topography and surface free energy, and all these factors affect cell adherence onto the material. In some cases, nano or micro level modifications of the bulk or surface of the medical devices and scaffolds are needed to enhance the biocompatibility in the desired direction.

Bulk nano modifications can be achieved by adding nano components (e.g. nanoparticles, nanoclusters, nanocrystals, nanofibres, nanotubes, nanowires, nanorods, nanomagnetic particles, nanocomposites, etc.) into the structure or by forming interpenetrating structures so that some macromolecules diffuse in the network and form another matrix with different properties. For surface modification, coating with self assembled mono layer, immobilizing bioactive molecules, adding a graft, doping with ions, forming a certain pattern are some techniques used to alter the surface chemistry or physical shape (Figure 1).

Gelatin and chitosan micro particles prepared in our labs were studied as carriers for cancer and thalasemia drugs. Depending on the preparation parameters, such as polymer concentration, amount of crosslinker, stirring rate, etc; the size and the properties of the particles were changed, and therefore the release rates of the drugs were adjusted from days to months. Conjugation of antibodies onto the surface made it possible to target the drugs to the specific cells [1].

Synthetic elastomeric polymers, like polyurethanes (PU) are commonly used in artificial vein production, and the most desired property is being antithrombogenic. This can be achieved by linking bioactive molecules like heparin to the surface and prevent blood coagulation. The PU polymers synthesized in our labs were activated with oxygen plasma and then heparin molecules were covalently linked on the surface (Figure 2). This monolayer heparin conjugation made the surface blood compatible causing minimum platelet adsorption [2,3].

For tissue engineering applications, scaffolds can be enriched with growth factor (GF) carrying nanoparticles so that the controlled delivery of GF would activate the healing process for both, soft and hard tissues [4,5]. Chitosan-gelatin scaffolds containing hydroxyapatite (HAp) micro and nano crystals demonstrated strong adherence of SaOs cells indicating good performance for bone tissue engineering applications (Figure 3).

This presentation will focus on the types of nano and micro systems as well as nano and micro modifications of the macro systems used in medicine by giving examples from novel applications.

Preparation of Lysozyme Loaded Chitosan Nanoparticles and Test of Their Antimicrobial Activity

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Abstract-The lysozyme loaded chitosan nanoparticles are prepared under different conditions. The release profiles of the lysozyme from the prepared nanoparticles are determined. The antimicrobial activity of the lysozyme loaded chitosan nanoparticles is tested against B. amyloliquefaciens and E. coli bacteria.

Chitosan, which is a partially deacetylated derivative of chitin, has an important biopolymer for pharmaceutical and biomedical applications. Especially in the drug and gene delivery applications, the use of chitosan, which is naturally cationic, as a carrier vector is preferable due to its ability to make ionic complexes with negatively charged substances, low immunogenicity, biocompatibility and biodegradability [1]. The use of nanoparticles to control the release of the bioactive substances has been well accepted as a delivery system. Therefore, the preparation and characterization of chitosan (CS) nanoparticles intended for delivery systems and controlled release applications has become a challenging area due to the difficulties in reducing the size of the prepared CS particles into the nano level (below 100nm). In addition to the size of the particles, the monodispersity of the prepared nanoparticles is also another challenge [2]. The main objective of this study is to prepare the lysozyme (LS) loaded CS nanoparticles for controlled release applications.

The CS nanoparticles were prepared by using ionic gelation method. The LS was loaded on CS nanoparticles via encapsulation as it is illustrated in Figure 1.

In the ionic gelation method, the formation of nanoparticles occurs as a result of the interaction between the negative groups of tripolyphosphate (TPP) and the positively charged amino groups of CS. The efficiency of the method in preparing the nanoparticles is determined by the size, size distribution of the particles as well as the encapsulation efficiency of the compound loaded into the nanoparticles. The CS/TPP ratio is considered as the most effective parameter in the formation of CS nanoparticles. The concentration of TPP, as a cross linking agent in the CS/TPP ratio plays an important role in the gelation which causes the formation of nanoparticles. The degree of deacetylation of CS also has an important effect on the size of the CS particles. The removal of acetyl groups from the chains of the CS and increase in the deacetylation degree leads to an increase in degree of cross linking between the positive and negatively charged groups. In addition, the pH and the temperature of the CS solution are also important parameters on the size of the formed CS nanoparticles since they have a significant effect on the charges of the CS particles which can determine the size of the particles.

In this study, low and high molecular weight chitosan were used for preparing the nanoparticles. The effects of CS molecular weight, CS/TPP ratio, degree of deacetylation, temperature and pH of the solution on the CS particle size and size distribution were investigated. Moreover, the effects of these parameters on the loading capacity (LC) and encapsulation efficiency (EE) of LS on the CS nanoparticles were determined. Finally, the release profiles of lysozyme from the nanoparticles prepared with the optimum conditions and the antimicrobial activity of these particles was measured against B. amyloliquefaciens and E. coli bacteria.

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The Role of Orosomucoid (ORM) in Vascular Endothelial Cells and Angiogenesis

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Abstract-We demonstrated that ORM, the acute phase protein, enhances endothelial migration and supports VEGF-A-induced endothelial tube formation. Our results let assume that ORM seems to be involved in regulation of angiogenesis.

Orosomucoid (ORM), also known as alpha1-acid glycoprotein (AGP) belongs to a group of lipocalin family and along with many other functions plays a role in the modulation of immune response to stress [1]. ORM is found to be increased in infection, inflammation and cancer. The structure of ORM is composed of polypeptide chain carrying about 45% carbohydrate residues including a large amount of fucosyl and sialic acid.

ORM is synthesized in liver and various extracellular cell types e.g. granulocytes and endothelial cells [2]. An induced expression of sialyl Lewis X (sLeX) on ORM during acute inflammation has been reported, leading to the speculation that it might influence the E- or P-selectin mediated influx of sLeX-expressing leukocytes into inflamed areas. It has been suggested that an increased level of sLeX-expressing ORM might have a feedback inhibitory effect on the extravasation of leukocytes, by competition for E-selectin [3]. By this mechanism and by interacting with the endothelial glycocalyx ORM is thought to modify the permeability of the vascular endothelium [4,5]. Thus far it has been shown that ORM binds to the vascular endothelial cell surface and subsequently causes transcytosis across the cell without passing the intercellular junction [6].

Angiogenesis is a prerequisite for tumor growth and metastasis and is regulated by angiogenic activators and inhibitors [7,8]. Among these factors VEGF is the key regulator of physiological and pathological angiogenesis and acts as a survival factor for endothelial cells (EC), both in vitro and in vivo [9].

Up to now, it is unclear which potential influence the constitutive expression of ORM or the application of ORM in vascular endothelial cells possesses during angiogenesis. Therefore, we aimed to analyze the potential role of ORM in angiogenesis. To this aim we performed endothelial overexpression versus silencing via siRNA of ORM and tested the effect on endothelial cells in vitro and in vivo angiogenesis assays such as endothelial tube assay, migration assay and CAM assay using recombinant ORM (ORM).

Employing ORM we show here that ORM increases endothelial cell migration in a dose dependent manner and supports the VEGF-A-induced endothelial tube formation in vitro when VEGF and ORM are applied simultaneously. While in vitro ORM alone does not induce endothelial tubes it increases the number of blood vessels in vivo in CAM tissue. Also, in CAM assay ORM enhances the VEGF-A-induced new vessel formation. These data suggest for the first time that ORM is involved in angiogenesis and supports VEGF-A mediated new vessel formation.

In conclusion, our results suggest that ORM produced by endothelial cells acts in an autocrine and the circulating ORM in paracrine manner on vascular endothelial cells, ORM is essentially involved in the regulation of angiogenesis, particularly in the presence of VEGF-A, and with these properties it might influence tumor vascularization. Further studies are needed to identify the interaction partners of ORM on the surface of endothelial cells.

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Self-Assembled Peptidic Nanostructures

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Abstract—This lecture will illustrate concepts of making materials which mimic the structure and function of the biological materials through programmed self-assembly of small molecules and their applications in functional materials. The self-assembly mechanism that forms the supramolecular aggregates involves non-covalent interactions such as hydrogen bonds, electrostatic and hydrophobic interactions. Diverse functional groups were incorporated into nanostructures, for example bioactive peptide sequences and metal chelating groups as well as hydrophobic motifs that include alkyl chains, steroid rings, and aromatic systems. The potential impact of these nanostructures on functional materials will be discussed.

Understanding interactions in assembly mechanisms of biological molecules has become a crucial factor in the design of nanoscale materials. For example, protein structure is defined by information encoded in the individual amino acids. The amino acids are joined together to form peptides which then fold into complex structures.1 A considerable number of the structural features in proteins consist of α-helix and β-sheet secondary structural components of peptides.2 Synthetic methodologies provide routes for synthesis of peptide sequences that are useful in the formation of one-dimensional nanostructures. Non-peptidic moieties can incorporate novel functionalities into supramolecular systems such as photoswitching units and ligands for recognition events.3-5

We study self-assembling peptidic molecules with various functional groups that assemble to form nanofiber networks under controlled conditions.6 These nanofibers mimic the architecture of natural extracellular matrix components and are ideal to encapsulate and signal cells in three dimensions through self-assembly of a nanostructure network. We were able to synthesize and characterize new molecules to enhance recognition of bioactive signals on the surfaces of nanofibers.6 There has been great interest in materials design with biological signals that can induce cellular events important in tissue regeneration. The use of self-assembly is particularly attractive, because it can allow biomolecular scaffold formation in situ by delivering liquids containing self-assembling molecules to a target tissue site. We also studied biotinylated model systems for recognition of biotin by avidin on the periphery of the nanofibers and enhanced recognition of biotin was observed by changing the packing density of molecules in the nanofibers.6 Various molecules containing the cell adhesion epitope RGDS were synthesized with branched architectures for enhanced recognition of biological signals for cellular adhesion.6 The self-assembling molecules were also labeled with gadolinium chelated magnetic resonance active groups for magnetic resonance imaging (MRI).6 Longer relaxation times were observed in the presence of the MRI active material we developed than the commercial MRI contrast agents. In addition, we exploited the nanostructures to present various other non-peptidic functional groups. For example, a peptide nucleic acid sequence on the periphery of the self-assembled supramolecular structures was presented to recognize RNA or DNA oligonucleotides.6 High epitope density on the surface of the self-assembled nanostructures was also exploited for catalysis applications. To investigate this, hydrolysis of esters such as 2, 4-dinitrophenyl acetate in the presence of imidazole functionalized nanofibers was studied.2 Enhancement in the hydrolysis rates was observed with imidazole groups presented on the nanofiber surface compared to imidazole in spherical nanostructures and in solution. In addition to functionalizing the periphery of these nanostructures, we also studied the possibility of encapsulating molecules in the hydrophobic interior of the nanostructures by encapsulating hydrophobic molecules, such as carbon nanotubes and pyrene in order to examine the potential use of these systems in biosensors and drug delivery.6

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Figure 1. Self-assembling peptide amphiphiles form nanofibers.6

References:
Abstract— Nanotechnology is the ability to measure, design, and manipulate at the atomic, molecular and supramolecular levels on a scale of about 1 to 100 nm in an effort to understand, create, and use material structures, devices, and systems with fundamentally new properties and functions attributable to their small structures[1]. In this respect we can built the objects to atom by atom specifications by the ability of nanotechnology. Therefore nanotechnology will make possible very big range of new products. On the other hand those products will be also inexpensive and clear products. As the industrial perspective it can be concluded that nanotechnology affects almost all of the industrial sectors (i.e., energy, electronic, communication, textile etc) due to its mentioned advantages. One of the most important application fields of nanotechnology is about the medicine and health sciences as biomedical applications. Biomedical applications of nanotechnology consists of the nanoscale principles and techniques to understanding and transforming the biomaterials and biosystems for medical purposes such as drug and gene delivery-controlled release systems, molecular imaging and diagnostics, cardiac therapy, dental care, orthopedics, tissue engineering applications and targeted cancer and/or gene therapies[2].

Biomedical applications of nanotechnology or using the nanotechnological approaches to maintain and improve human health at the molecular scale can be called as “nanomedicine”. The main goal of nanomedicine is to diagnose as accurately and early as fast as possible and to treat as effectively as possible without side effects, and to evaluate the efficacy of treatment noninvasively by using the nanotechnological approaches.

Mainly nanotechnological approaches in biomedical sciences can be categorized as diagnosis or treatment or therapy. Different types of nanomaterials and/or nanoplatforms can be used in both of these approaches such as nanoparticles, nanofibres, nanopores, nanowires, magnetic nanoparticles, metallic nanoparticles and quantum dots as QDots, dendrimers, nanotubes (i.e., carbon nanotubes,CNTs and peptide-protein nanotubes) etc.

System design and production by using mentioned nanoplatforms for diagnosis and therapy in biomedical applications can be categorized into three main sections such as nanocolloidal systems, surface modifications of biomaterials at molecular level and nanodevices.

One of the primary objectives of this investigation was to explore applications of nanomaterials to develop intelligent systems to treat complex diseases and enhance health-related quality of life (HRQL) index[2].

On the other hand biomedical applications of nanotechnology can be summarized as time-controlled and targeted release of drug regimen/gene delivery; diagnostic imaging; molecular diagnostics; bio-molecular detection; cardiac therapy; tissue engineering and nerve regeneration; dental care; orthopedics; ophthalmology; artificial tissues/organs; and antibacterial/ antimicrobial surfaces of biomaterials.

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The Raman Spectroscopy refer to the inelastic scattering of photons upon collision with molecules [1,2]. The scattering process may result with the photons’ energy gain or loss when interacting with the molecules. The photons’ energy change is reflected as the change in the photons’ frequency relative to the exciting laser light’s frequency. On Raman spectra, several bands pertaining to different vibrational modes of bonds in a molecule appear and are specific for a molecule, which is considered “fingerprint”. Raman scattering is inherently weak and a typical cross-section is in between $10^{-30}$–$10^{-25}$ cm$^2$ per molecule. When compared to a fluorescence cross-section, whose typical value is between $10^{17}$ and $10^{16}$ cm$^2$, Raman scattering is quite weak. Thus, in Raman experiments, large amount of molecules is necessary to achieve the same level of signal intensity in fluorescence. Therefore, it is necessary to have a powerful light source and very sensitive detectors for the measurement of Raman scattering. This is the reason why the development of sensitive Raman spectrometers is delayed until recently.

With the discovery of the enhancement in Raman scattering by Jeaninaire and Van Duyne and Fleischman in mid 1970s on noble metal surfaces [3,4], a technique called Surface-enhanced Raman scattering (SERS) was born and it is now considered an exceptionally powerful vibrational spectroscopic technique that competes with the highly sensitive techniques such as fluorescence and mass spectroscopy. The enhancement mechanism is now better understood and is considered due to the formation of surface plasmons on metal nanostructured surfaces upon interaction of laser light and the nature of chemical interaction of molecule with noble metal surfaces.

In a SERS experiment, it is necessary to consider a number of parameters for a healthy interpretation of spectra. A few are substrate nature, the laser wavelength, molecule size-substrate relationship, and chemical structure of molecule. It is now clear that the molecules or molecular structures must be as much as in contact with nanostructured surfaces to feel the surface plasmons. When a small molecule is considered, there is mostly no problem as long as the molecule remains in the same location and position near the plasmonic structure. However, when the size of a molecule increases, a special attention must be given to the size relationship between plasmonic structure and the molecules. A large molecule such as a protein or microorganism may not come into contact from all points with the nanostructured surfaces and the obtained SERS spectra remains limited to the parts of the molecule interacting with the surface of the plasmonic structure. Figure 1 shows an example SERS conditions for proteins. As seen, when protein/AgNP structure are well controlled, the SERS spectrum much improved with new spectral features as compared to uncontrolled protein/AgNP structures.

**Abstract**— The fundamentals of Raman scattering and surface-enhanced Raman scattering (SERS) are discussed. The requirement of bringing molecules and molecular structures in close vicinity of nanostructured surfaces of noble metals such as gold and silver in reproducible manner is one of the fundamental limitations of SERS. When the problem of generation of reproducible surfaces is considered, the design of a SERS experiment gains vital importance for healthy interpretation of results. In this lecture, after a brief introduction of Raman scattering and SERS phenomenon, the experimental parameters in play for analysis of biological molecules and structures such as DNA, proteins, microorganisms and living cells will be presented.
Photodynamic Therapy: New Directions

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Abstract—The current practice of photodynamic therapy and our contributions will be discussed.

Photodynamic therapy (PDT) is a noninvasive method of treating malignant tumors and age-related macular degeneration, and is particularly promising in the treatment of multidrug-resistant (MDR) tumors. The PDT strategy is based on the preferential localization of certain photosensitizers in tumor tissues upon systemic administration. The sensitizer is then excited with red or near infrared (NIR) light, generating reactive oxygen species (ROS) including singlet oxygen (¹O₂) and thus irreversibly damaging tumor cells. Current practice of PDT is limited to a few functionalized porphyrins, however these compounds are not considered to be ideal drugs for use in PDT. Among the limitations, the most prominent is the low extinction coefficient of porphyrins in the body’s therapeutic window (650–800 nm, low absorptivity region in typical mammalian tissues). As a consequence many research groups worldwide are engaged in efforts to develop better sensitizers. One important aspect is the tight control of the delivery of cytotoxic singlet oxygen to be produced. In our latest design[1], a sensitizer which behaves as an “AND” logic gate was proposed. Singlet excited state of the sensitizer dye can take a number of different paths for de-excitation (returning to the ground state), through competing processes. Among these processes, photoinduced electron transfer (PeT), intersystem crossing (ic), fluorescence (fl), non-radiative de-excitation (nr) are the most prominent ones. The rates of fluorescence or non-radiative process are not affected by the modulators such as Na⁺ ions increases the rate of singlet oxygen generation. H⁺ ions influence the same rate by a different mechanism, the added acid causes a bathochromic (red) shift in the absorption spectrum. This shift moves the absorption peak to the peak emission wavelength of the LED used in the excitation. Thus, the sensitizers are more efficiently excited when there is acid around. Although this is a proof of principle study, we firmly established the fact that, molecular logic holds a greater promise than previously recognized. They are not merely bad copies of semiconductor logic gates, they are molecular systems which have the potential to combine sensing, computing and actuation, all in sub-nm³ volume.

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CONFINING RESONANT PHOTONS TO THE NANO-GOLD LENGTH SCALE: THE NEW PROPERTIES AND APPLICATIONS IN MATERIAL SCIENCE, NANO-BIOLOGY AND CANCER NANO-MEDICINE.

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Abstract

New fields such as optoelectronics, sensors, nanocatalysis, nanomotors and nano-medicine use the new exciting properties 1-3 of gold and silver nanoparticles. Some of the most exciting properties arise when resonant photons are captured by these nanoparticles of the right size and shape. This excites the localized surface plasmon oscillation resulting from the coherent excitation of the free electrons in the conduction band. This greatly enhances the electromagnetic fields of the captured photon on the surface of the nanoparticle which strongly enhances their Radiative properties as well as that of any electronic system that falls within the range of this field. The effect of the coupling between close nanoparticles change their color (used as nano-ruler4), increase or decrease the Raman scattering intensity of adsorbed molecules5, enhance the nonradiative properties of near electronic systems like the relaxation of hot electrons in semiconductors6, the rate of exciton annihilation in conducting polymers7 or the rate of retinal photo-isomerization and proton pump in Bacterio-Rhodopsin photosynthesis8.

The strong Radiative properties of gold nano-particles are used in imaging and the sensitive detection of cancer cells in vitro9 and in-vivo11. The strongly absorbed photon energy is rapidly converted into heat. This localized heating of the gold nanoparticles can heat and destroy attached cancer (or sick) cells and is thus used in Vitro and in-Vivo cancer therapy 10,11. Very recently, non-photo-thermal techniques of using gold nano-particles in Cancer Therapy have been developed.12

References:

8. Arianna Biesso, Wei Qian, Mostafa A. El-Sayed, "Gold nanoparticle plasmonic effect on the retinal photo-isomerisation and the proton pump in the photocycle of the other photosynthetic system in nature, bacteriorhodopsin," Journal of the American Chemical Society, 130(11), 3258-+, (2008); 131,2442, 2009
**Advances (Innovations) in Neural Engineering**

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**Abstract:** Neural Engineering is a new discipline which unites engineering, computer science, physics, chemistry, and mathematics with cellular, molecular, cognitive and behavioral neurosciences, to understand the organizational principles and underlying mechanisms of the biology of neural systems, and to study the behavior dynamics and complexities of neural systems in nature. Therefore, it deals with many aspects of basic and clinical problems associated with neural dysfunction including the representation of sensory and motor information, the electrical stimulation of the neuromuscular system to control the muscle activation and movement, the analysis and visualization of complex neural systems at multi-scale from the single-cell and to the system levels to understand the underlying mechanisms, the development of novel electronic and photonic devices and techniques for experimental probing, the neural simulation studies, and the design and development of human-machine interface systems and artificial vision sensors and neural prosthesis to restore and enhance the impaired sensory and motor systems and functions from gene to system.

Furthermore, the neuroscience has become more quantitative and information-driven science since emerging implantable and wearable sensors from macro to nano and computational tools facilitate collection and analysis of vast amounts of neural data. Complexity analysis of neural systems provides physiological knowledge for the organization, management and mining of neural data by using advanced computational tools since the neurological data are inherently complex and non-uniform and collected at multiple temporal and spatial scales. The investigations of complex neural systems and processes require an extensive collaboration between biologists, mathematicians, physicists, computer scientists and engineering to improve our understanding of complex neurological process from system to gene.

To highlight this emerging discipline, we devote this talk to the neural engineering related research including the recent advances in neural prostheses, nano neuro-implants, neuro-robotics, cognitive engineering, neural signal and image processing and modeling, and brain computer interface from hardware to experimentation.
In-vitro Study of Interaction of Dopamine Analogues with the Most Bio-essential Metal, Iron

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Abstract-Dopamine has a significant role in the proper function of central nervous system which is responsible of body movements. Dopamine is a strong chelator of Fe, which is the most essential element in the all living systems. The stability of complex is evaluated by the formation constant values using a computational graphical program. Present study will explore interesting aspects about the interaction of dopamine and its analogous molecules with iron.

Iron is required for normal brain and nerve function through its involvement in cellular metabolism, as well as the synthesis of neurotransmitters and myelin. The disorder of the Central Nervous system, results from destruction of the substantia nigra causes Parkinson’s disease. It is a chronic ailment due to the progressive degeneration of those neurons that normally produce the neurotransmitter dopamine, leading to a dopamine deficiency in the basal ganglia. The treatment of this disease for most patients entails long term exposure to multiple agents, including dopamine receptor agonists, levodopa and carbidopa.

Dopamine molecules are combination of amino acid derivative (alanine), and a catechol analog. Catechols are reported to form stable complex with Fe2+ and Fe3+. Due to catecholic functional group dopamine assume to bind Fe2+/Fe3+ and may cause deficiency of this most required metal. Dopa of dihydroxyphenyl alanine is a precursor of dopamine and norepinephrine. It replaces missing dopamine in the brain [6]. It reduces the symptoms of Parkinson’s disease because it is changed into dopamine after it enters into the brain cells.

In the present study the complex formation of Levodopa, Methyldopa and Carbidopa (Parkinson’s disease drug components) with iron has been carried.

Iron formed intense colored complex with these molecules having absorbance maxima in visible region with high molar extinction coefficients were found. Stoichiometry of the examined complexes is explored by various methods including cyclic voltammetry. Kinetics of the said complexes will also be discussed in this presentation.

Dopamines chelates the iron strongly, there stability constants are evaluated by computational method were found in high range. pH effect on the complex formation will also provide valuable information.

It has been suggested that dopamine may exert a protective effect by chelating iron in dopaminergic neurons and that this system might be at fault in Parkinson disease.

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Improved Neuron Cell Adhesion and Proliferation on Engineered Surfaces


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Abstract— We report our preliminary investigations of the influence of the topography on neuronal growth and the formation of neuronal networks. In this work neurons isolated from rat dorsal root ganglion (DRG) were cultured on Al/Al2O3 core/shell nanowires by CVD of [\text{\text{BuOAlH2}}]$^2$ and femto second (FS) laser treated surfaces. Both nanostructures supported neuron cell adhesion and axonal growth. Isolated neuron clusters were observed along the FS laser treated surface.

Since nanotechnology evaluates materials which are in the range of nanometers, there are a lot of similarities between nanophase materials and elements of biological organs [1]. Especially, the development of surfaces which improve the biocompatibility and cell-substrate adhesion is of great interest in bio-applications. The high surface area to volume ratio of the one dimensional (1D) nanostructures combined with their micro porous structure favors cell adhesion, outgrowing, and differentiation, all of which are greatly desired properties for tissue engineering application [2]. For instance, 1D structures such as micropette electrodes and micro fabricated electrodes are well established tools to explore the electrical behavior of individual neuron and neuronal network [3]. Interest in carbon nanotubes (CNTs) has been growing exponentially due to their exceptional electrical, mechanical, and surface properties since CNT was discovered [4]. Furthermore such as carbon nanotubes or fibers hold much promise for orthopedic and neural applications [5]. Despite this promise, their properties in biochemical and biological environments are however less known as is the biocompatibility of the material. For those reason aluminium oxide is a good candidate for this area because of its excellent biocompatibility. There is increasing interest in use of alumina nanofibers in tissue engineering since they provide unique biocompatibility with a high surface-to-volume ratio. Webster et al. demonstrated that the alumina nanofibers enhanced the cell adhesion and synthesis of osteoblast phenotypic markers [6]. On the other hand, using cells alone, a scaffold provides a 3D matrix on which the cells can proliferate and migrate, produce matrix, and form a functional tissue with a desired shape. Veith et al. showed that while osteoblast adhesion is increased on Al2O3 core/shell nanowires, the fibroblast adhesion reduces on the same surface [7]. This indicates clearly that different cell types show diverse responses to the topography independent from bulk biocompatibility of the material. Current research in this area is driven towards the fabrication, characterization, and applications of nanostructured systems as scaffolds for tissue engineering.

In this current work we present the synthesis of Al2O3 core shell nanowires by chemical vapour deposition (CVD) of a single precursor [\text{\text{BuOAlH2}}]$^2$ on glass substrates. Afterwards such surfaces were treated by FS laser. Laser modification leads to the oxidation of the Al core within the nanowires. This opens up a possibility to fabricate different morphologies of alumina. In case of polymer like surfaces laser treatment yields both chemical and physical changes on the surface. In our approach we aim altering only topography while keeping the surface chemistry identical. In this context, this approach is beneficial for studying the topography effect on the cell response. Depending on the preparation, different topography was synthesized with hydrophilic nature. Following the detailed surface characterizations, the neuron cells isolated from dorsal root ganglia (DRG) of Neonatal Sprague-Dawley rats were seeded on both substrates and the interaction of neuron cells with the nanostructured surface was studied in detail.

It is observed that neurons formed dense networks on both Al2O3 nanowires and FS laser treated surfaces compared to control glass substrates. Significantly enhanced attachment of isolated neuron clusters were observed on FS laser treated area and similarly neuron activity increases on FS laser treated Al2O3 nanowires. Especially, different than as-deposited layers, laser treated nanowires lead to alignment of neurons.

References:
Nanoparticles as Drug Carriers for the Treatment of Skin Diseases

Abstract-Polymeric nanoparticles and liposomes were used in the design of a drug delivery system for the treatment of skin diseases. Both types of nanoparticles were able to encapsulate the bioactive agents and did not present any adverse effects in vitro. Polymeric particles penetrated the cell membranes and accumulated near the nucleus. The nanoparticles prepared in this study revealed a great potential for use in gene therapy in addition to the personalized skin treatment for which they were originally designed.

Economics and health aspects of skin problems are rapidly becoming major issues in Europe, following the remarkable extension in life expectancy in western countries together with the increased awareness of UV radiation risks. The aim of this study was to develop nanocarriers as drug delivery systems to achieve personalized treatment of selected skin disorders like psoriasis, contact dermatitis, and UV related photo-aging.

Retinyl palmitate (RP) and Dead Sea Water (DSW) (AHA VA, Israel) were selected as the bioactive agents and loaded into nano and microcarriers. Poly(lactic acid-co-glycolic acid) (PLGA) (50:50), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (HV 8% molar) and (HV 5% molar) nanocapsules and nanospheres were prepared by w/o/w emulsion techniques, respectively. Phosphatidylcholine (PC): Cholesterol (CHOL): RP (7:3:1 molar) were used in the preparation of DRV s. Dicetylphosphate (DCP) and phosphatidyl ethanolamine (PE) were used to prepare negative and positive charged DRV s, respectively. Encapsulation efficiency, size distribution, and release kinetics of the carriers were determined. Presence of liposomes was verified with fluorescence microscopy after applying gel permeation chromatography (Figure 1a).

SEM micrographs of PHBV and PLGA capsules revealed that particles with smooth surfaces suitable for use as carriers of drugs are obtained (Figure 1b, 1c).

Size distribution of PHBV and PLGA particles were found to be in the range of 200 nm - 10 μm. Diameter of RP loaded and DSW loaded PHBV capsules were 4.3±0.1 and 6.3±0.0 μm, respectively and for PLGA these were ca. 6.5 μm. Size of DRV s were calculated as 200 n.m. DSW encapsulation efficiency of particles was determined with a chloride specific electrode and ionmeter (Cole-Parmer Instrument Company, USA) as 4.0% and 5.5% for PHBV8 and PHBV5, respectively. For DRV s rehydration was done with DSW and encapsulation efficiency was calculated as 15%.

Uptake of polymeric capsules was studied in vitro with human osteosarcoma cells (Saos-2). It was observed with Nile red stained PHBV and PLGA capsules that the particles were located near or on nuclei implying that they could also serve as carriers of agents for gene therapy because they seem to be able enter the cells (Figure 2).

Figure 1. SEM and Fluorescence micrographs of carriers. (a) Neutral DRV s, (b) PHBV5, (c) PLGA.

Figure 2. Fluorescence micrographs of nanocapsules incubated with Saos-2 cells. (a) PHBV5 (4 h) (x40), (b) PHBV5 (24 h) (x40), (c) PLGA (4 h) (x20).

Hemolytic activities of DRV s, PHBV and PLGA particles were studied and according to the released hemoglobin absorbance at 540 nm, the particles do not have any hemolytic activity.

L929 cells were used in the toxicity study; their proliferation rates were determined by MTS test. Cells were treated with medium containing different amounts of liposome suspension. DRV s had no effect on the cell proliferation rate (Figure 3).

Figure 3. Cell toxicity test of DRV s.

Polym eric nanoparticles taken up by the cells could be an advantage in medical applications such as gene therapy due to their penetration ability. Absence of hemolytic activity indicates the suitability of these particles to use in drug release studies. The variety of the systems prepared and the different release rates and stabilities of the bioactive agents will allow the patient to have a personalized treatment.

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Smart Golden NanoCapsules for Controlled Release with Near-Infrared Light

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Abstract- We explored the laser triggered release of an anti cancer drug, doxorubicin, from gold nanocages to destruct the breast cancer cells via using different duration of a constant power density of laser beam. Upon laser irradiation with increasing exposure time, a remarkable increase in the number of deaths of cells was observed.

Recently, the photothermal therapy has attracted the interest, mainly the use of lasers for thermally destruction of tumors or cancers [1]. The near infra-red (NIR) laser light can penetrate to the deeper tumor tissues and be absorbed by the photosensitizers without damaging the healthy cells due to the high transparency of soft tissue in the NIR region. Injectable NIR photosensitizers such as gold nanoshells, nanocages, and nanorods have been applied as cancer therapy agents to absorb the low energy laser light and convert it into heat which will destruct selectively and efficiently the tumor cells [2-4]. Despite of the powerful photothermal cancer therapy, these NIR absorbers suffer from the following obstacles; i) the requirement for continuous high power NIR irradiation, ii) exposure to radiation for extended periods and iii) the use of high concentration of gold nanostructures.

Thermosensitive smart hydrogels, poly(N-isopropyl acrylamide) (polyNIPAAm) in this case, show a reversible volume phase transition from expanded form to shrunken one upon heat treatment. These materials were incorporated with gold nanocages (AuNCs) (shown in the inset of Figure 1) which were designed to strongly absorb NIR light. Upon irradiation, the AuNCs absorbed the light and then converted to heat which initiates the volume phase transition process via temperature change in the surroundings. In this paper, we demonstrated a laser-triggered controlled release of doxorubicin (Dox), a cancer drug for breast cancer, from polyNIPAAm-covered AuNCs.

Disulfide- and thiol-based molecules are well-known for their strong binding to the gold surfaces. Disulfide containing polyNIPAAm was synthesized according to literature report by using disulfide initiators in atom transfer radical polymerization technique which result one disulfide group in every chain of polyNIPAAm [5]. Excess amount of polyNIPAAm was mixed with AuNCs in order to replace the polyvinylpyrrolidone (PVP) on the surface. After exchanging process, free PVP and extra polyNIPAAm were discarded during washing steps.

The release process was demonstrated with a real cancer treatment, breast cancer cells, by using Dox, well-known commercial chemotherapy cancer drug. In this study, the breast cancer cells were transferred to 24-well plates. Dox-loaded AuNCs solution was injected to the rows of well plates except two rows which was used as controls. After the irradiation experiments were done, the solutions from the well plates were replaced with MTT assay and medium solution.

Irradiation duration dependent release of Dox with constant power density of the laser beam at 20 mW/cm² was demonstrated as shown in Figure 1. The breast cancer cells without both adding AuNCs and irradiation of laser beam were used as a reference written “control”. The laser exposure without AuNCs, called “laser control”, gave very small deaths of cells could be due to a small heat generation during the laser process.

A small number of deaths of cancer cells after treating with Dox-loaded AuNCs without irradiation, named “gold nanocages control”, were observed. This can be due to remaining very small amount of extra Dox in the solution after washing steps. On the other hand, the first fifteen seconds irradiation was the huge difference in cell death as compared to the controls due to the release of Dox from AuNCs. Further irradiation with a longer time (30 and 60 seconds) gave more release of Dox from AuNCs with a decreasing slope due to less remaining Dox inside the AuNCs.

In summary, the gold nanocages (AuNCs) were functionalized with polyNIPAAm which can be used as drug delivery devices with a remote control release capability. The AuNCs are strong absorbers of NIR laser beam and then they converted to heat which makes again the shrinking of polyNIPAAm. Laser triggered release of Dox was demonstrated with different duration of a constant power density of laser beam. Depending on the duration of irradiation, the deaths of cells were observed which were treated with Dox-loaded AuNCs. These results suggest that AuNCs are a promising new class of drug delivery devices for cancer treatment. This work was supported by a 2006 Director’s Pioneer Award from the NIH (DP1OD000798).

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References:
**Biosensing platform based on the manipulation of functionalized superparamagnetic beads**

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**Abstract**—Highly sensitive and rapid detection of DNA hybridization, anti-body-antigen type coupling, and other biorecognition processes within less than two minutes.

Points of care protocols using ferrite nanometer sized magnetic beads (labels/tags) are being developed for early diagnosis of life threatening diseases. Target biomolecules are ‘tagged’ with functionalized superparamagnetic beads of less ~250 nm in diameter. The ‘tags’ are detected using magnetic sensors or optical transmission. Our approach enables the highly sensitive and rapid detection of DNA hybridization, anti-body-antigen type coupling, and other biorecognition processes within less than two minutes. Our approach overcomes the limits of conventional ‘fluorescent’ based biosensing, and has a wide range of applications including early detection of biomarkers for prostate cancer, and development of drugs by the pharmaceutical industry.

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Figure: Rotating magnetic bead biosensor (left) and quantitative measurement of avidin concentration (right)

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Investigation of Shell Characteristics of Contrast Agents for Ultrasonography

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Abstract – Contrast agents for ultrasound such as microbubbles have been proven to be very efficient in many clinical studies to enhance the image quality in ultrasonography. Contrast agents comprise micron-sized bubbles of gas stabilized by a shell of biocompatible material. The shell characteristics of such contrast agents needs to be understood for their in-vivo effectiveness and storage stabilities. In this study, the effects of shell composition, various salt concentrations and ionic strengths on the formation and morphology of monolayers were investigated.

Ultrasound relies on the difference in acoustic impedance at the interface between two different types of cells to produce the image. In a situation where there is a small impedance mismatch, such as a tumor and healthy cell, administration of contrast agents can provide contrast enhanced image (Fig.1), enabling the clinicians to better visualize the organs and tissues for early and accurate diagnosis of diseases [1-3]. In contrast to conventional CT or MRI agents, the ultrasound contrast agents do not diffuse into the extra cellular fluid compartment. Ultrasound contrast agents are administered intravaneously to the systemic circulation during examination. Upon destruction, the gas is eliminated by exhalation via the lungs, and the shell material is metabolized through the kidney and spleen. The use of microbubbles is highly encouraged by FDA in clinical settings when needed because of their advantages compared to other imaging modalities [4].

Figure 1. Image of the tumour under ultrasound (a) before and (b) after administration of microbubbles [1].

Design of stable and robust contrast agents, however, remain as a critical issue because microbubbles should be able to circulate in the blood stream without bursting and also resist shear forces and pressure differences in arteries until the imaging is complete [1,3]. The shell material is composed of monolayers of DSPC (1,2-diacyl-sn-glycerol-3-phosphatidylcholine) as the main lipid component and polyoxyethylene 40 stearate (PEG40 S) as the emulsifying agent. The effectiveness of microbubbles can therefore be correlated to the magnitude of intermolecular cohesive forces between monolayer components and degree of packing of components around the gas bubble. Effect of mixing ratio of lipid:emulsifier and electrolyte solution on monolayer packing was investigated using Langmuir-Blodgett (LB) film technique on different subphase solutions. The monolayers were also transferred to a solid substrate and characterized by atomic force microscopy (AFM).

Figure 2 shows the surface pressure-area isoterms of DSPC and PEG40 mixture both in pure water and phosphate buffered solutions at pH=7.2. DSPC:PEG40 S ratio (a) 9:1 and (b) 8:2

Figure 3 shows the topographies of the mixed monolayer films of DSPC and PEG40 S at different ratios and on different subphases at 5 μm in the tapping mode.

These results indicated that DSPC:PEG40 S mixture with a ratio of 8:2 forms more homogeneous and compact monolayer than that of 9:1 both in the pure and buffered solution at 110 mM salt solution. Further discussions will be given during the presentation.

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Nanoscale Cues and Astrocyte Responses in Neural System Regenerative Medicine

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Abstract—The goal of regenerative medicine is to create a scaffold which is biomimetic for the native attachment environment of a given cell group, which, prompted by nanoscale cues, re-colonizes the scaffold. In the present investigation, astrocyte responses are correlated with the mechanical, topographical and biochemical cues of a prosthetic environment.

Recent efforts in the field of regenerative medicine have yielded impressive successes [1]. The basis of regenerative medicine is to create a scaffold which is biomimetic for the native extracellular matrix (ECM) of a given tissue and to then expose it to a desired cell population, which may contain adult, embryonic or pluripotent stem cells. If the scaffold has the correct nanoscale cues, the cells will naturally re-colonize the prosthetic ECM matrix and resume normal functions. Fundamental understanding is still needed to design scaffolds with appropriate mechanical, topographical and biochemical cues for particular cell classes. Understanding the nature of the physical interactions of cells within these biomimetic structures and deriving information that would correlate mechanical, topographical and biochemical properties of the scaffolds with the promotion of specific cellular responses would have a major impact on their design and utility.

This study is focused on astrocyte response to different types of scaffolds. Astrocytes are members of the neural cell system that provide nutrients to and remove wastes from neurons [2]. They also provide mechanical support for neurons both directly and through the expression of interstitial extracellular matrix molecules. Furthermore, astrocytes express directive growth factor proteins that promote neuron axon growth and alignment, resulting in healthy synaptic transmission. Astrocytes are themselves in contact with a dense ECM termed the basement membrane, which surrounds each capillary at the blood-brain barrier. We will report our recent research that indicates that astrocytes respond strongly to the mechanical, topographical and biochemical cues provided by the native, or by a prosthetic, ECM.

The prosthetic scaffold currently under investigation is a synthetic electrospun polyamide dense nanofibrillar matrix that has demonstrated promise for the repair of the injured spinal cord and is architecturally mimetic for the capillary basement membrane at the blood brain barrier [3]. The mechanical, topographical and biochemical cues of the prosthetic nanofibrillar matrix are investigated at the nanoscale using atomic force microscopy techniques, including a new technique developed by this group, scanning probe recognition microscopy [4], which enables property measurements along individual nanofibres within a scaffold, followed by compilation of results into statistical representation for the nanofibrillar matrix as a whole.

The astrocyte responses to the nanoscale cues presented by three different substrates are investigated. The substrates are: an unmodified nanofibrillar matrix (NANS), a surface activated nanofibrillar matrix (SANS) which been treated with a polyaniline coating, and a nanofibrillar matrix which has been covalently modified with the directive growth factor fibroblast growth factor-2 (FGF-2). A fourth 2D plastic substrate is used as a control.

Atomic force microscopy images (tapping mode) that show details of the topographical differences amongst the nanofibrillar substrates are shown in Figure 1. The topological differences translate into several physical differences. As further shown in Figure 1, the frictional environment is changed by the addition of the growth factor. We have recently reported additional nanoscale physical changes in surface roughness and elasticity caused by the FGF-2 growth factor modification [5]. Astrocyte responses to nanofibrillar versus 2D substrates include both differences in cell morphology and, significantly, up-regulation of FGF-2 expression by astrocytes. The latter induces longer and more branched axon development by neurons in co-culture [6].

Figure 1. AFM investigation of unmodified, coated, and FGF-2 growth factor modified nanofibrillar matrices shows topographical differences. Friction, assessed by lateral force microscopy, is one of several changed nanoscale physical properties.

Friction, surface roughness and elasticity have all been recently shown to influence cell morphogenesis and differentiation Therefore, when performing growth factor modification of prosthetic tissue scaffolds to achieve a desired biochemical directive goal, it is necessary to consider the changed nanoscale physical environment presented to re-colonizing cell groups.

Nanofibrous scaffolds for repair of cranial bone defects

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Abstract-The objective of this study was to evaluate reconstruction of critical-size (non-self-healing) calvarial bone defects using electrospun nanofibrous poly(caprolactone) scaffolds. Electrospinning was used to fabricate biodegradable nanostructured matrices from blends of high and low molecular weight poly(ε-caprolactone) (PCL). The matrices were spiral-wound to reach 3D structure. The matrices were implanted in cranial bone defects of rats either plane or with stem cells or with the drug simvastatin. This presentation reviews the results of a series of experiments demonstrating the performance electrospun biodegradable matrices in the repair of cranial bone defects.

Electrospinning is a unique method that produces polymer fibers with usually diameters in nanoscale and non-woven fibrous structures compose of these fibers using electrically driven jet of polymer solution or melt. The electrospinning method has been used for the nanofibrous fabrication from many biodegradable natural/synthetic polymers such as collagen, poly(α-hydroxy acids) [1]. Due to unique properties of the materials produced from these nanofibers, several novel applications in biomedicine including tissue engineering, controlled release systems, soft and hard tissue repair materials have been proposed [2,3,4]. In this work, we used electrospun biodegradable 3-D nanofibrous PCL matrices in the repair of cranial bone defects.

PCL with two different molecular weights (high and low) were synthesized and the blends of these two were electrospun into nonwoven membranes composed of nanofibers (Figure 1a). The matrices were then spiral wound to produce scaffolds with 3D structures (Figure 1b). 8 mm diameter critical size cranial defects were created in rats and scaffolds in the same size of the defects were implanted into these defects (Figure 1c). Reconstruction of the cranium and bone formation were evaluated 1, 3 and 6 months post implantation. Bone regeneration, the amount and mineralization of newly formed bone was evaluated by using x-ray microcomputed tomography (microCT) analysis. Tissue response to the biomaterial and defect healing was histologically evaluated.

In vivo results indicated osseous tissue integration within the electrospun PCL matrices (Figure 1d) and functionally stable restoration of the calvarium. MicroCT analysis results found that degradeable release of the drug simvastatin from PCL scaffolds, promoted effective bone mineralisation within the defect. A significant ingrowth of connective tissue cells and new blood vessels allowed new bone formation especially in the group of scaffolds used with stem cells.

This study demonstrated that biodegradable 3D electrospun PCL scaffold may be used to deliver simvastatin or combined with stem cells and enhance bone formation within critically-sized defects in vivo and offers new possibilities in the functional and aesthetic reconstruction of craniofacial defects.

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Nanoparticle drug delivery systems for Topoisomerase I anticancer drugs

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Abstract—The current study investigated stability, release and in vitro bioactivity of CPT- anticancer drugs- iron oxide micro and nanoparticles. We achieved CPT anticancer drugs- magnetite micro and nanoparticles with nearly 100% lactone form and sustained release profile. In addition, we obtained prominent and superior in vitro cytotoxic activity than free drug in the representative study.

The research that has been conducted on camptothecin (CPT) anticancer drugs (Camptothecin, Topotecan, Irinotecan, SN 38) has shown that there is significant antitumor activity involving various tumors including lung, ovarian, breast, pancreas, and stomach cancers. However, the lactone ring opening causes a reduction in cytotoxic activity and severe side effects to physiological conditions (pH:7.4, 37°C), which is due to decreased cell membrane binding, decreased membrane diffusibility, and decreased intrinsic potency against the topoisomerase target (Scheme 1). Although, various chemical modification approaches and different CPT anticancer drug delivery strategies were applied for improved chemotherapeutic activity, targeted and controlled release, improved stabilization of the lactone moiety, and solubility in water, the handicaps of CPT and the use of its analogs in clinical conditions that result from the lactone ring opening have not yet been overcome.

In order to contribute to the removal of these handicaps, this study investigated the stability, release, and in vitro bioactivity of CPT anticancer drugs. Several types of iron oxide micro and nanoparticles with various surface properties were synthesized without changing the magnetic properties of the particles. The fluorescence emission properties of drug containing magnetoliposomes was measured in phosphate-buffered saline (pH 7.4). In vitro release of CPT and TPT-HCl from magnetoliposomes were analyzed by membrane dialysis methods against phosphate-buffered saline (PBS, pH:7.4) at 37°C and monitored by fluorescence spectroscopy. The methods of dynamic light scattering (DLS), fluorescence microscopy (FM), and transmission electron spectroscopy (TEM) were used to determine the physical properties of the nanoparticles.

The analysis of encapsulated CPT anticancer drugs illustrated that over 99% of the lactone form was protected in the magnetic particles and the release of CPT anticancer drugs from the magnetic micro and nanoparticles lasted for 9 and 4 days, respectively. In addition, in a representative in vitro cytotoxicity study, an approximately 65% free CPT anticancer drugs concentration was sufficient to inhibit 100% of the cell growth of three different human liver carcinoma cell lines when we used CPT encapsulated magnetite microparticles. Therefore, the iron oxide formulation is ideal for achieving stable and sustained CPT delivery in order to overcome its handicaps in clinical conditions.

Figure 1. In vitro release profiles of CPT-loaded OA-PL stabilized magnetite micro (A) and nanoparticles (B) in a PBS (pH 5, 0.01 M) solution at 37°C.

Figure 2. a) CPT and TPT magnetoliposomes. b) Camptothecin encapsulation in oleic acid-pluronic F127 coated iron oxide formulation.

Production of Zeolite Including Microfluidic Channels for Advanced Biosensor Applications

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Abstract—In recent studies, most of the researchers suggesting the usage of microfluidic systems in biosensors for the accuracy of detection, reduction of the sample volume, a fast response time, and for their capabilities of being used as portable and disposable chips. Also zeolites are lately being investigated and being used in nanotechnology applications due to their high surface area per volume ratio, stability and biocompatibility. It is believed that the combination of both zeolites and microfluidic systems will result improvements in biosensor applications. In this study, mono-double layers of zeolite microfluidic channels with dimensions of ~35 x 100 μm were formed on Si wafer by the combination of e-beam lithography and direct attachment techniques for the first time. These studies are believed to open new routes for more advanced applications in this area.

Integrating nano-microparticles as well as biomolecules onto different compounds and forming ordered crystalline films are of great interest for multipurpose systems in the area of electronics, optoelectronics, sensor, and biomedical applications. Controlling the position of these elements on the finalized system is crucial so that the integrated system will serve for its designed purpose. The real challenge is to assemble nanoelements, which have many useful properties by themselves, into different compounds for manufacturing advanced materials. The structural order with a broad range of diversity, which exists in those nanoelements can be hypothesized to bring multi-functionality and enhanced selectivity to the manufactured device.

Zeolites are known with their structural order in nanometer scale. Typically, zeolites are crystalline aluminosilicates consisting of an anionic framework and charge-compensating cations [1, 2]. Their 3-dimensional (3D) framework is build up by corner-sharing TO₄ tetrahedra (T = Si, Al) leading to materials featuring defined channels and cavities in nanometer scale. This high degree of open porosity gives rise to an exceptionally high-surface area. In addition to this, zeolites are preferred due to biocompatibility, high mechanical, thermal, and chemical stability, large surface areas, and unique hydrophilic and electrostatic properties. However, the fact that the synthesized zeolites are in powder form restricts their potential usages in advanced application areas. The most important aspect to make zeolites’ application areas more efficient in that sense is to assemble the micron and nano sized zeolites on substrates in a controlled manner. Such control might lead to brand new research objectives in order to obtain more efficient biosensors over zeolite thin films.

A facile and efficient method in order to assemble zeolites for the purpose of generating zeolite nano-micropatterns on the Si wafer is e-beam lithography (EBL) technique, which does not require the use of chemical linkers [3]. For that purpose, in the current study microfluidic channels were produced using poly(methyl methacrylate) PMMA as a photosresist on the Si wafer combining EBL and direct attachment methods for zeolite assembly (Figure 1).

The channel dimensions were ~35 x 100 μm. It was shown that zeolite patterns with 2.5 μm in width can be efficiently formed of mono-multilayers.

Figure 1. Zeolite microchannel production procedure

In summary, it was shown that integrating zeolites of nano to micron sizes onto different compounds was feasible by the shown technique. It is believed that the production of zeolite microchannels with different zeolites and different patterns will improve the multipurpose use, sensitivity and detection limits of biosensors.

This study was partly supported by Scientific and Technical Research Council of Turkey (TÜBİTAK) and partly by a European Union project with the project number PIRSES-GA-2008-230802. The support provided by METU-Central Laboratory is greatly acknowledged.

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[2] (a) H. Robson and K. P. Lillerud, Verified Syntheses of Zeolitic Materials, 2nd revised edn, Elsevier, Amsterdam, 2001 (see also: www.iza-structure.org);
Resonant Nano-Biosensor for Multi-Analyte Screening using optical MEMS

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Abstract— A biosensor is developed for the real time multi-analyte screening point-of-care diagnostics applications. Biological mass is sensed by monitoring the shift in the resonance frequency of the microcantilever arrays. Both the actuation and sensing are remote and wireless, making it attractive as a low-cost disposable and portable biosensor platform. The cantilevers are suitable for thin film magnetic actuation. The optical interference principle is utilized for sensing with sub nanometric precision. With closed-loop control of the oscillations, magnetic actuator optimization, and the electronic compensation of the coil, resonant deflection of the microcantilever and the signal to noise ratio (SNR) are improved significantly. Current application focus is on detection of Hepatitis biomarkers and increasing the selectivity of the sensors.

Progress in sensing methodologies and sensitivity leads to real-time parallel multi-analyte screening with low cost, power efficient, miniaturized, portable, remote platforms. Micro-cantilever arrays which are fabricated through single mask lithography are excellent transducers for biological mass sensing [1].

The concept drawing of the point-of-care (POC) diagnostics system developed in this research is illustrated in Figure 1. Current application focus is the detection of Hepatitis markers by monitoring the shift in the resonance frequency of an array of cantilevers. Figure 2 illustrates the double-layered (Nickel and Gold) T-shaped microcantilevers with diffraction gratings which are suitable for thin film magnetic actuation and monitoring deflection from the optical interference. This platform is superior to conventional biosensor platforms since the multi-analyte screening is remote, wireless and free from the electrostatic actuation problems such as stiction and charging.

Biosensing requires operation in viscous environments, where SNR, quality factor of the arrays and, therefore, the sensitivity decrease substantially. In this work, SNR is enhanced by amplifying the deflection of cantilevers. In order to increase deflection of the nickel cantilevers, in and out-of-plane AC magnetic field generated by the electrocoil is amplified by electronic compensation. Current through the coil is tabulated in Table 1 and shows that electronic compensation increases current by a factor of 10 at 500kHz with less than 1/20 of the coil voltage of the uncompensated case. After choosing the best geometry of the coil, two magnets are placed and optimized to generate a DC magnetic field.

Table 1. Current through the coil with and w/o compensation

<table>
<thead>
<tr>
<th>Current (rms) [mA]</th>
<th>Frequency [kHz]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>(V_{coil} = 20\text{V (pk-pk)}) No compensation</td>
<td>8</td>
</tr>
<tr>
<td>(V_{coil} = 700\text{mV (pk-pk)}) Compensated</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 2 shows the amplification of deflection of a cantilever, resonating at 261kHz, by a factor of 100 with compensation and magnetic optimization.

Table 2. Amplification of deflection of a cantilever

<table>
<thead>
<tr>
<th>Deflection (pk-pk) [nm]</th>
<th>No Compensation</th>
<th>Compensated</th>
<th>Magnetic Optimization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0,23</td>
<td>5,2</td>
<td>23,3</td>
</tr>
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</table>

Using closed-loop control with optical feedback, the brownian motion observed from the optical interference is amplified and phase shifted for self-oscillation of the cantilevers. This approach also enables operation of multiple microcantilevers using a single drive coil. Using this method, the quality factor is increased 80 times in water and 200 times in air [2].

The biosensing with this platform allows for real-time monitoring as the readout circuitry can operate much faster than the biological mass binding events.

Using closed-loop control of the oscillations, magnetic actuator optimization, and the electronic compensation of the coil, resonant deflection of the microcantilever and the signal to noise ratio (SNR) are improved significantly.
Studying Activity Of A Water Origin Antibacterial Product Of Nanosilver Solution Which Constituted By Nano Technologies In The Critical Areas Of Cardiac Surgery Operating Room And Forensic Science Institution

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Abstract. Nanotechnology is a most promising field for generating new applications in medicine. However, only few nanoproducts are currently in use for medical purposes. A most prominent nanoproduct is nanosilver. Silver compounds and ions are historically recognized for their effective antimicrobial activity. At nanoscale, silver exhibits remarkably unusual physical, chemical and biological properties. Nanosilver particles have been applied as a biocide in many aspects of disinfection, including healthcare products. Due to its strong antibacterial activity, nanosilver coatings are used on various textiles and surfaces. Recently silver nanoparticles have come up as a potent antimicrobial agent and are finding diverse medical applications ranging from silver based dressings to silver coated medical devices and marketed as a water disinfectant to room spray for aerosolize usage. Disinfectant resistance of microorganisms and short influence time of potent products is one of the major problems faced in the field of sterilization. In the conducted study all the culture samples have been taken and proliferated agents are recorded before the application of nanosilver particles to the three different area (Intensive care unit, operating rooms, microbiology laboratory). We demonstrated in this study, bacterial culture results of these areas were still sterile in monthly controls within the six months period. The study results suggested that these nanosilver solutions could be used for sterilization of critical areas such as operating rooms, intensive care and transplantation units.

Keywords: colloidal silver, nanosilver, antibacterial solutions, critical area disinfection.

PACS: 87.85.Rs

INTRODUCTION

There has been an increased awareness for the role of contaminated environmental surfaces within the home and health care settings as a potential vector of disease transmission. The use of routine disinfection procedures has been used to address this issue and has contributed significantly in the prevention of the spread of infectious disease. The effectiveness of these standard disinfection technologies on product application is well-characterized. However, microbial contamination of environmental surfaces occurs more frequently than standard disinfection habits and practices within residential and institutional settings can prevent(1). Surface disinfection and decontamination provide temporary amelioration against bacterial colonization. Disinfected surfaces eventually become contaminated, thus, mitigating the benefit of the initial disinfection. Contaminated surfaces can act as a reservoir for pathogenic microorganisms and potentially exacerbate the risk of Infection in the critical areas such as operating room, intensive care unit. As a result, environmental surfaces can harbor bacteria and other pathogens, and, consequently, contaminated surfaces have been linked to disease transmission. Many disinfectants contain nonvolatile antimicrobial agents such as a quaternary ammonium compound (QAC) that can leave an antimicrobial residue on treated surfaces. The potential of these agents to prevent bacterial colonization is limited because of their lack of substantivity, or ability to persist on surfaces after some environmental insult, such as water contact or rubbing. These moist environments and physically contacted surfaces are most likely to be contaminated, to allow bacterial proliferation, and to act as a pathogenic reservoir(2). For a disinfectant technology to realize a significant residual antimicrobial benefit, it must persist under
Self-assembled alkyl-sulfide monolayers on Au(111): an ab-initio study

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Abstract. We describe how computational modeling based on ab-initio molecular dynamics, combined with experimental data, has been applied to solve the long-standing problem of the adsorption geometry of self-assembled monolayers of alkyl sulfides on the Au(111) surface.

Self-assembled monolayers of alkyl sulfides (-S-(CH₂)n-CH₃) on gold surfaces have potential applications in molecular electronics, biosensors, and nanopatterning.

In the high-coverage regime, the molecules are anchored to the metal substrate through their sulfur termination, and the alkyl chains point away from the surface. Interpretation of conductance data has been hampered by the insufficient characterization of the atomic structure of the sulfide-gold interface and in particular of the role of disorder. Molecular dynamics is ideally suited to explore the phase space associated with molecular adsorption, particularly when the interatomic potential is determined “ab-initio”, i.e. by solving the quantum mechanics of the electrons on the fly during the atomic dynamics. Here we will describe how computational modeling based on ab-initio molecular dynamics, combined with experimental data, has been applied to solve the long-standing problem of the adsorption geometry of the sulfides on the Au(111) surface. The analysis of molecular dynamics trajectories and the relative energies of possible interface structures suggest a competition between molecular ordering, driven by the lateral van der Waals interatomic forces between alkyl chains, and disordering of interfacial Au atoms, driven by the sulfur-gold interaction.

We find that the atoms of the sulfur molecules bind at two distinct surface sites, and that the first gold surface layer contains gold atom vacancies as well as gold adatoms that are laterally bound to two sulfur atoms. Our study suggests that Au adatoms and vacancies play an unexpected but fundamental role [1,2].

These findings strongly underscore the importance of the underlying Au-S interactions and support some recent single-molecule conductance measurements of the Au-dithiol system that have shown junctions differing in space and time because of both static and dynamic disorder. As a consequence of these findings, studies regarding the formation, growth, diffusion, and mechanical properties of these films may need to be revisited in order to properly account for the influence of Au-S interactions and the presence of the sulfide-Au-sulfide structural motifs. From a theoretical perspective, this gives paramount importance to the development of empirical potential models that include not only molecule-molecule interactions but explicitly the Au-sulfide interactions, which are often neglected. In addition, our findings indicate that the adatom structures will alter the local density of states at the Fermi energy and will affect the interpretation of electronic and magnetic properties of these materials.

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