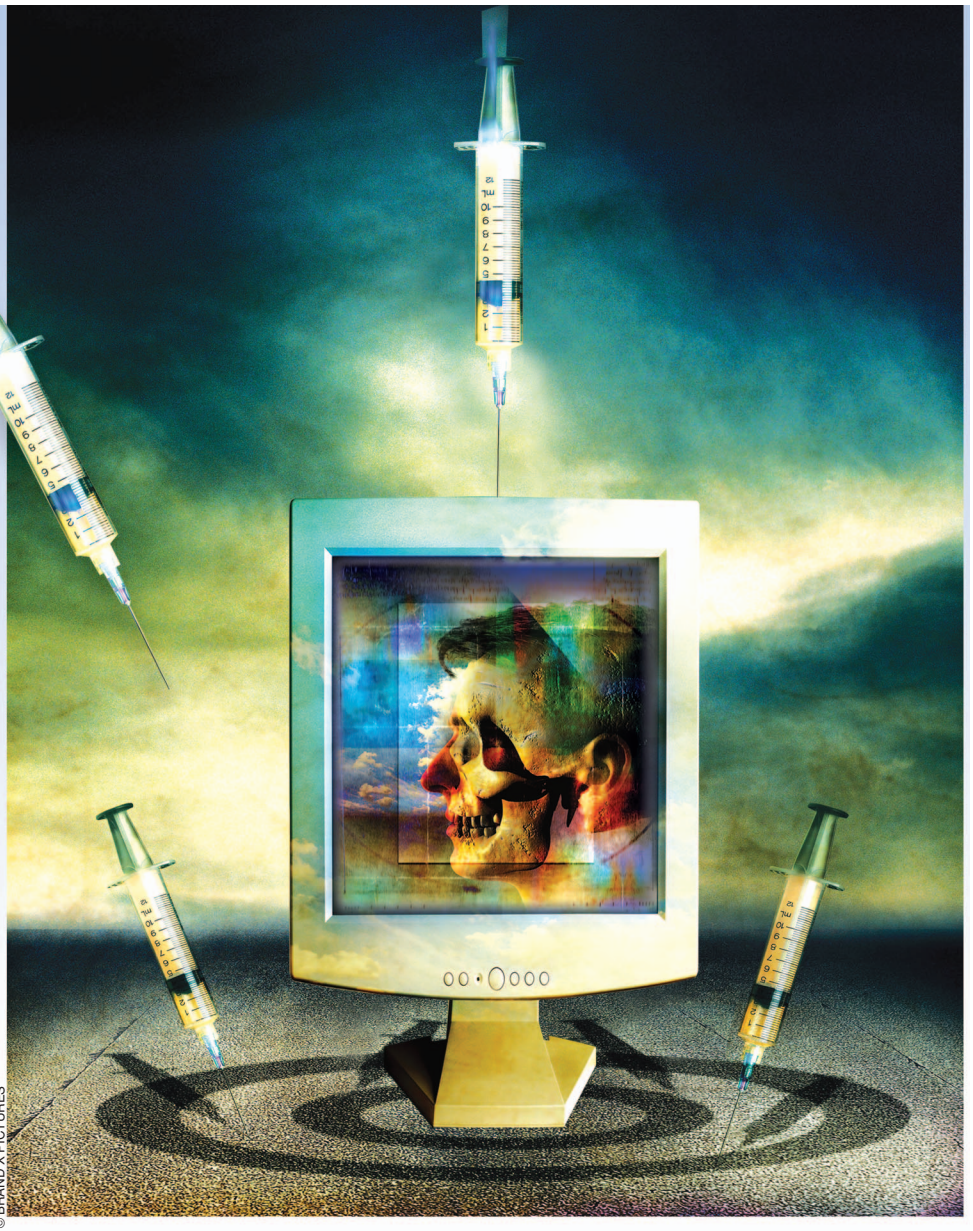
THE FUNDAMENTAL CHALlenges in modern biomedical science often arise from the inherent complexity of the biomolecular systems that serve as the foundations from which sensing and therapeutic platforms are developed to address. For instance, the molecular biomarkers indicating the biological information of interest often exist in many different levels (e.g., DNA, RNA, protein, and other biological activities).

The abundance of these biomarkers may span across many orders of magnitude, which makes quantitative measurements extremely difficult. At the same time, these molecules can be closely similar (e.g., different by as little as one nucleotide). Furthermore, the large number of biomarkers involved presents a major obstacle for obtaining a systematic perspective of the complex architecture.



The New Interface of Technology and Medicine

Emergent diagnostic and therapeutic platforms for nanoengineered medicine

PAK KIN WONG AND DEAN HO

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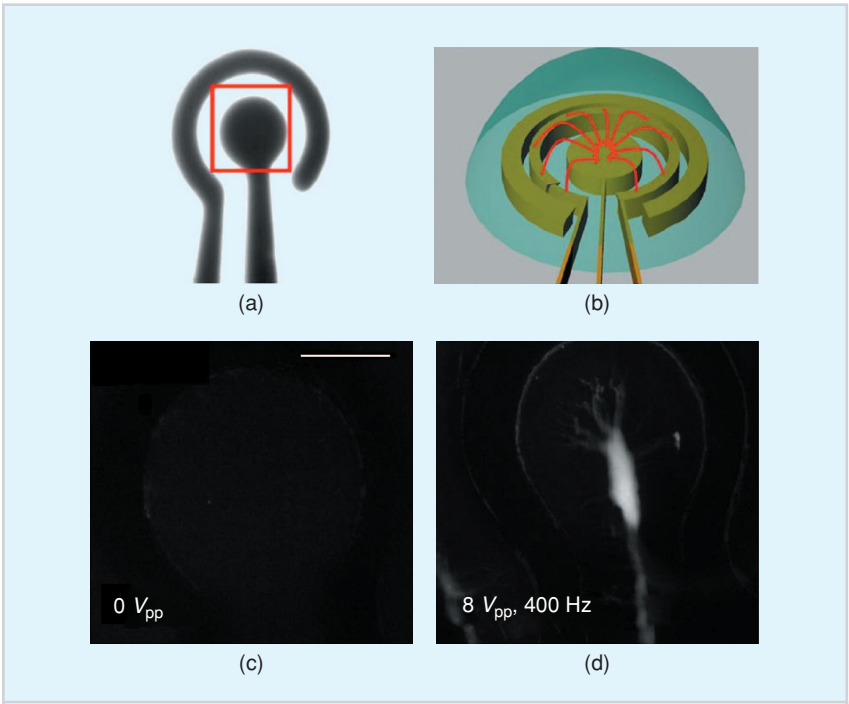

FIGURE 1 (a) and (b) Photograph and schematics of an electrokinetic bioprocessor. The red square indicates the region of observation. The outer diameter of the electrode is 1.5 mm. (c) and (d) Fluorescence images of nanoparticle concentration on the inner electrode surface at different operating conditions. Scale bar represents 200 μm.

The development of a rapid, quantitative biosensor that allows large-scale analysis of these molecular biomarkers will represent an initial step toward future predictive, preventive, and personalized medicine [1], [2]. For example, systematic interrogation of complex biological networks will be facilitated by the development of a quantitative biosensor capable of large-scale measurements of multiple biomarkers simultaneously and a multiplexed platform that facilitates automated analysis. A bioassay that is rapid and high throughput will also be invaluable for screening of chemopreventive compounds. Moreover, a rapid, specific biosensor will be beneficial for point-of-care diagnosis of pathogenic agents.

Toward the development of a complementary approach to utilize information gleaned from genomic and proteomic screening, nanomaterial-based technologies for the efficient elution of a broad arrange of therapeutics can be engineered. Utilizing inflammation and cancer as model disorders, localized as well as particle-based methodologies have been engineered to provide a dual-delivery approach that is tailored to specific clinical requirements. For example, due to the innate hydrophilic and hydrophobic properties inherent to a block copolymer structure, virtually any drug can be carried and delivered locally when the material is utilized as a coating.

> The advent of nanodiamond hydrogels addresses a critical need for methodologies that can deliver therapeutic systems in a highly targeted fashion while also enabling controllable dosing capabilities.

In the case of mitigating medical implant-induced inflammation to prolong device functionality, these nanofilms can serve as powerful strategies that are noninvasive and biocompatible. Additionally, as systemic toxicity resulting from uncontrolled delivery is a factor contributing to treatment complications in cancer, bioamenable materials for the localized and sustained elution of chemotherapies would serve as important components of optimized patient care. Due to their favorable aspect ratios and surface properties, nanodiamond hydrogels have been explored as next-generation carriers for nanomedicine. Our studies have demonstrated the nanodiamond-mediated switchable release of the doxorubicin chemotherapeutic while remaining biocompatible as confirmed through gene expression analysis of multiple inflammatory cytokines [e.g., interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), etc.] and an apoptosis regulator Bcl-x. Coupled with highly sensitive and rapid biomolecular screening assays, nanomaterial-based therapeutic delivery technologies represent a collectively powerful foundation for clinically significant nanoengineered medicine.

GENOMIC ANALYSIS

Several powerful techniques are available for large-scale genomic analyses [3]–[6]. However, these techniques typically require a large amount of cell lysates and cumbersome procedures. More importantly, the time-intensive nature of these techniques often creates a bottleneck in high-throughput drug screening. On the other hand, molecular beacons have been developed for rapid detection of specific oligonucleotides [7]. A molecular beacon is an oligonucleotide probe that self-hybridizes to form a stem-and-loop structure and can undergo a spontaneous fluorogenic conformational change upon hybridization to its complementary nucleic acid target.

The molecular beacon design provides a mechanism for both molecular recognition and transduction of the hybridization events in one single step and thus dramatically accelerates the genomic analysis process. The molecular beacon and other related molecular probe designs represent a promising approach for high-throughput screening of chemotherapeutic and chemopreventive compounds. In general, the technique has been applied in various biological studies.

For instance, our team has previously demonstrated rapid detection of the low abundance of nucleic acids by incorporating molecular beacons and an ultra-sensitive single-molecule detection system [8], [9]. Recently, we have developed a molecular probe biosensor for screening of chemopreventive compounds targeting the Nrf2 pathway. In addition, we have developed a spectrum of electrokinetic and microfluidic technologies for automated biomedical analysis[10]–[13]. Figure 1 shows an electrokinetic bioprocessor, which is capable of concentrating nanoscale particles in less than 1 min and increasing the concentration by two orders of magnitude.

PROTEOMIC AND TRANSCRIPTION FACTOR ANALYSIS

Protein detection typically relies on antibody-based strategies. Similar to genomic analysis, these assays usually require a large amount of sample and complicated sample preparation processes. On the other hand, recent developments in molecular designs and in vitro selections have provided new probes, such as molecular aptamers [14], [15]. An aptamer is an oligonucleotide (DNA or RNA) that binds a specific target molecule.

A variety of molecular aptamers for binding proteins, peptides, small organic compounds, and even entire organisms have been developed. Several groups have designed aptamer sensors that allow conformation changes upon molecular bindings (similar to a molecular beacon) [16]–[19]. For transcription factors, electrophoretic mobility shift assays and reporter gene assays are currently applied for measuring transcription factor activities. These assays, however, could be time/labor/cost intensive for high-throughput and multiplex applications.

The molecular beacon and other related molecular probe designs represent a promising approach for high-throughput screening of chemotherapeutic and chemopreventive compounds.

These shortcomings present major roadblocks for performing a screening of compounds targeting specific transcription factor activities. A simple, convenient, and high-throughput detection scheme for transcription factors is therefore highly desirable. Several homogeneous assays have been developed for studying protein–DNA interactions [20]–[22]. In our laboratory, we have developed a homogeneous assay (i.e., mix and measure) for transcription factors NF-κB and Nrf2.

The technology is well suited for high-throughput screening as it avoids cumbersome and time-consuming steps, such as washing, filtration, and separation. These developments represent the foundation of the future nanoengineered diagnostic platform.

NANOFILM DEVICES FOR LOCALIZED THERAPY

The versatility of block copolymers has been applied toward a spectrum of nanomedicine and biological studies with translational potential [23]–[25]. We have previously demonstrated the application of triblock nanopolymers for the highly efficient suppression of adverse macrophage recruitment and the ability to tune drug release depending on the number of layers of drug–nanopolymer composites. This technology successfully eliminated implant-mediated macrophage inflammation while possessing drug delivery tuning capabilities with dimensions that were ~10,000 times thinner than existing technologies with comparable or better efficacy (e.g., pacing lead/stent coatings).

We have conducted numerous studies to confirm the efficacy of the nanopolymer-based thin film toward reducing cellular inflammation/macrophage recruitment both in vitro and in vivo. We first demonstrated polymer-mediated drug deposition using dexamethasone (Dex) via conventional self-assembly methods to enable orderly film deposition/robust substrate adsorption. Also, the Langmuir-Blodgett (LB) deposition method allowed for layer-by-layer deposition to tune and control drug release rates, which was a major achievement. For hydrophilic drugs such as Dex, ordered deposition on the LB trough was enabled by using the polymer as a "buoy" to suspend the drug on the surface of the water. Following polymer/drug transfer to the solid substrate (glass), lipopolysaccharide-stimulated RAW 264.7 macrophages incubated atop the uncoated (cntrl) substrates resulted in cellular inflammation indicated through reverse transcription polymerase chain reaction. Polymer-drug films generated a significant reduction of mRNA levels as large as 20-fold. We examined the mRNA levels of several cytokines including tumor necrosis factor-alpha (TNFα), IL-6/12, IP-10, and iNOS to provide a comprehensive assessment of film efficacy [26].

As such, we showed that the composite material is extremely effective at reducing cellular inflammation in vitro. We further tested the ability to tune the nanopolymer-drug film dosing capabilities by examining the effects of depositing varying layers of the material on the resultant suppression of inflammation. We compared the mRNA levels of cultures that contained no film (cntrl), three layers, and seven layers, which showed a gradually decreasing level of mRNA and demonstrated tailorable drug release and polymer-mediated drug tethering [26].

Coupled with highly sensitive and rapid biomolecular screening assays, nanomaterial-based therapeutic delivery technologies represent a collectively powerful foundation for clinically significant nanoengineered medicine.

In vivo studies examined the material-mediated blockage of cell aggregation. Histological and fluorescent tissue staining analysis was performed using hematoxylin and eosin (H&E) to image cell recruitment activity. Untreated tissue, tissue containing implanted uncoated disks, as well as Dex-copolymer nanofilms were stained to examine cellular recruitment to the implant surface, which is a commonly observed mechanism of foreign body formation [Figure 2(a) and (b)]. C57bl/6 mice ($n = 6$) were subcutaneously implanted dorsally with two polyethylene disks (uncoated or polyDex coated). Following seven days, disks were stained with CD11b and 4′,6-diamidino-2-phenylindole and analyzed at 10x magnification. Figure 2(b) shows the interface between the implant and tissue. Coated implants showed that the tissue at the implant–tissue interface exhibited suppression of tissue inflammation, opening up possibilities of translational/clinical applicability of the composite materials following the in vivo efficacy demonstration.

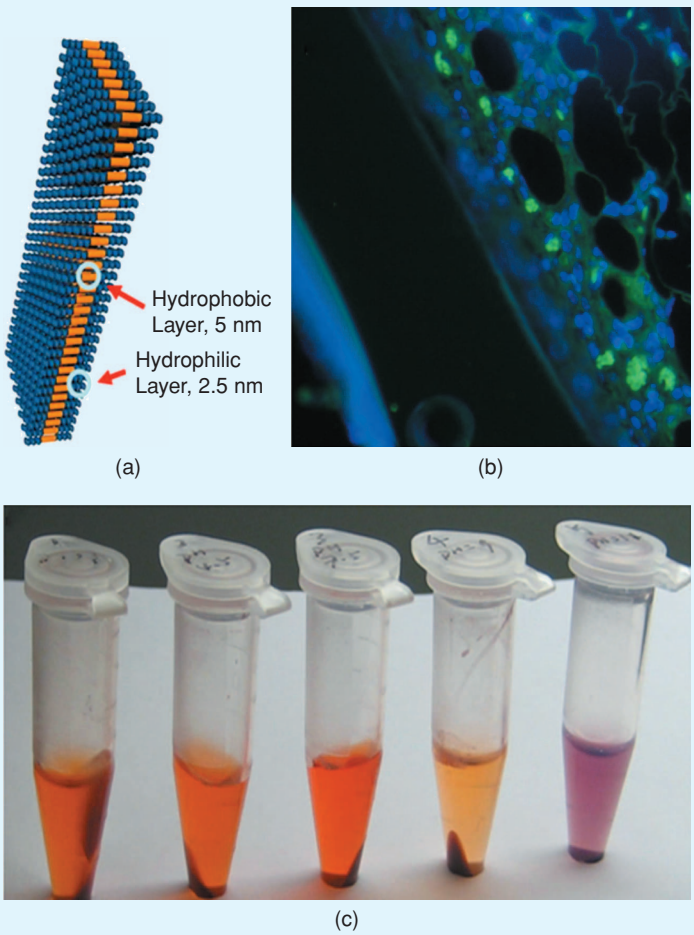

FIGURE 2 (a) The block copolymer structure is shown here containing hydrophilic and hydrophobic elements for versatile drug carrying. (b) Copolymer nanofilm-mediated suppression of inflammation at the implant–tissue interface in vivo is shown. (c) Nanodiamond hydrogels serve as versatile platforms for the controlled and sustained elution of chemotherapeutics while remaining innately biocompatible.

NANODIAMOND HYDROGELS AS BIOCOMPATIBLE THERAPEUTIC DELIVERY STRATEGIES

The advent of nanodiamond hydrogels addresses a critical need for methodologies that can deliver therapeutic systems in a highly targeted fashion while also enabling controllable dosing capabilities [27]–[33]. A major drawback at the foundation of many cancer therapeutics resides with their generic level of activity/inability to slow release their cytotoxic functionality toward only cancer cells. As such, a therapeutic vector that will enable persistent cancer cell targeting while not killing healthy cells remains a critical component of optimized cancer treatment. A key element of the nanodiamond–drug hybrids that we have developed is that they are capable of trapping/releasing almost any type of drug due to the stabilizing abilities of the nanodiamond surface, making them a platform system. Addressing the issue of targeted cellular functionality, we have engineered the nanodiamonds to sequester (inactivate the drug) or release therapeutics depending on tuned salt conditions where drug release may be directed to occur around cell regions depending on environmental modifications. Fluorescence readouts as well as quantitative cellular response measurements have been utilized to analyze drug internalization/transfer efficiency. Furthermore, in vitro studies showed that the functionalized nanodiamonds efficiently preserved drug

activity, while bare nanodiamonds generated no adverse impact upon gene expression. IL-6, TNFα, iNOS, and Bcl-x levels were unchanged, which is an important characteristic of biocompatibility [34]. This collective suite of characterization strategies will provide important drug–nanodiamond fabrication principles as well as foundations for optimized therapeutic transfer in a translational context.

> We have conducted numerous studies to confirm the efficacy of the nanopolymer-based thin film toward reducing cellular inflammation/macrophage recruitment both in vitro and in vivo.

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ABOUT THE AUTHORS

Pak Kin Wong (pak@email.arizona.edu) is an assistant professor with the Aerospace and Mechanical Engineering Department, Biomedical Engineering IDP, and Bio5 Institute at the University of Arizona, Tucson.

Dean Ho (d-ho@northwestern.edu) is an assistant professor in the Biomedical and Mechanical Engineering Departments and the Robert H. Lurie Comprehensive Cancer Center at Northwestern University in Evanston, Illinois. N