Accounts

Nano-Combinatorial Chemistry Strategy for Nanotechnology Research

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Nanotechnology refers to the creation of functional materials, devices, and systems through the control of matter on the nanometer scale, and the exploitation of novel phenomena and properties at that scale.[1] Advances in nanotechnology will have tremendous impacts on every aspect of our society.[2−4] The discovery and optimization of novel nanomaterials with unique properties require a time-consuming research effort. Parallel reactions and screenings are deemed to be more efficient than conventional linear operations. Combinatorial chemistry has already revolutionized drug discovery and the discovery of materials, catalysts, polymers, and pesticide. It has recently also made a significant impact on nanotechnology.

Combinatorial and high-throughput approaches afford several advantages in discovery research, including decreased costs and increased efficiency. This perspective will review diverse applications of combinatorial and high-throughput approaches in nanotechnology research (Figure 1). Combinatorial approaches applied in nanotechnology are reviewed in three aspects: First, combinatorial libraries are developed for optimization of synthesis conditions, such as combinatorial catalyst libraries developed to optimize the growth conditions of carbon nanotubes (CNTs). Second, synthesis of library of nanomaterials with different chemical compositions or sizes to discover materials with desired properties, such as combinatorial thin film libraries screened for unique properties. Third, library made by surface chemistry modifications to discover materials with suitable properties for applications in certain field such as medicine and diagnostics. As important as the library synthesis methods, high-throughput screening (HTS) methods have also been developed to evaluate the targeted properties of the libraries efficiently. Significant opportunities and challenges of applying nano-combinatorial chemistry approaches are also discussed.

Combinatorial Approaches in Nanomaterial Discovery

Combinatorial approaches employ parallel and high-throughput methods for discovery of nanomaterials with unique properties. The successful application of combinatorial approaches in nanomaterial discovery are demonstrated in three examples: catalysts for carbon nanotubes growth, thin films, and novel polymer carriers for gene delivery.

Combinatorial Catalyst Libraries for Controlling the Growth of CNTs. CNTs are grown mainly by chemical vapor deposition (CVD) on substrates with transition metal catalysts. Controlling the growth of CNTs is essential for their nanoscale manipulation and their applications.[5] The growth of singlewalled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs) were optimized by catalyst libraries produced by tuning composition of three components: metal salts as catalysts, $SiCl_4$ or $AlCl_3$ as substrate-forming components, and triblock copolymers as "structure-directing agents".[6,7] Another similar study demonstrated that the structuring agent was not controlling the size of the catalyst particle by encapsulating the metal salts in micelles.[8] These solution-based catalyst preparation techniques are not amenable for growth of CNTs on small patterns. So physical techniques for catalyst preparation are developed. Ion beam sputtering and pulsed laser deposition were used to fabricate catalyst libraries and growth conditions of MWCNTs were successfully optimized.[9,10] These works demonstrated the effectiveness of the combinatorial approaches.

A simple combinatorial masked deposition (CMD) method was developed for screening nominal thickness of deposits.[11] In this method, a mask with holes is placed above a substrate during vapor deposition to dilute the deposition flux, in which the degree of dilution can be controlled by the hole size and the gap between the mask and substrate. Metal nanoparticles are generally believed to catalyze the growth of CNTs to a diameter similar to the nanoparticles' size.[12−14] By using the CMD method, a library of sputter-deposited cobalt patterns with nominally 0.001−1 nm thickness was developed. It was discovered that Co nanoparticles spontaneously forming from a nominal Co submonolayer on SiO_2 can catalyze the growth of high quality SWCNTs.[15] This CMD method was also extended to binary systems and was used to examine a Co−Mo binary library. A catalyst library with Mo (0.2−4 nm) and Co (0.2−8 nm) thickness profiles on a SiO_2/Si wafer was prepared (Figure 2) and optimal active

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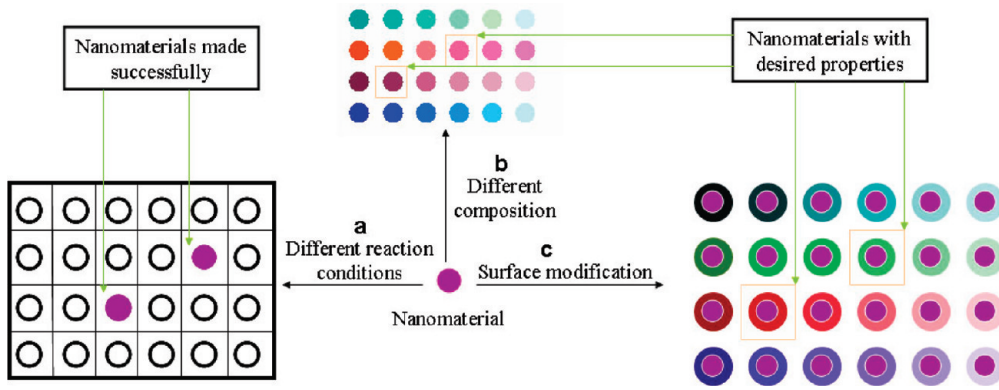

Figure 1. Diverse applications of combinatorial approaches in nanotechnology research. (a) Combinatorial libraries are developed for optimization of synthesis conditions. (b) Synthesis of library of nanomaterials with different chemical compositions or sizes to discover materials with desired properties. (c) Library made by surface chemistry modifications to discover materials with suitable properties for applications in certain field such as medicine and diagnostics.

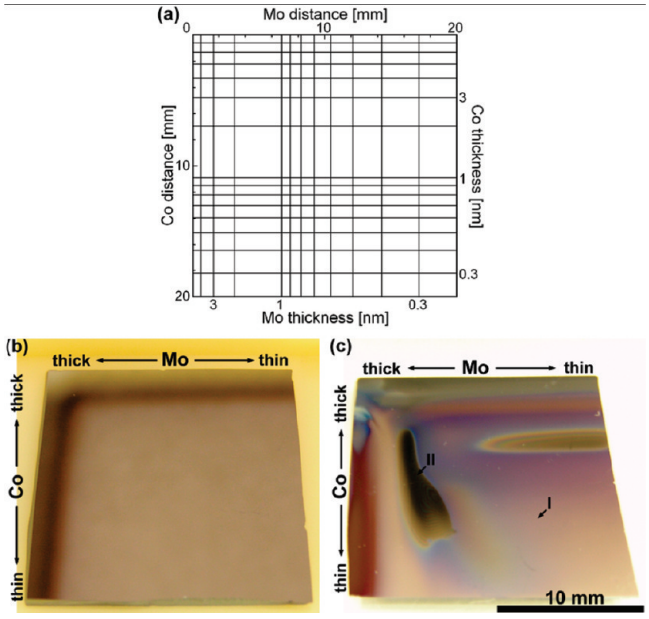

Figure 2. Nominal thickness profiles of Mo and Co in the catalyst library (a) and photographs of the catalyst library before (b) and after (c) alcohol catalytic CVD. Reprinted with permission from ref 16. Copyright 2006 Elsevier.

catalysts that grow SWCNTs vertically aligned by alcohol catalytic chemical vapor deposition were discovered.[16]

Combinatorial Thin Film Libraries. Combinatorial techniques are especially beneficial in searches for material properties that might depend on many different compositional and microstructural factors. A method was developed to make phosphor thin-film libraries by depositing multiple-layered precursors using a movable mask and a thermal annealing step.[17] Other methods include various high-vacuum thin-film deposition approaches, such as sputtering, laser ablation, ion-beam deposition, and thermal and electron beam evaporation. Different mask strategies, such as shadow masks, lithographic masks, and movable shutter masks, have also been developed to create composition variations. Solution-based parallel synthesis is another combinatorial strategy to develop thin film libraries.[18−20] To discover film materials with various unique properties, the screening methods are correspondingly very flexible and diverse. Properties investigated in a high-throughput format include the crystallinity,[21] mechanical,[22] thermoelectric,[23] semiconductor electronic,[24] piezoelectric,[25,26] photochemical,[27] and magnetic properties.[28,29] Combinatorial arrays of polymer thin films were also prepared for transmission electron microscopy (TEM) analysis.[30]

Combinatorial Polymer Libraries for Gene Delivery. Genetic diseases can be treated with gene therapy. However, the major challenge of gene therapy is the development of safe and effective gene delivery systems. There are two approaches to gene delivery: viral and nonviral. Viruses are effective in delivery efficiency but they are not safe by inducing acute toxicity and the induction of cellular and humoral immune responses.[31] Intensive research efforts have been focused on nonviral approaches for gene therapy. Synthetic polymers are one of the promising nonviral gene delivery vectors because they allow a high level of design flexibility for biomaterial construction. Typically, cationic polymers are used to bind and condense anionic DNA molecules to form nanometer-sized particles. Polymerization chemistry is well suited for combinatorial approaches because it is relatively easy to vary many parameters during the synthesis, processing, blending, and compounding.[32]

Combinatorial libraries of poly-β-amino esters (PBAEs) were synthesized (Figure 3a) to evaluate their gene delivery efficiency.[33−35] A large library of 2350 structurally unique PBAEs was created using automated high-throughput synthesis.[36] Forty-six PBAEs were found to be superior to polyethylenimine (PEI). A next-generation library of 486 polymers was synthesized to optimize the polymers and investigated the structure-function relationships.[37] The top performing polymer complexes had sizes smaller than 150 nm and positive ζ-potentials in buffer. PBAEs was used to deliver DNA to primary human endothelial cells.[38] To investigate a larger PBAE chemical space, libraries of end-modified PBAEs were also synthesized by using various end-capping reagents.[39−41] Two modified polymers (C32-103 and C32-117, Figure 3b) with greatly improved transfection efficiency both in vitro and in vivo were discovered.

Studying another combinatorial polymer library,[42] several highly effective and less toxic cross-linked PEIs were identified. Two nontoxic PEIs derivatives (Figure 3c) medi-

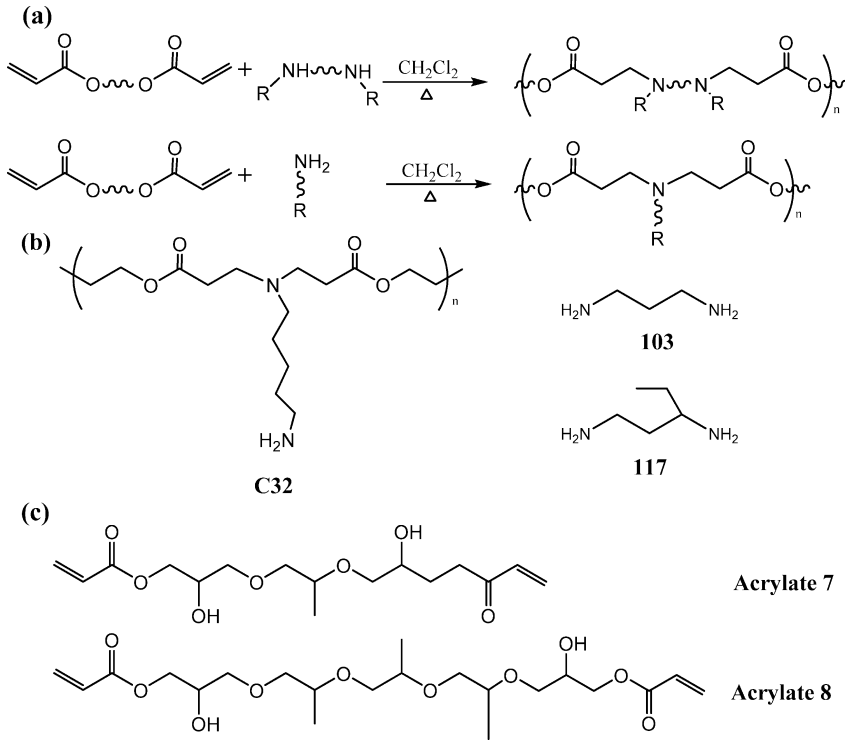

Figure 3. (a) Poly β-amino esters were synthesized by the conjugate addition of primary or bis(secondary amines) to diacrylates. (b) Structure of C32 and structures of amine-capping molecules 103 and 117. C32-103 and C32-117 were synthesized from lead polymer C32 was end-capped with 103 and 117. (c) Structures of acrylate cross-linking agents. Acrylate 7-cross-linked PEI and acrylate 8-cross-linked PEI perfomed high levels of lucifase expression in the lung in vivo.

ated high levels of luciferase expression in the lungs in vivo upon systemic delivery of the complexes.

Parallel and Combinatorial Surface Modifications on Nanomaterials

Reduction of a nanoparticle's toxicity is a critical component in nanomedicine, nanodiagnosics, and the general manufacturing of environmentally friendly nanoproducts. Nanoparticles have a large surface to volume ratio. Suface chemistry modification plays an important role in regulating nanoparticle's bioactivity and toxicity. Nanoparticle sruface modifications can be done in two ways. Parallel random synthesis is usually done by reactions between a set of available chemicals with a surface molecule. Combinatorial synthesis is carried out by varying two or more dimensions on a linker molecule on nanoparticle surface to generate a nano-combinatorial library. The library is designed based on diversity or the knowledge of targets and the building blocks can be selected by in silico calculations.

High-Throughput Surface Modification to Improve Cell Targeting. Iron oxide-based magnetic nanoparticles (MNPs) have become an important tool for sensing, separation, and imaging. Strategies have been developed to make MNPs target tissues of interest by conjugating antibodies,[43,44] peptides[45] or small molecules to the surface of MNPs.[46,47] A collection of MNPs comprising 146 nanoparticles modified by different synthetic small molecules was synthesized and screened against different cell lines (Figure 4).[47] Nanoparticles were discovered with high specificity for endothelial cells, activated human macrophages, or pancreatic cancer cells. Different synthetic routes to attach small molecules with anhydride, amine, hydroxyl carboxyl, thiol, and epoxy handles were utilized. These approaches can potentially generate large and diverse libraries of functional nanomaterials toward numerous biological targets.[46] Surface characterization was carried out by determining the loss of amine groups on the surface.

Combinatorial Surface Modifications to Reduce Toxicity of MWCNTs. CNTs can be used as drug and gene delivery carriers, imaging, or therapeutic agents. At the same time, they are very active binding cellular proteins, activating cellular signaling pathway, and causing toxicity in vivo. To discover biocompatible MWCNTs without a priori knowledge of the related targets, our laboratory developed a nano-combinatorial library strategy.[48] Combinatorial modifications of the nanotube's surface would enable us to map unknown chemical space more effectively and rapidly. Computer-aided design was used to select surface molecules to present the most diverse molecular and physicochemical properties. A combinatorial MWCNTs library containing 80 functional MWCNTs was designed, synthesized (Figure 5a), and investigated in biological screenings such as protein binding, cytotoxicity, and immunological perturbations (Figure 5b). A comprehensive evaluation of screening results identified biocompatible MWCNTs. In order to test the improved molecular recognition after surface modifications on MWCNTs, this library was screened against α-Chymotrypsin (ChT) to compare MWCNT's interactions with the enzyme catalytic site and the fluorescence-quenching sites. Four MWCNTs out of 80 were found to significantly affect the catalysis with much less effect on fluorescence quenching. They showed competitive inhibition of the enzyme activity indicating a specific binding to the catalytic site.[49]

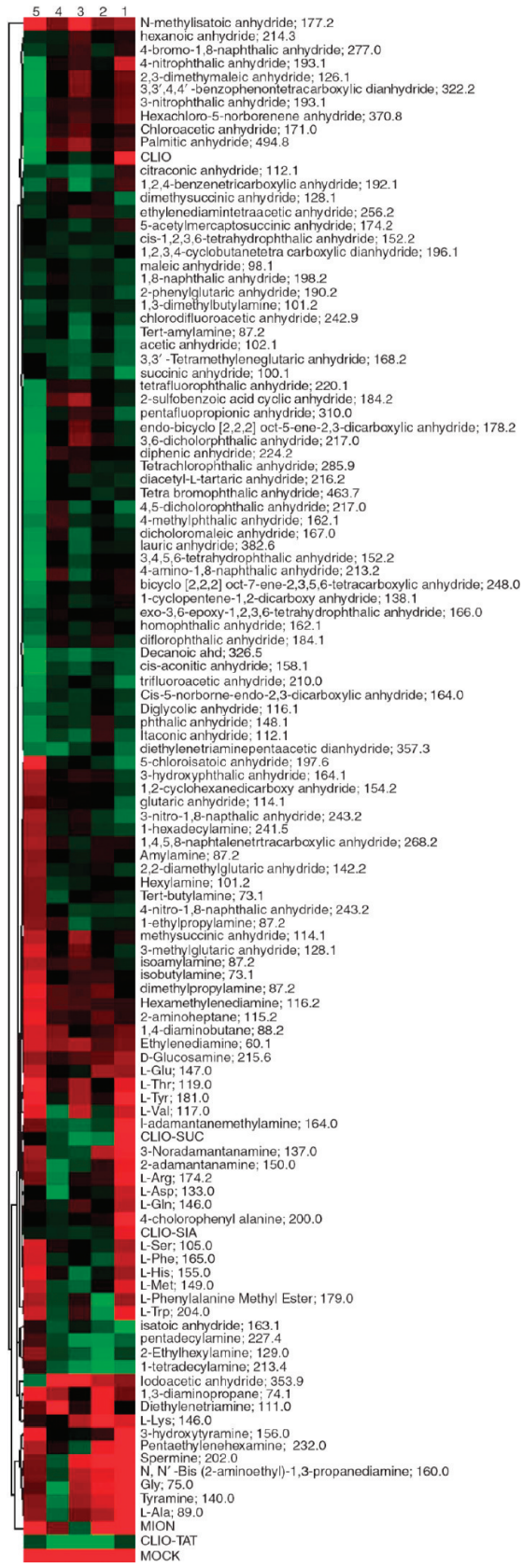

Figure 4. Heat map representing cellular uptake of different nanoparticles. Columns from right to left: 1, pancreatic cancer cells (PaCa-2); 2, macrophage cell line (U937); 3, resting primary human macrophages; 4, activated primary human macrophages; 5, human umbilical vein endothelial cells (HUVEC). Each column represents mean values from six different experiments. Red refers to the lowest accumulation and green refers to the highest accumulation. Reprinted with permission from ref 47. Copyright 2005 Macmillan Publishers Ltd.

Combinatorial Surface Modification to Prevent GNP Aggregations. A pentapeptide CALNN is able to convert citrate-stabilized gold nanoparticles (GNPs) into more stable and water-soluble GNPs.[50] A combinatorial GNP library containing 58 members was synthesized. The stability of GNPs was studied by electrolyte-induced aggregation. Results showed that the stability of GNP required a capping peptide with optimal length, hydrophobicity, and charge. Better GNP-capping ligands were discovered and detailed design criteria for peptide capping ligands were established.

High-Throughput Screening of Nanomaterials

The development of HTS methods to identify desired nanomaterials is as important as the synthesis of nanocombinatorial libraries. HTS methods used in other research areas can be employed in the screening of functional nanomaterials. For example, fluorescent activated cell sorter (FACS) is a high-throughput cell-based screening method, and it was used in screening cell lines that interact with fluorescent nanoparticle libraries.[45,47] High-throughput *Vibrio fischeri* luminescence assay, which was used for toxicity analysis of environmental and other samples, was also adapt to screen the toxicity of nanoparticles.[51]

High-Throughput Reaction Optimization. Arrays with various catalyst particle sizes were fabricated using a vapor deposition system. Simultaneous electrochemical measurements at the arrayed electrodes, together with determination of the actual particle size distribution on each electrode by TEM allowed rapid determination of the activity of the materials as a function of size.[52] Metal arrays with various sizes on both carbon or substoichiometric titania (TiO_x) supports were also fabricated and characterized[53] using conventional voltammetry method to verify the results.

High-Throughput Nanomaterial Screening. A HTS method was developed for automatic measurements of ferroelectric thin film samples with piezoresponse force microscopy.[54] Samples with varying chemical compositions, produced by high throughput experimentation via the sol−gel route, were screened by recording piezoresponse images. Samples with optimum piezoelectric properties were identified. The method was validated through comparison with the macroscopic permittivity measurements.[55] A HTS system using porous photoelectrode was developed for visible-light-responsive semiconductors.[56] In this work, an automated semiconductor synthesis system for making porous thin-film photoelectrodes of various materials was developed and photoelectrochemical measurement was selected to evaluate visible-light responsiveness. The potential of low numerical aperture photothermal microscopy as a detection scheme for HTS of GNPs was also reported.[57]

High-Throughput Screening of Nanomaterial Toxicity. Oxidative stress exerted by nanomaterials has been suggested as a plausible metric to evaluate the toxicity associated with nanomaterials.[58−61] A ferric reducing ability of serum (FRAS) assay as a HTS tool was developed to quantify the degree of oxidative damage induced by nanomaterials in human blood serum.[62] Results showed that the antioxidant capacity of nanomaterial treated serum was

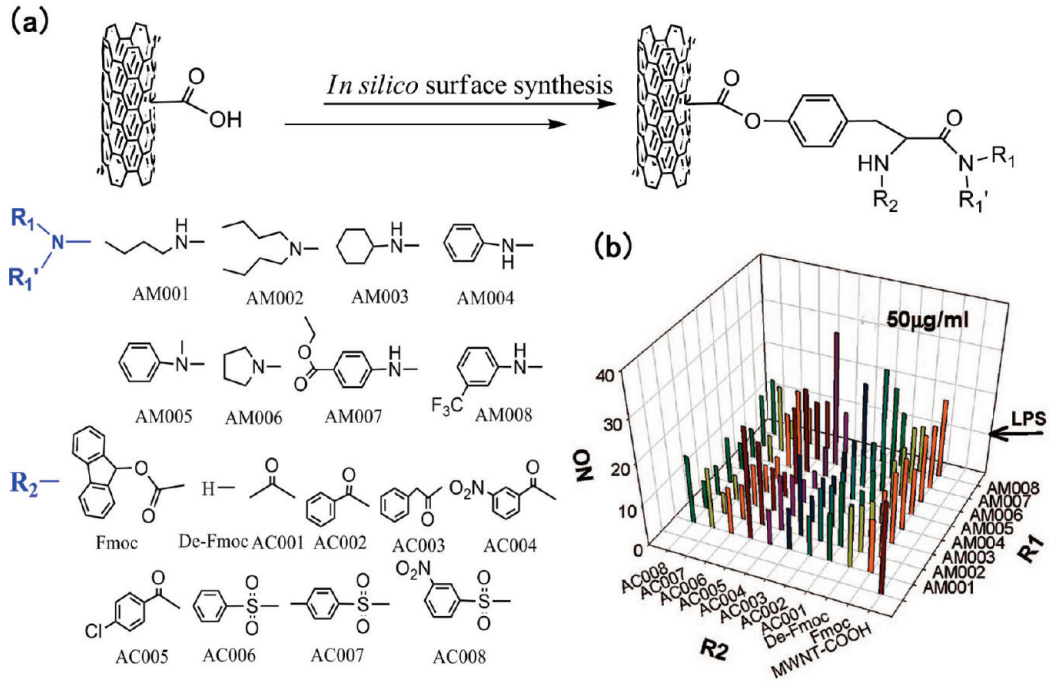

Figure 5. (a) MWCNT library containing 80 members and building blocks selected according to computer-aided design. (b) Immune responses induced by the functionalized MWCNT library at 50 μg/mL. The functionalized MWCNT library-induced NO release in the presence of LPS (100 ng/mL) was shown as vertical bars. Reprinted with permission from ref 48. Copyright 2008 American Chemical Society.

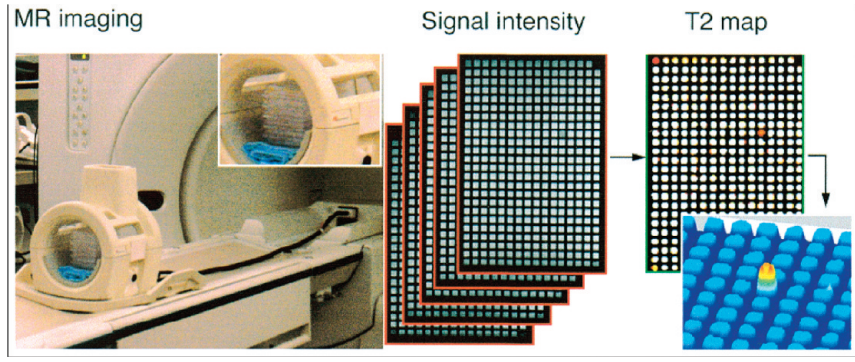

Figure 6. Use of a MR imager for HTS. Microtiter plates were imaged in a quadrature coil with different pulse sequences (variable TE) to yield a series of images for each plate. Signal intensity data were processed to yield a T2 map indicating wells with "hits". Reprinted with permission from ref 65. Copyright 2002 American Chemical Society.

significantly decreased by nanosilver, nanocarbon blacks, fullerene soot, and nano-TiO_2 (anatase, $p < 0.05$).

Most cytotoxicity assays used to evaluate nanotoxicity are end-point assays. They cannot provide real-time cellular information. Many of these assays use optical read-out and interfered by nanoparticles, which are also optical active. A real-time cell electronic sensing (RT-CES) method was initially used to study drug and small molecule-induced cellular responses. The method successfully monitor nanoparticle's cell responses and toxicities.[63,64]

High-Throughput Screening of Interactions between Nanostructures and Biological Samples

Magnetic resonance imaging (MRI) was developed and validated as a HTS method for examining the interactions between superparamagnetic nanoparticles and cells in the wells of microtiter plates (Figure 6).[65] MRI can evaluate large number of samples simultaneously providing accurate measurements. The method also provided receptor binding/internalization data as validated by radioactive assays. The technique allows the screening of libraries of peptide−nanoparticle conjugates against target cells and the identification of conjugates that may be subsequently used as reporter agents in vivo.

Understanding and controlling the protein−nanomaterial interaction is crucial for designing functional nanomedicine or nanodevices. A high-throughput technique to quantitatively characterize the protein-nanostructured surface interaction was developed by protein−surface interaction microarrays. This technique was employed to compare substrates with different surface roughness and with different postdeposition treatments using streptavidin and protein arrays.[66] Results showed that nanostructured surface provided an optimal balance between adsorption efficiency and protein functionality. A layer of adsorbed streptavidin was stably maintained on a cluster-assembled

TiO_x surface under cell culture conditions, and streptavidin retained its biological activity in the adsorbed layer.

Perspectives and Future Challenges

Combinatorial chemistry has made a significant impact on drug discovery, and the discovery of catalysts, polymers, and new materials. In comparison, its application in nanotechnology is still at its infancy. Design, synthesis, and screening of nanomaterials all need to be improved. Combinatorial approach is expected to apply to more areas in near future.

As nanomedicine research progresses, toxicity study of biofunction nanomaterials has become more important. However, the relationships between various toxicological effects and the properties of nanomaterials are still poorly understood.[67,68] Furthermore, the call for nanotoxicity regulation also comes from concerns on the burgeoning nanotechnology-based consumer product and the released nanomaterials in the environment. Nanoparticles have a large surface to volume ratio. Chemical modification will improve many crucial properties. Although random parallel chemistry conversions can modify molecular diversity on nanoparticle's surface, combinatorial library design of molecules on nanoparticle surface will map larger chemical and diversity space. Therefore, combinatorial design, synthesis, and screening nanomaterial libraries with modifications of surface will play a crucial role in future.

Nanoparticle computer modeling and sophisticated HTS all pose challenges for nano-combinatorial chemistry. However, the more challenging task is the development of analytical methodologies[69,70] to characterize surface chemistry of nanomaterials. The strategy to overcome these difficulties bears some similarity to early analytical issues in combinatorial chemistry.[71] More and more analytical methods have been reported or in the process of active research and development. These methods are expected to identify molecular structures and quantify the absolute amounts of compounds on nanoparticle surface, including multifunctionalized nanoparticles. In spite of enormous challenges ahead, nanotechnology will be greatly benefited by the application of nano-combinatorial chemistry methodologies.

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