Perspective

Research in Bioengineering and Nanotechnology

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Introduction

Research at the interface between bioengineering and nanotechnology presents exciting opportunities for creating novel technologies that can make a significant impact, both scientifically and commercially. This new field is characterized by its multidisciplinary nature, cutting across science, engineering and medicine. This article describes how research in bioengineering and nanotechnology comes together to bring about novel materials and processes to tackle challenges in protein delivery, tissue engineering, artificial implants, biological and biomedical imaging, and nanobiotechnology. It discusses the importance of designing nanoscale features and surface reactivities for deriving unique systems with tailored properties, and illustrates the significance of controlling multiple length scales in fabricating hierarchical structures, and the flexibility of nanocomposite processing in creating complex materials with multiple functionalities.

Protein Delivery

Current treatment of bone defects due to trauma, cancer or degenerative spine diseases involves the implantation of a bone graft. Autografts, which are harvested from the patient's own body, are associated with problems of limited availability and surgical morbidity. The use of allografts obtained from donors is also not desirable due to the risks of disease transmission, and the costs of maintaining bone banks. The ideal solution would be to regenerate native bone to fill the defects. A group of potent growth factors known as bone morphogenetic proteins (BMPs) have been hailed as alternatives to bone grafts due to their ability to elicit new bone formation. Clinical use of BMPs involves loading the protein solution onto collagen sponges, which are subsequently implanted. However, these conventional collagen carriers show rapid clearance of BMPs within approximately 2 weeks, whereas bone healing requires a much longer time frame, especially in higher mammals.

An ideal carrier for BMP should exhibit (1) sustained release to maintain the response and activity of bone-forming cells, (2) low initial burst to avoid adverse effects of a bolus administration and to conserve the expensive growth factor, and (3) tunable release to meter out BMPs at the desired rate. In particular, tunable release and low-burst release have long been challenges in controlled delivery systems. A carrier that can offer such temporal control will be highly valuable to the delivery of other therapeutic proteins, as wells as drugs and genes.

To this end, we have devised a novel nanocomposite of poly(lactic acid-coglycolic acid) (PLGA) and apatite.[1] The controlled release strategy is based on the use of a biodegradable polymer with acidic degradation products to manipulate the dissolution of the basic apatitic component. Proteins are preadsorbed onto the apatitic nanocrystals such that, as the apatite is dissolved, proteins would be released. In contrast to the conventional polymeric microparticles, the apatite-PLGA nanocomposite microparticles exhibit zeroth-order, low-burst release. Low-burst release is attributed to the affinity of the apatite for the protein; until the apatite is dissolved, the protein is sequestered and prevented from premature release. Accordingly, the use of apatite singly as a carrier would have led to extremely slow release.

Recombinant human BMP-2 (rhBMP-2) has been effectively encapsulated in the apatite-PLGA nanocomposite particles.[2] Its release profile can be systematically modified by changing variables that affect polymer degradation and apatite dissolution, such as polymer molecular weight, polymer hydrophobicity, apatite loading, and apatite particle size. Using an optimized formulation of apatite nanocrystals embedded in PLGA microparticles, a novel system has been successfully engineered to provide sustained rhBMP-2 release over 100 days. The bioactivity of the therapeutic protein is preserved in this nanocomposite carrier, as demonstrated by the rhBMP-2's ability to induce the differentiation of mesenchymal stem cells toward the osteoblast lineage.

Tissue Engineering

Serious bone injuries are typically repaired using bone grafts or artificial implants, which can cause problems, such as rejection and nonideal healing. To address these challenges, we have created a porous bioresorbable scaffold using type 1 collagen and nanocrystalline apatites.[3] Our nanocomposite

scaffold has been optimized to match natural trabecular bone in terms of composition, crystalline phase, grain size and chemical functionalities. We have successfully synthesized hydroxyapatite and carbonated apatite in the form of nanocrystals of 15–50 nm, which are comparable to those of natural bone apatite.[4] Compared to the commercially available apatites, our nanocrystalline apatites have a much finer grain size and greater chemical uniformity.

Through nanocomposite processing, we have derived a collagen-apatite foam-like scaffold that matches both the microstructure and mechanical properties of trabecular bone. Our nanocomposite scaffold consists of nanometer-sized pores, which are important for cell adhesion and proliferation, as well as micron-sized pores, which are necessary for vascularization. The hierarchical nature of the pore structure of this nanocomposite scaffold is very similar to that of trabecular bone. Other commercially available bone scaffolds are not able to match the pore structure of natural bone as closely due to their processing limitations.

In vivo studies have shown that our scaffold is osteoconductive, and can heal a nonunion fracture effectively.[5] There is also evidence of bone mineralization even when our scaffold is implanted ectopically, suggesting that stem cells in the blood supply under the skin can be converted to bone cells. Thus, unlike conventional bone scaffolds, our nanocomposite scaffold may be osteoinductive. Detailed studies are under way to evaluate the nature and quality of bone healing using our nanofoam bone scaffolds.

Artificial Implants

Nanostructure processing has also been applied to hydroxyapatite-based bioceramics to achieve the desired mechanical characteristics and enhance the surface reactivities for orthopedic implant applications.[6] Hydroxyapatite is of great interest as a bioactive ceramic material since it elicits a favorable biological response and forms a bond with the surrounding tissues. However, its applications have been limited to powders, coatings, porous bodies and nonload-bearing implants, due to processing difficulties and the poor mechanical properties of conventional hydroxyapatite. Hydroxyapatite is highly sensitive to nonstoichiometry and impurities in its synthesis and processing due to its complex composition and crystal structure ($Ca_{10}(PO_4)_6(OH)_2$, $P6_3/m$). As a result, conventionally processed hydroxyapatite lacks phase purity and chemical homogeneity. It is also very challenging to sinter; densification has typically required high temperatures,[7] which result in grain growth and decomposition into undesired phases with poor mechanical and chemical stabilities. To circumvent densification at high temperatures, glassy additives have been introduced to promote liquid-phase sintering at a lower temperature.[7] However, the presence of a secondary glassy phase gives rise to poor mechanical properties.

Nanostructure processing has allowed chemical homogeneity and microstructural uniformity to be achieved for hydroxyapatite, so that fully dense bioceramics can be generated at low sintering temperatures with a significant reduction in flaw size. Our nanocrystalline hydroxyapatite powders can be densified easily to >98% theoretical density by pressureless sintering at 1,000°C or by pressure-assisted sintering at 900°C. Translucent compacts constituted of ultrafine grains of <125 nm (see Figure 1a) thus obtained exhibit superior compressive strength (900 MPa), bending strength (200 MPa) and fracture toughness (1.3 MPa · $m^{1/2}$) compared to conventional coarse-grained hydroxyapatite compacts (see Figure 1b). The ultrafine grain size and the high volume fraction of grain boundaries of nanostructured hydroxyapatite are also found to enhance osteoblast adhesion, proliferation and mineralization.

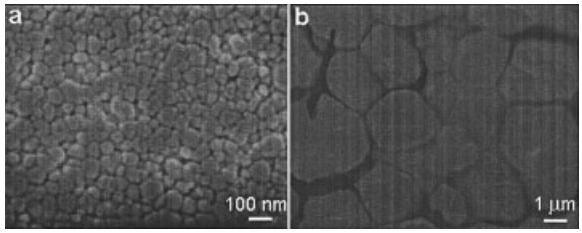

Figure 1. Scanning electron micrograph of (a) nanostructured hydroxyapatite compact (adapted from[6]), and (b) conventional coarse-grained hydroxyapatite compact.

Using nanocomposite processing, we have introduced secondary phases to mechanically reinforce hydroxyapatite-based ceramics.[8] The ultrahigh dispersion yields uniform microstructures and excellent mechanical reinforcement. In particular, ≤3 wt% zirconia dispersoids have been used to effectively toughen the hydroxyapatite matrix by transformation toughening and/or crack deflection. The resulting nanocomposite ceramic system remains easily sinterable and retains the excellent bioactivity of the hydroxyapatite matrix. It achieves >98% theoretical density from pressure-assisted sintering at 1,000°C, while maintaining an ultrafine grain size of <125 nm. The incorporation of highly dispersed zirconia nanocrystals significantly enhances the fracture toughness (2.0 MPa · $m^{1/2}$) and bending strength (280 MPa) of hydroxyapatite, allowing this bioceramic system to be developed for load-bearing implant applications.

Biological and Biomedical Imaging

Quantum dots (e.g., CdSe, CdTe and CdS) are fluorescent semiconductor nanoparticles that display narrow emission, tunable emission band and broad absorption. They also demonstrate superior photostability over conventional organic dyes. These characteristics make quantum dots promising fluorescent labels[9–11] for cellular imaging, immunoassays and other biomedical applications. However, existing quantum dots are toxic and water-insoluble. For biological applications, quantum dots need to be water-soluble, and must exhibit high colloidal stability, efficient fluorescence and low nonspecific adsorption in aqueous biological media. Recent advances in synthesis using less toxic precursors (e.g. CdO) have made it possible to produce highly photoluminescent CdSe nanocrystals.[12] However, it remains a challenge to prepare plain CdSe quantum dots that are soluble in water.

We have developed a facile and effective strategy for the synthesis of highly luminescent and photostable quantum dots.[13] The approach involves the silica encapsulation of quantum dots by reverse microemulsion synthesis (Figure 2a). With this strategy, water-soluble plain CdSe quantum dots (i.e., without ZnS capping) have been successfully derived with a

higher quantum yield (20%) in water compared to ZnS-capped CdSe (17%).[13] The SiO_2-coated quantum dots are also much less cytotoxic than the conventional organically coated water-soluble quantum dots. This combination of properties opens up new possibilities for quantum dot applications, especially in biological labeling and quantum dot-glasses for light-emitting devices, where photostability is of great importance.

The SiO_2 encapsulation approach is not limited to CdSe quantum dots. Our current work is focused on developing photostable visible quantum dots (e.g., CdTe) and near-infrared quantum dots (e.g., PbSe, PbS and PbTe) for biological applications, as well as light-emitting devices, photodetectors and solar cells. The strategy can also be broadly applied to other hydrophobic materials, such as metallic and magnetic particles synthesized in non-aqueous solvents. We have already demonstrated the synthesis of a novel water-soluble, silica-encapsulated hybrid material consisting of quantum dots and magnetic nanoparticles (see Figure 2b).[14] This material has potential in bioimaging, drug targeting, biosensing and biolabeling applications.

Nanobiotechnology

In the multibillion-dollar chiral pharmaceuticals industry, the manufacturing of chiral ingredients accounts for 10–40% of the total cost due to the inefficient, energy-intensive batch processes involved. Chiral pharmaceuticals syntheses are mostly catalyzed by asymmetric homogeneous catalysts, which are organometallic compounds, wherein transition metals are coordinated to chiral ligands. The transition metals and chiral ligands are very expensive, and are often air- and moisture-sensitive. The homogeneous catalysts are also difficult to separate from the liquid-phase products, and cannot be easily recycled for reuse. The transition metals are toxic, and may contaminate the pharmaceuticals when they are not completely removed.

To counter the above problems, much effort has been devoted to immobilizing homogeneous catalysts onto polymeric supports.[15] However, polymer-supported catalysts suffer from swelling problems, and the embedded catalysts are not always easily accessible to the substrates.

Our research is targeted towards improving the efficiency of chiral pharmaceuticals syntheses through the development of novel heterogenized catalysts. Supramolecular templating is employed to generate novel nanoporous silica supports that can

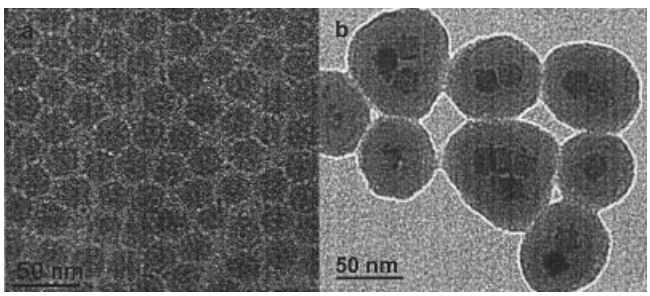

Figure 2. Transmission electron micrographs of (a) SiO_2-coated CdSe nanoparticles (adapted from[13]), and (b) SiO_2-coated nanocomposite particles of γ-Fe_2O_3 and CdSe (adapted from[14]).

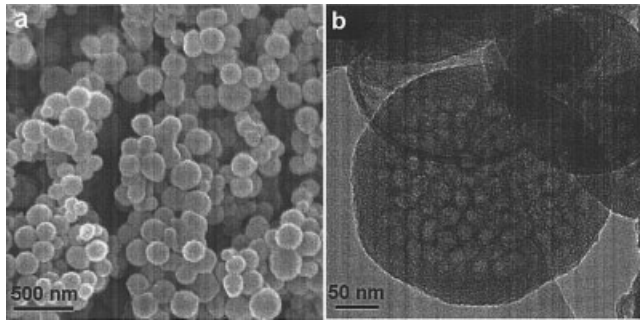

Figure 3. (a) Scanning electron micrograph, and (b) high-resolution electron micrograph of nanoparticulate siliceous mesocellular foam (IBN-3) (adapted from[16]).

immobilize chiral ligands for enantioselective catalysis. We have developed synthesis schemes that provide for the simultaneous control of pore size, pore structure, particle size and particle morphology.[16] For example, we have derived siliceous mesocellular foam (MCF) with controlled particle size and morphology (see Figure 3). MCF is templated by triblock copolymers in the presence of mesitylene. It has a high surface area (814 m^2/g), and open, ultralarge pores of 25 nm, which are interconnected by windows of 11 nm. Such a pore structure would prevent any steric effects associated with the immobilization of bulky ligands, and minimize any diffusion limitation of large substrates. Experiments have also been conducted to assess the effect of free silanol groups on the support surface, and the impact of modifying the environment around the catalytic sites. Specifically, different silanol-capping agents have been introduced to the MCF surface, and various linker groups have been employed for the fixation of the chiral organometallic ligands onto the MCF support.[17] These studies have shown that the catalyst immobilization scheme, the modification of the support surface, and the interaction between the chiral ligand and MCF support are critical towards achieving successful heterogenized catalysts. By optimizing these parameters, we have attained heterogenized catalysts that retain the excellent enantioselectivity of the original homogeneous catalysts, while providing for superior activity and allowing for catalyst recycling and reuse. This presents a major breakthrough compared to the conventional silica-supported chiral catalysts, which typically suffer from low enantioselectivity and/or activity.

Our approach is now being broadly applied to a variety of key reactions involved in chiral pharmaceuticals syntheses. We are also working on novel schemes for immobilizing asymmetric catalysts and controlling the catalyst loading. These features would further improve the general applicability of our approach, and lower the cost associated with heterogenized catalysts preparation. Our ultimate goal is to develop heterogenized chiral catalysts for packed-bed reactor applications. By enabling efficient, continuous processes, heterogenized catalysts can reduce the manufacturing cost and increase the productivity in pharmaceuticals syntheses.

Conclusions

This article has described a few examples of research at the interface of bioengineering and nanotechnology. The examples

illustrate the importance of manipulating length scales at the nanometer level to achieve nanocrystalline and nanoporous materials. In addition, they demonstrate the potential of nanocomposite engineering, which presents the possibility of dispersing different compositions at the nanometer-scale to achieve multiple functionalities and synergistic effects. The examples further reveal the importance of assembling nanostructured materials at the macroscopic level, so as to achieve unique hierarchical structures with controlled porosity, particle size, particle morphology, and component dispersion. These studies also indicate the significance of tailoring surface chemistry, reactivity and stability of nanostructured materials to realize their full potential.

Research in bioengineering and nanotechnology presents exciting possibilities for biomedical, biochemical and pharmaceuticals applications. To advance this research frontier, it is critical to establish close collaborations between different disciplines in engineering, science and medicine. By developing molecular sciences and nanotechnology as our knowledge base and tool box, and by integrating nanoscopic and macroscopic engineering, many novel materials, devices, systems, and processes can be generated with unique functionalities.

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