Nanomaterials at work in biomedical research

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With some nanomaterial-based medicines having entered the marketplace, and more on the verge of doing so, nanomedicine is expected to become an exciting playground for chemists and material scientists.

The past decade has witnessed steady growth in investment in nanotechnology by both government and industry around the world. In the United States alone, the budget for 2009 provides $1.5 billion for the National Nanotechnology Initiative (NNI), a programme that began in 2001 with a budget of $494 million. Over time the focus of nanotech research has gradually shifted from development of high-quality nanomaterials and investigation of their physical properties to the application side. Biomedical research has been identified as one of the fields that can greatly benefit from the advancement in nanotech. In particular, nanomedicine — an offshoot of nanotech that refers to highly specific medical intervention at the nanoscale for curing disease and repairing damaged tissues such as bone, muscle or nerve — is emerging as an exciting playground not just for biomedical researchers but also for chemists and material scientists.

The power of nanomedicine lies in its ability to operate on the same small scale as all the intimate biochemical functions involved in the growth, development and ageing of the human body. It is expected to provide a new framework for diagnosing, treating and preventing disease (Fig. 1). The National Institutes of Health (NIH) has recognized the promise of nanomedicine for basic and applied biomedical research and included it as a priority area in the Roadmap for Medical Research in the twenty-first century[1]. Many new demonstrations of potential applications are creating a paradigm shift for biomedical research. It is envisioned that rapid advances in different frontiers of nanomedicine will soon spawn a multibillion-dollar industry, transforming basic research discoveries into tangible benefits for people.

NANOMATERIALS CAME FIRST

Nanomaterials started to impact biomedical research long before the term nanomedicine was coined. Superparamagnetic nanoparticles and their derivatives, for example, have been marketed commercially for purification, separation and detection of biological species since the early 1980s. This class of nanomaterials has also been extensively exploited as vectors for drug delivery and as molecular contrast agents for magnetic resonance imaging[2] (MRI). Exemplified by the Guerbet Group's Endorem, iron-oxide-based nanoparticles have been commercialized as MRI contrast agents for clinical use by several companies.

About one decade ago, it was discovered that semiconductor quantum dots (QDs) could serve as fluorescent tags for *in vitro* imaging with a number of advantages (for example, high emission intensity, superb photostability, and a single excitation wavelength for multiple colours) over conventional organic dyes[3]. Commercial kits have been developed and marketed (for example, by Invitrogen) for a variety of applications that range from labelling and tracking to imaging. At the same time, both gold and silver nanoparticles have received renewed interest because of their fascinating localized surface plasmon resonance properties, which can generate a strong electromagnetic field in the vicinity of a particle surface on irradiation with light. This light-induced field can enhance the intensity of Raman scattering by up to a billion times, enabling the development of optical probes for detecting biomarkers indicative of specific diseases at low levels[4]. Recently, these nanoparticles (and their derivatives) have been further developed into colorimetric sensors (by Nanosphere); barcoding tags (by Oxonica); contrast agents for imaging modalities based on optical coherence, photoacoustics, and two-photon fluorescence; and photothermal agents for cancer treatment and controlled release of drugs (by Nanospectra Biosciences). According to a 2006 report[5] more than 130 nanotech-based drugs and delivery systems and 125 devices or diagnostic tests are in preclinical, clinical or commercial development. These figures are up from the 61 drugs and 91 devices a year earlier. All these applications require the development of nanomaterials or nanostructures with controlled size, shape, composition, surface chemistry and other physicochemical properties.

TWO DIFFERENT APPROACHES

In an ideal world, one would develop a particular material system for a specific biomedical application. This approach has been made with electrospun nanofibres, a class of nanomaterials that is increasingly gaining interest for use in biomedical research, such as that related to the extracellular matrix (ECM; the medium surrounding cells in animal tissue). The ECM can have many different roles, such as providing support and anchorage for cells, segregating tissues from one another, and

regulating intercellular communication. In addition, it sequesters a broad range of cellular growth factors, and acts as a local depot for them. Changes in physiological conditions can trigger protease activities, causing local release of such depots. This mediation by a growth factor allows rapid and local activation of cellular functions, without new synthesis.

One of the challenges in tissue/neural engineering is to design a scaffold with hierarchical structures capable of replacing the ECM. Owing to the high porosity and large surface area, electrospun nanofibres have recently been explored as scaffolds for mimicking the ECM required for cell attachment and nutrient transportation[6]. The nanofibres can be routinely prepared from a variety of biocompatible and biodegradable polymers (both natural and synthetic), as well as composites containing inorganic solids such as hydroxyapatite, the major component of bone. The nanofibres can also be conveniently functionalized by encapsulation or attachment of bioactive species such as ECM proteins, enzymes, DNA and growth factors to control proliferation and differentiation of cells seeded on the scaffolds. In addition, the nanofibres can be assembled into an array of hierarchically structured films by manipulating their alignment, stacking and/or folding. All these attributes make electrospun nanofibres a class of attractive scaffolds for tissue or neural engineering.

In a recent study, we examined the use of electrospun nanofibres of biodegradable polymers as scaffolds for enhancing the differentiation of mouse embryonic stem cells into neural lineages (Y. Xia *et al.*, unpublished work). As shown in Fig. 2, the use of uniaxially aligned nanofibres can also promote and guide the neurite outgrowth. It is anticipated that a combination of nanofibre-based scaffolds and neural progenitor cells derived from embryonic stem cells could lead to the development of a better strategy for the repair of nerve injuries.

What happens more frequently at present is that nanomaterials developed in the past are directly applied to a new biomedical application. A clear example is represented by gold nanocages[7]. These hollow and highly porous nanostructures can be synthesized via galvanic replacement, starting from silver nanocubes and a salt precursor to gold (for example, $HAuCl_4$ or $HAuCl_2$). The nanocages are essentially cubic boxes with truncated corners and can be as small as a few tens of nanometres, with single-crystal walls as thin as 30 atomic layers. These structures have unique optical properties: they can strongly scatter and absorb light

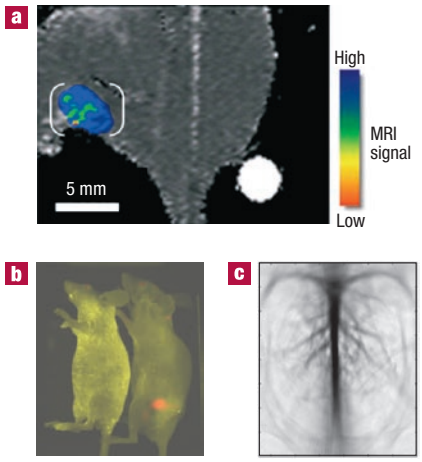

Figure 1 Examples demonstrating the use of nanomaterials in biomedical engineering. **a**, MRI of a very small tumour (~50 mg) with cancer markers in a mouse, which is selectively detected by using intravenously injected 12-nm MnMEIO-herceptin (antibody) conjugates.Reprinted with permission from ref. 2. © 2007 NPG. **b**, Spectral imaging of QD–PSMA Ab (prostrate-specific membrane antigen antibody) conjugates in live animals harbouring C4-2 tumours induced in an animal by the injection of tumour cells. Right image: orange–red fluorescence signals indicate a prostate tumour growing in a live mouse. Left image: control studies using a healthy mouse (no tumour); the same amount of QD injection showed no localized fluorescence signals. Reprinted with permission from ref. 3. © 2004 NPG. **c**, PAT of a rat's cerebral cortex two hours after the injection of gold nanocages with polyethyleneglycol attached to the surface, whose absorption peak had been tuned to the wavelength of laser. At this point, a 80% enhancement in contrast was observed. Reprinted with permission from ref. 10. © 2007 ACS.

with resonant wavelengths precisely tunable across the visible and near-infrared regions. The nanostructures were initially studied purely from a materials science perspective, that is, with the aim of understanding the reaction mechanisms and of fine tuning the optical properties. Only at a later stage was it realized that the nanostructures could be immediately applied to biomedical applications. For instance, they can serve as contrast agents for optical imaging, as therapeutic agents for cancer treatment, and as carriers for drug delivery[8]. Current efforts are focused on the exploration of gold nanocages as exogenous contrast agents for biomedical imaging modalities such as optical coherence tomography (OCT) and photoacoustic tomography (PAT) — with a focus on early cancer diagnosis, and as photothermal agents (based on their absorption features) for cancer therapy. Another possible use of these nanostructures is in drug delivery. To this end, functionalization of their surfaces with smart polymers that responsd to a near-infrared laser could lead to the possibility of externally and precisely controlling the location and dosage of drug release.

THE SAFETY ISSUE

Although the potential of nanomedicine is tremendous, questions remain about the long-term safety of nanomaterials and the risk-benefit characteristics of their usage. For *in vitro* applications such as diagnostic detection, commercialization of a product derived from nanomaterials is relatively straightforward. For *in vivo* applications, however, things could become very complicated owing to potential difficulties in gaining regulatory approval. Parallel to the development of nanomedicine, a field known as nanotoxicology has also recently emerged, which refers to the study of the potential negative impacts of the interactions between nanomaterials and biological systems[9]. An understanding of the relationship between the physicochemical properties of a nanomaterial and its *in vitro* and *in vivo* behaviour would provide a good basis for assessing the toxicity. Specifically, studies with animal models will identify the organs of interest, in turn leading to identification of the best cell types for *in vitro* and cytotoxicity studies to further understand how these cells molecularly respond to the nanomaterial. A number of research centres have been established in the United States, with an aim to assess the potential toxicity of nanomaterials that have been developed in the nanotech community. Despite the tremendous effort in recent years, a reliable database of the toxicology tests still needs to be constructed to provide materials safety sheets for nanomaterials as well as providing a basis for risk assessment and management. Therefore, potential hazards caused by nanomaterials represent a possible obstacle for the development of nanomedicine. The necessity for investigating the safety of particular applications provides further opportunities not only for biomedical researchers but also for chemists and materials scientists.

OPPORTUNITIES AND OBSTACLES FOR FUNDING

As described so far, there are enormous prospects for scientists to use their expertise in chemistry or materials science in biomedical applications. Considerable opportunities also exist as far as funding is concerned. In fact,

there is a huge difference in resources available for research in the physical and life sciences. In the United States, the National Science Foundation (NSF) is the major sponsor for research in the physical sciences. At present, a typical NSF grant could only support one or two graduate students per year for up to three years. Other funding agencies include the Department of Defense (DOD) and Department of Energy (DOE), but most of their programmes have a specific mission and the funded research has to be in line with that. There are also a number of private foundations for sponsoring basic research, with notable examples including the Dreyfus, the Sloan and the Packard foundations. The opportunities in biomedical research are significantly more. The NIH has an annual budget substantially higher than the sum of NSF, DOD and DOE. The two major funding mechanisms for NIH are the so-called R21 and R01 schemes. The overall size of an R21 grant is on the same level as an NSF grant, albeit it is set for two years. The size of an R01 grant is typically 3–5 times the size of an NSF grant, and could cover up to five years. In addition, there are many private foundations for sponsoring biomedical research; examples include the Howard Hughes Medical Institute, the Keck Foundation, the Beckman Foundation, the Coulter Foundation, Burroughs Wellcome Fund, Pew Scholars and Searle Scholars among others. Importantly, most of these foundations have special programmes to support faculty members who want to switch from physical to life science. Still, there are many opportunities to seek funding from the local government (for example, most states in the USA have life-science initiatives as a result of the Tobacco Settlement[11]) and many biotech companies.

Recognized as one of the major components of the Roadmap for Medical Research, the NIH began to fund large-scale research efforts on nanomedicine about six years ago. In 2005 they started an $80 million Nanomedicine Initiatives, and the National Cancer Institute (NCI) started a $144.4 million Cancer Directed Nanotechnology Initiative in 2004. Earlier, in 2002, the NIH had established a normal programme (including both R01 and R21 funding mechanisms) on nanomedicine, which is set to run for a number of years. It is expected that such a trend of funding will continue to grow and spread to many other areas of biomedical research.

It should, however, be kept in mind that despite the opportunities there is an intrinsic element of risk involved. There are clear differences in philosophy, approach, expectations and language between

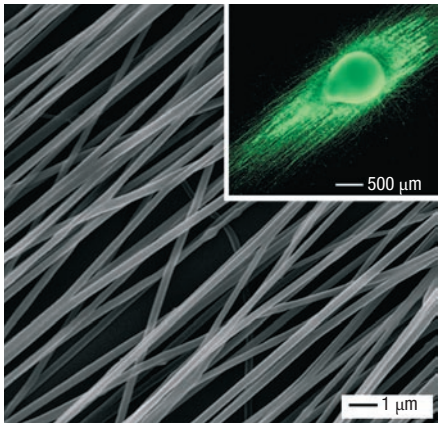

Figure 2 Scanning electron microscope image of uniaxially aligned nanofibres (~250 nm in diameter) of poly(ϵ-caprolactone) that were prepared by electrospinning. The inset is a fluorescence micrograph of RW4 mouse embryoid bodies cultured on the nanofibres for 14 days. The sample was stained with Tuj1, which highlights neurons. Images by Jingwei Xie.

materials science and biomedical research. For example, biomedical engineering is mainly driven by problem-solving capability. The research result must lead to the solution of a real medical problem, or at least to a better understanding of the problem. This is a clear impact on how proposals are prepared. For biomedical research, the issue that the project is going to address has to be specified, followed by the hypothesis (or approach), the targets and milestones. In contrast, most of the research in physical sciences is mainly driven by curiosity and a desire for new knowledge. In many cases, interesting results that may eventually lead to new research developments simply emerge while a certain research project is ongoing. It is therefore quite difficult to predict in advance what kind of real problem fundamental research will help to solve. It is therefore extremely important that a critical mass of fundamental research is maintained. Unfortunately, most of the funding agencies are moving away from basic research nowadays. There is no doubt that this change in funding mechanism has driven and is driving more scientists into applied disciplines such as biomedical engineering.

COLLABORATION IS THE KEY

The disparity between the approaches in different disciplines is of course a more general problem than just applying for funding. It is surely necessary to acquire some fundamental knowledge in the new field but it is hard to compete with colleagues who have years of expertise. The most effective way is to build connections with experts in the field and start interdisciplinary collaborations. A clear example of how this can lead to success is the interaction between George Whitesides of Harvard University and Don Ingber of the Children's Hospital, Boston, USA, who have been working together for more than a decade and have developed a new platform based on surface patterning for investigating the behaviour of cells under physical confinement. The collaboration has not only created a new research direction for chemists, material scientists and cell biologists, but has also helped to train a new generation of students and postdocs capable of tackling interdisciplinary problems.

For my own research group, we have established productive collaborations with a number of others to explore the use of gold nanocages as contrasts agents for biomedical imaging such as OCT and PAT and as therapeutic agents for photothermal cancer treatment. We have also been able to work with several groups to explore the potential of electrospun nanofibres as scaffolds for tissue/neural engineering with an initial focus on musculoskeletal and peripheral nerve repairing. As expected, this kind of collaboration is also critical for the success of a grant application as the review panel always pays close attention to this aspect of the proposed work.

Although the opportunities for discovery have never been greater, the complexity of biological systems remains a daunting challenge. Today, the scale and complexity of a biomedical problem demands that scientists move beyond the confines of their own discipline and work together in synergistic teams. As an interdisciplinary field, nanomedicine must engage people from a variety of different disciplines including chemistry, physics, engineering, genetics, proteomics, cell biology, neuron biology, musculoskeletal biology, radiology, oncology and public health among others.

References

1. http://nihroadmap.nih.gov/nanomedicine
2. Lee, J.-H. *et al. Nature Med.* **13,** 95–102 (2007).
3. Gao, X., Cui, Y., Levenson, R. M., Chung, L. W. K. & Nie, S. *Nature Biotechnol.* **22,** 969–976 (2004).
4. Anker, J. N. *et al. Nature Mater.* **7,** 442–453 (2008).
5. Moran, N. *Nature Biotech.* **24,** 121 (2006).
6. Xie, J., Li, X. & Xia, Y. *Macromole. Rapid Commun.* (in the press).
7. Sun, Y. & Xia, Y. *J. Am. Chem. Soc.* **126,** 3892–3901 (2004).
8. Skrabalak, S. E., Au, L., Lu, X., Li, X. & Xia, Y. *Nanomedicine* **2,** 657–668 (2007).
9. Jiang, W., Kim, B. Y. S., Rutka, J. T. & Chan, W. C. W. *Nature Nanotech.* **3,** 145–150 (2008).
10. Yang, X., Skrabalak, S. E., Li, Z.-Y., Xia, Y. & Wang, L. V. *Nano Lett.* **7,** 3798–3802 (2007).
11. www.tobaccofreekids.org/reports/settlements