

group from The Institute for Genomic Research (Ravel and Eisen), and in the course of our studies we rationally identified and heterologously expressed the patellamide pathway [9]. The patellamides are not made by an NRPS; therefore, these studies illuminate the potential pitfalls of the homology-based approach. However, they do constitute the first whole pathway discovery and expression from symbionts.

### Prospects for the future

The three reported bacterial symbiotic pathways were cloned using different techniques and reveal the promise and pitfalls of gene cloning to access drug candidates from marine organisms. The long-awaited proof-of-concept experiments have been concluded successfully, opening the floodgates to a new era of drug discovery and research in the marine environment.

### References

- Newman, D.J. and Cragg, G.M. (2004) Marine natural products and related compounds in clinical and advanced preclinical trials. *J. Nat. Prod.* 67, 1216–1238
- Faulkner, D.J. (2000) Marine pharmacology. *Anton. Leeuw. Int. J.G.* 77, 135–145
- Faulkner, D.J. *et al.* (1993) New metabolites from marine sponges: are symbionts important? *Gazz. Chim. Ital.* 123, 301–307
- Kobayashi, J. and Ishibashi, M. (1993) Bioactive metabolites of symbiotic marine organisms. *Chem. Rev.* 93, 1753–1770
- Unson, M.D. *et al.* (1994) A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. *Mar. Biol.* 119, 1–11
- Hill, R.T. *et al.* (2004) Manzamine-producing actinomycetes. Patent WO2004013297
- Hildebrand, M. *et al.* (2004) *bryA*: an unusual modular polyketide synthase gene from the uncultivated bacterial symbiont of the marine bryozoan *Bugula neritina*. *Chem. Biol.* 11, 1543–1552
- Piel, J. *et al.* (2004) Antitumor polyketide biosynthesis by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei*. *Proc. Natl. Acad. Sci. U. S. A.* 101, 16222–16227
- Schmidt, E.W. *et al.* (2005) Patellamide A and C biosynthesis by a microcin-like pathway in *Prochloron didemni*, the cyanobacterial symbiont of *Lissoclinum patella*. *Proc. Natl. Acad. Sci. U. S. A.* 102, 7315–7320
- Pettit, G.R. *et al.* (1982) Isolation and structure of bryostatin 1. *J. Am. Chem. Soc.* 104, 6846–6848
- Kerr, R.G. *et al.* (1996) *In vitro* biosynthetic studies of the bryostatins, anti-cancer agents from the marine bryozoan *Bugula neritina*. *Tetrahedron Lett.* 37, 8305–8308
- Kumar, P. *et al.* (2004) Manipulation and analysis of polyketide synthases. *Methods Enzymol.* 388, 269–293
- Woollacott, R.M. (1981) Association of bacteria with bryozoans larvae. *Mar. Biol.* 65, 155–158
- Haygood, M.G. and Davidson, S.K. (1997) Small-subunit rRNA genes and *in situ* hybridization with oligonucleotides specific for the bacterial symbionts in the larvae of the bryozoan *Bugula neritina* and proposal of 'Candidatus Endobugula sertula'. *Appl. Environ. Microbiol.* 63, 4612–4616
- Davidson, S.K. *et al.* (2001) Evidence for the biosynthesis of bryostatins by the bacterial symbiont 'Candidatus Endobugula sertula' of the bryozoan *Bugula neritina*. *Appl. Environ. Microbiol.* 67, 4531–4537
- Wu, K. *et al.* (2000) The FK520 gene cluster of *Streptomyces hygroscopicus* var. *ascomyceticus* (ATCC 14891) contains genes for the biosynthesis of unusual polyketide extender units. *Gene* 251, 81–90
- Piel, J. (2002) A polyketide synthase-peptide synthetase gene cluster from an uncultured bacterial symbiont of *Paederus* beetles. *Proc. Natl. Acad. Sci. U. S. A.* 99, 14002–14007
- Piel, J. *et al.* (2004) Targeting modular polyketide synthases with iteratively acting acyltransferases from metagenomes of uncultured bacterial consortia. *Environ. Microbiol.* 6, 921–927
- Sings, H.L. and Rinehart, K.L. (1996) Compounds produced from potential tunicate-blue-green algal symbiosis: a review. *J. Ind. Microbiol. Biot.* 17, 385–396
- Schmidt, E.W. *et al.* (2004) Genetic evidence supports secondary metabolic diversity in *Prochloron* spp., the cyanobacterial symbiont of a tropical ascidian. *J. Nat. Prod.* 67, 1341–1345

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## Developing implantable optical biosensors

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**Nanobiotechnologists are developing devices that can measure specific enzymes and proteins. These devices are expected to detect single enzyme or protein molecules accurately, providing highly sensitive biosensing applications. A recent study by Strano and co-workers shows that single-walled carbon nanotubes (SWNTs) hold great promise as implantable biosensors. Although most researchers have focused on substrate-oriented biosensors, Strano and colleagues have shown**

**that the inherent fluorescent properties of suspended individual SWNTs can be used for solution-phase  $\beta$ -D-glucose sensing.**

### Introduction

Nanotechnology has the potential to enable crucial new inventions in a wide variety of fields. At the nanometer scale, material dimensions lead to quantum confinement effects that give rise to unique electronic and optical properties that are useful for a variety of new

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technologies, including electronic, optical, medical, catalytic, memory and sensor applications. This scale is also relevant to biotechnology because elementary biomolecules such as enzymes, proteins and DNA have dimensions of 1–1000 nm. Researchers have already demonstrated that nanomaterials can be used to treat cancer tumors with specificity, thereby preventing unwanted damage to healthy cells [1]. Other researchers have used nanomaterials as scaffolding for the growth of neurons [2] or tissue [3].

We have all probably seen the doctor in Star Trek pass a medical device over a patient and instantly obtain an analysis of the problems ailing the patient, and we have dismissed the technology as sci-fi fantasy. However, nanobiotechnologists are attempting to turn this sci-fi fantasy into reality. In a recent study, Strano and co-workers [4] demonstrated that detection of proteins and enzymes, such as  $\beta$ -D-glucose, can probably be measured without removing a sample from the body. The authors use the inherent fluorescence from single-walled carbon nanotubes (SWNTs) as a relatively simple biosensor that can monitor glucose levels in the blood. In a related study, Weisman and co-workers [5] show that the inherent fluorescence from SWNTs can be used for imaging biological processes. These studies highlight the promising role that these nanomaterials have in understanding *in vitro* or *in vivo* bioprocesses. Here, the use of SWNTs as implantable biosensors will be discussed.

## Design of SWNT-based biosensors

### Fluorescence of SWNTs

Many researchers have been using nanomaterials for their fluorescent properties in biological imaging and sensing because of their photostability. Standard organic-based dyes typically undergo photobleaching, quickly diminishing their capability at extended analysis. In addition, most organic dyes only fluoresce in the visible wavelength region with low quantum yields. By precise control over the dimensions of nanomaterials, high quantum efficiency fluorescence can be achieved easily at tunable wavelengths. Previous work has focused on the use of semi-conducting quantum dots (QDs) owing to the ability to control their dimensions and properties.

Smalley, Weisman and co-workers [6,7] recently showed that individual semi-conducting SWNTs encased in surfactant micelles fluoresce at near-infrared (nIR) wavelengths. Furthermore, SWNTs of different chirality yield different wavelength emission (Box 1). The fluorescence emission from these one-dimensional nanostructures has narrow-band emission owing to the high fidelity of the quantum states. This narrow-band emission is in contrast to fluorescence from most QDs, which have a broader emission band as a result of slight variations in their dimensions in addition to the presence of surface or trapped states. The unique properties of SWNT fluorescence has led to increased interest in the use of these one-dimensional nanostructures in optical and biological applications.

### Optical sensors

SWNT-based optical biosensors can potentially be more sensitive owing to their increased scattering from adsorbates. The nIR fluorescence of SWNTs also makes them ideal candidates for implantable devices because of the increased transparency of tissue and biological fluids at these wavelengths [8] where the signal might be able to penetrate several centimeters. The use of SWNTs in solution-phase biosensors described by Strano and co-workers [4] offers several advantages over more-common substrate-based biosensors. Substrate-based devices will probably require mapping and deposition of electrodes to achieve ordered alignment of SWNTs. These substrate-based biosensors require multiple biocompatible materials, and bio-fouling can be problematic. By contrast, the solution-phase biosensor will not require any alignment or electrode deposition. The sensor is completely encased with an enzyme, minimizing biocompatibility and bio-fouling problems.

To serve as a biosensor, SWNTs need to be functionalized with ligand specificity. However, covalent sidewall functionalization of SWNTs diminishes their optical properties owing to the disruption of the one-dimensional electronic structure. Therefore, non-covalent binding is required for optical SWNT biosensors. In addition, the SWNTs must be suspended as individuals to maintain the fluorescence. Strano and co-workers show that the surfactant used to suspend the nanotubes can be exchanged with glucose oxidase to suspend the SWNTs in buffered solutions. Ferricyanide is also irreversibly adsorbed onto the nanotube sidewall and acts as an electron-withdrawing group, diminishing the inherent fluorescence of the nanotubes. As  $\beta$ -D-glucose reacts with the enzyme surrounding the nanotube, hydrogen peroxide ( $H_2O_2$ ) is generated, which then reduces ferricyanide. The reduction transfers electrons back into the nanotube, thereby increasing the fluorescence. By monitoring the modulation of the fluorescence caused by changes in the electron transfer, the reaction with glucose oxidase can be monitored.

The enzyme-functionalized SWNTs are placed inside a porous dialysis capillary where the analyte is free to diffuse across the membrane, whereas the SWNT biosensors are retained within the capillary. The fluorescence from the capillary was shown to penetrate a skin sample easily and it was possible to measure glucose concentration with sensitivity in the range required for diabetic patients [4]. Furthermore, no degradation of the enzyme was witnessed, suggesting that the biosensors can operate for extended periods of time.

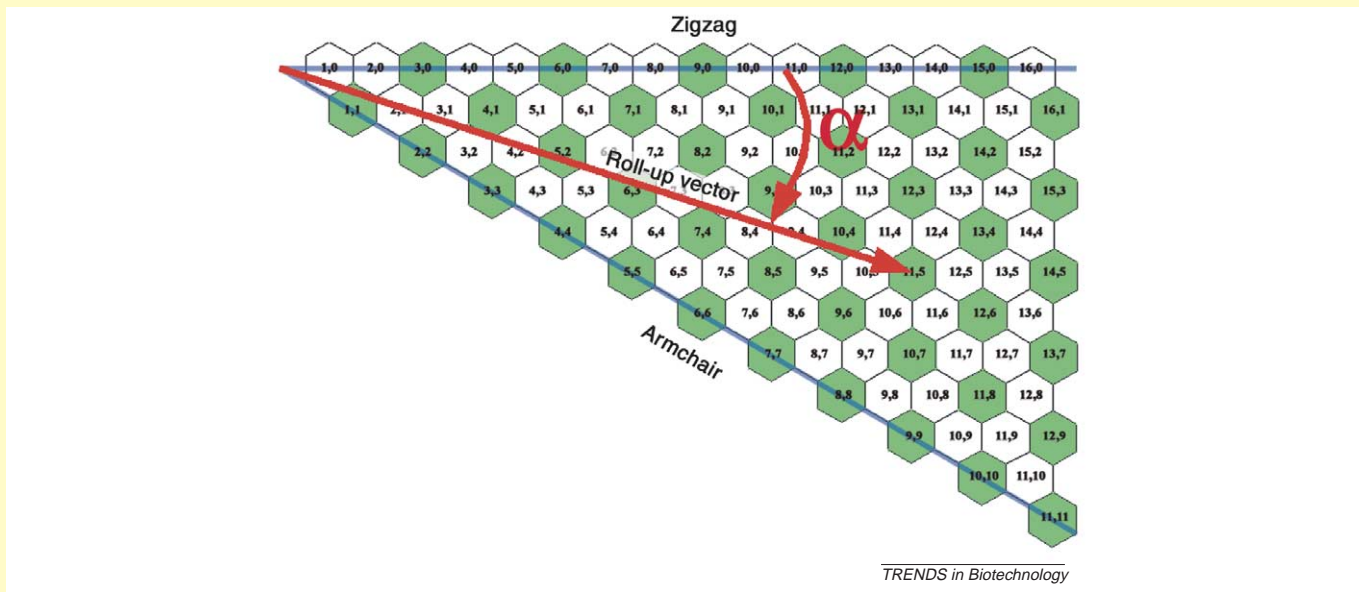
In a related study, Weisman and co-workers [5] showed that low concentrations of Pluronic-suspended SWNT could be used to image bioprocesses *in vitro*. The authors demonstrated that macrophage cells ingested the SWNTs contained within the incubation growth medium through phagocytosis. By monitoring the fluorescence from the SWNTs, the ingestion rate could be calculated as 1 nanotube per second per cell. Interestingly, the cells demonstrated no short-term cytotoxicity. This study highlights that SWNT fluorescence can be used to monitor *in vitro* or *in vivo* bioprocesses.

### Box 1. The effect of SWNT chirality on fluorescence

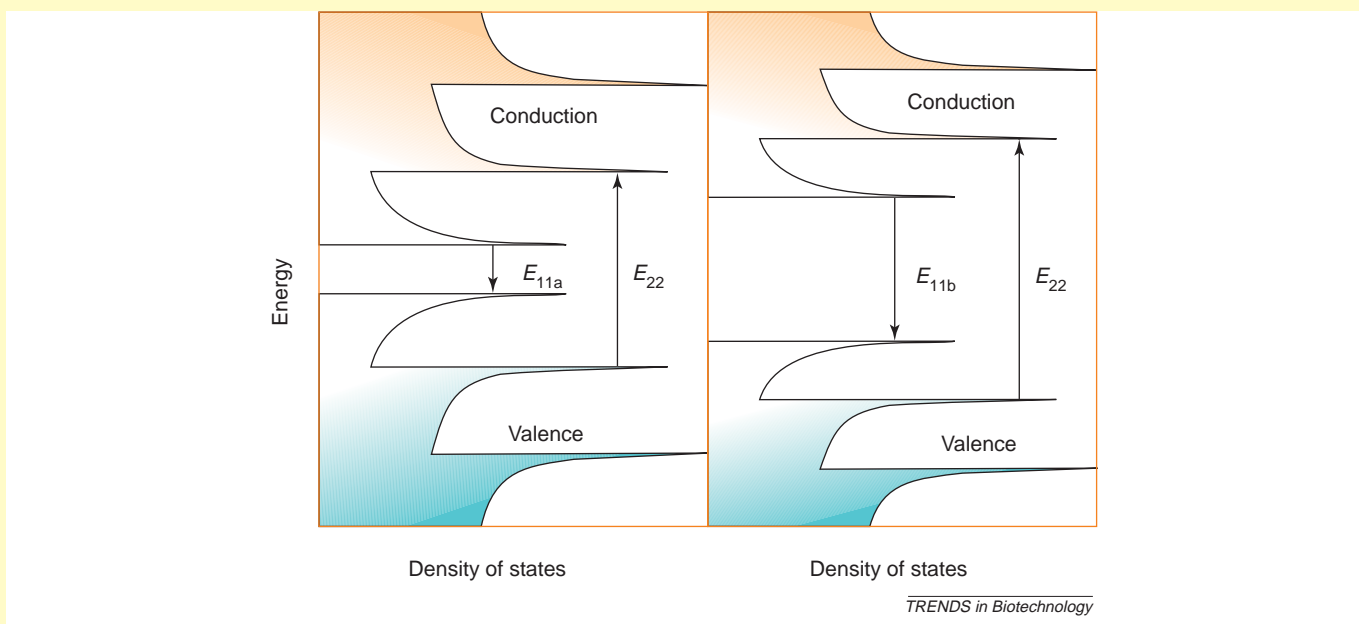
SWNTs can be viewed as a graphene layer that is rolled up into a tubular structure. However, the graphene layer can be rolled up at different vectors around the circumference of the nanotube designated by the indices  $(n,m)$ , as seen in Figure I. This has important implications for the electronic structure of the SWNTs. Slight differences in the electronic states result in some SWNTs being semi-conductors with different band gaps. Other SWNTs are metallic or semi-metallic and can be described by  $|n-m|=3q$  and are shown in green in Figure I. The quasi one-dimensionality of the nanotubes causes the electronic states to exhibit sharp van Hove peaks at energies dependent on the diameter and chirality of the nanotubes. As seen in Figure II, light absorption at  $E_{22}$  or greater will yield

fluorescence emission near  $E_{11a}$  and  $E_{11b}$  for semi-conducting nanotubes of different  $(n,m)$  type. The high fidelity of the crystalline structure of each SWNT also results in narrow band emission, allowing fluorescence to determine analytically the presence of each  $(n,m)$  type.

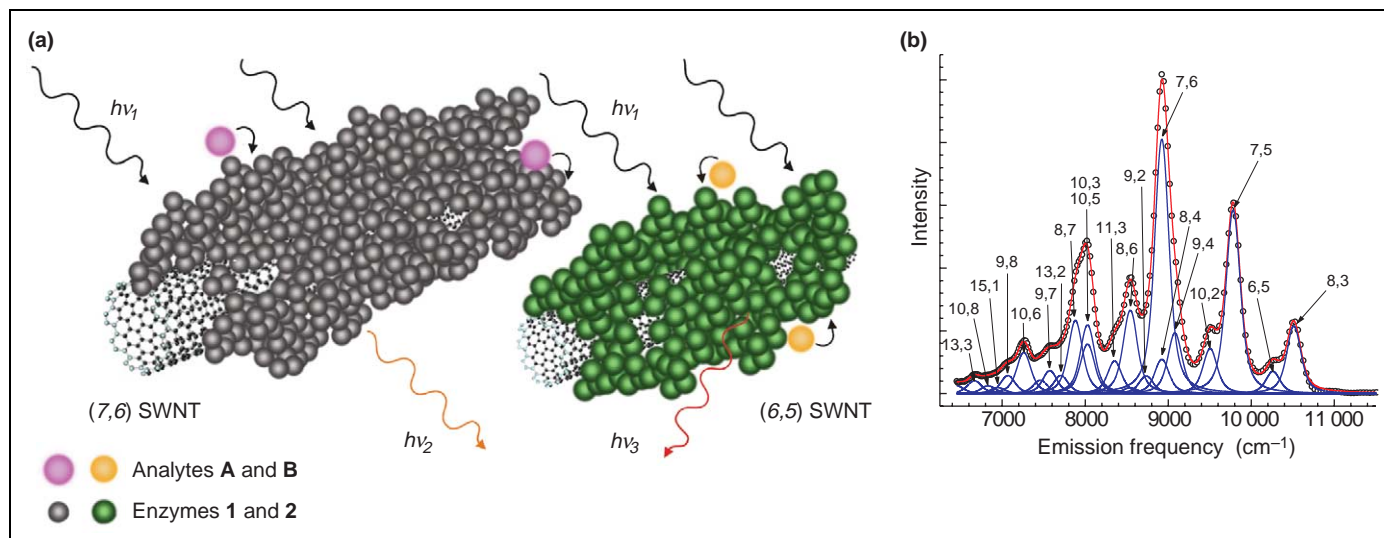
All SWNT synthesis methods yield a distribution of nanotube  $(n,m)$  types that are difficult to separate as a result of their similar diameter and lengths. Some researchers have demonstrated narrower distributions of SWNT types by taking advantage of the differences in electronic structure through electrophoresis or chromatography separations, whereas others have demonstrated the ability to synthesize fewer  $(n,m)$  types.



**Figure I.** A graphene sheet can be rolled at different vectors designated by the indices  $(n,m)$ . The nanotube circumference is determined by the length of the vector, and the chirality is measured from the zigzag axis.



**Figure II.** The electronic density of states for two different nanotubes. Different roll-up vectors result in different electronic states for each  $(n,m)$  type. Semi-conducting nanotubes, therefore, exhibit unique fluorescence dependent on their  $(n,m)$  type.



**Figure 1.** (a) SWNT-based multi-analyte biosensors. Nanotubes of a given  $(n,m)$  type are surrounded by a specific enzyme designed to interact with a specific analyte. These enzymes modulate the transfer of electrons in and out of the nanotube, thereby affecting the fluorescence intensity. Each  $(n,m)$  type will give a characteristic nIR fluorescence with narrow band emission that can be collected with a multichannel detector. (b) Deconvolution of the emission spectrum by  $(n,m)$  type will then enable simultaneous measurement of the concentration of each analyte. The deconvoluted spectrum was reproduced with permission from Applied NanoFluorescence, LLC (<http://www.appliednanofluorescence.com/>).

### Future directions

The obvious next step is to extend this type of biosensor to the measurement of other analytes. Fortuitously,  $H_2O_2$  is an intermediary product to many enzyme reactions, enabling these biosensors to be tailored easily to other analytes. Enzyme reactions where  $H_2O_2$  is not a by-product will probably require more-creative solutions to modulate the transfer of electrons in and out of the SWNT biosensor. The measurement of other analytes will require the successful exchange of surfactant for the specific enzyme required of each analyte. However, researchers have already demonstrated that other biomolecules, including DNA [9] or peptides [10], can suspend nanotubes. It is conceivable that, in the near future, it might be possible to monitor a multitude of analytes. Those individuals prone to certain ailments can then have the appropriate biosensor implanted to monitor their condition.

The ultimate goal will be to develop a multi-analyte sensor that can simultaneously monitor each target analyte. Researchers have demonstrated that the emission from SWNTs red shifts when surrounded by enzymes by as much as 20 nm [4,5]. Therefore, if different enzymes result in distinguishable shifts in fluorescence, it might be possible to monitor each analyte by monitoring the intensity of the shifted fluorescence spectra. However, to achieve higher fidelity signals from a particular analyte, the SWNTs should be separated by their chirality or  $(n,m)$  type and then selectively functionalized with the appropriate enzyme. Although researchers have not yet been able to obtain a pure sample of a particular  $(n,m)$  type, several groups have demonstrated some promising initial results [9,11,12]. Once semi-conducting SWNTs of a specific  $(n,m)$  type can be synthesized or separated, multi-analyte biosensors can be developed. Figure 1 demonstrates how the combination of multiple SWNT types could be used for an implantable multi-analyte biosensor. Each SWNT  $(n,m)$  type would be encoded with a

specific enzyme designed to monitor a specific analyte and then later mixed to form the multi-analyte biosensor. For example, the figure shows that a  $(7,6)$  SWNT would be surrounded with Enzyme 1 designed to interact specifically with Analyte A, whereas Enzyme 2 surrounding a  $(6,5)$  SWNT interacts specifically with Analyte B. A single excitation light source can be used for each individual sensor ( $h\nu_1$ ). The reaction of each analyte with the enzyme would modulate the fluorescence of the specific  $(n,m)$  SWNT. However, because each SWNT type yields characteristic nIR light ( $h\nu_2$  and  $h\nu_3$ ) with a narrow band emission, each analyte can be monitored simultaneously using a single multichannel detector. The resulting signal can then be deconvoluted to obtain the intensity change for each analyte. Applied NanoFluorescence (<http://www.appliednanofluorescence.com>) has begun to market a product capable of identifying the individual fluorescence from each  $(n,m)$  SWNT within seconds. The development of a portable light source and detector would make 24 h monitoring possible. These continuous monitoring biosensors can then aid medical researchers as they uncover the factors that forewarn of eminent disease, leading to better diagnosis and treatment.

In a similar fashion, these multi-analyte biosensors can be used for bioimaging or contrasting agents. Here, each  $(n,m)$  type would be surrounded with an enzyme designed to target a specific biomolecule. By monitoring the fluorescence associated with specific biomolecules, complex biological processes can be monitored or detailed images of tissues can be obtained.

However, before these promising applications come to fruition, many questions need to be answered. The most important factor will be the toxicity effects that these biosensors pose. Early studies have shown conflicting results. Warheit *et al.* [13] and Lam *et al.* [14] have shown that SWNTs were detrimental to the lung tissue of mice, whereas others [5,15] have shown that the toxicity is negligible. Other factors that need to be addressed will be

the specificity of each biosensor to the target analyte. The proximity of the generated  $H_2O_2$  to the electron-withdrawing group suggests that these biosensors should have high specificity. However,  $H_2O_2$  is generated by many enzymatic reactions and could result in false readings as the concentration of other proteins and enzymes change.

### Conclusions

The recent work [4,5] using the fluorescent properties of SWNTs suggests that biosensors can soon be applied *in vivo* using an external miniaturized excitation and detection device. However, long-term studies on toxicity need to be conducted before their introduction into the body. The ability to synthesize or separate nanotubes by their  $(n,m)$  chirality has the potential to aid the development of an implantable multi-analyte biosensor. In the near term, the fluorescence properties of SWNTs can be used as imaging markers and biosensors for *in vitro* studies, particularly in cases where traditional dyes suffer from bleaching, degradation and toxicity problems.

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### References

- 1 Loo, C. *et al.* (2004) Nanoshell-enabled photonics-based imaging and therapy of cancer. *Technol. Cancer Res. Treat.* 3, 33–40
- 2 Hu, H. *et al.* (2004) Chemically functionalized carbon nanotubes as substrates for neuronal growth. *Nano Lett.* 4, 507–511
- 3 Correa-Duarte, M.A. *et al.* (2004) Fabrication and biocompatibility of carbon nanotube-based 3D networks as scaffolds for cell seeding and growth. *Nano Lett.* 4, 2233–2236
- 4 Barone, P.W. *et al.* (2005) Near-infrared optical sensors based on single-walled carbon nanotubes. *Nat. Mater.* 4, 86–92
- 5 Cherukuri, P. *et al.* (2004) Near-infrared fluorescence microscopy of single-walled carbon nanotubes in phagocytic cells. *J. Am. Chem. Soc.* 126, 15638–15639
- 6 Bachilo, S.M. *et al.* (2002) Structure-assigned optical spectra of single-walled carbon nanotubes. *Science* 298, 2361–2366
- 7 O'Connell, M. *et al.* (2002) Band gap fluorescence from individual single-walled carbon nanotubes. *Science* 297, 593–596
- 8 Frangioni, J.V. (2003) *In vivo* near-infrared fluorescence imaging. *Curr. Opin. Chem. Biol.* 7, 626–634
- 9 Zheng, M. *et al.* (2003) Structure-based carbon nanotube sorting by sequence-dependent DNA assembly. *Science* 302, 1545–1548
- 10 Zorbas, V. *et al.* (2004) Preparation and characterization of individual peptide-wrapped single-walled carbon nanotubes. *J. Am. Chem. Soc.* 126, 7222–7227
- 11 Bachilo, S.M. *et al.* (2003) Narrow  $(n,m)$ -distribution of single-walled carbon nanotubes grown using a solid supported catalyst. *J. Am. Chem. Soc.* 125, 11186–11187
- 12 Krupke, R. *et al.* (2003) Separation of metallic from semiconducting single-walled carbon nanotubes. *Science* 301, 344–347
- 13 Warheit, D.B. *et al.* (2004) Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol. Sci.* 77, 117–125
- 14 Lam, C.W. *et al.* (2004) Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol. Sci.* 77, 126–134
- 15 Lu, Q. *et al.* (2004) RNA polymer translocation with single-walled carbon nanotubes. *Nano Lett.* 4, 2473–2477

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# A new source of endothelial progenitor cells – vascular biology redefined?

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**The initial discovery of endothelial progenitor cells (EPCs) in tandem with emerging concepts in stem cell biology has generated enormous interest and excitement in fields as diverse as tissue engineering, regenerative medicine, and tumor and vascular biology. A recent paper by Ingram *et al.* identifies a complete hierarchy of EPCs in the vessel wall, providing a new framework for classification of cells supporting endopoiesis akin to that previously established for hematopoiesis. This could have fundamental implications for our understanding of the role of the endothelium and, more specifically, the role of EPC interfacing with the vessel wall in health and disease.**

### Introduction

Peripheral blood was first described by Asahara and colleagues [1] to contain a circulating bone-marrow-derived cell termed an endothelial progenitor cell (EPC) that shared surface antigen characteristics with embryonal angioblasts and possessed the ability to differentiate into an endothelial-like cell. Since this initial observation in 1997, more than 2500 articles have been published describing various aspects of EPC biology, yet even today we know relatively little about the ontogeny and functional identity of these cells, and even less about their regulation. This provides both a challenge and an opportunity: answering these questions is likely to shed light on a hitherto unknown biology that spans a crossroads between the vasculature and blood. Moreover, elucidation of the full extent of EPC interfacing with the vessel wall might shake the foundations of our current

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