Box 1. Biogenesis and function of microRNAs

miRNAs are small noncoding RNAs between 18 and 24 nucleotides in length that are transcribed either individually or in clusters from RNA polymerase II (RNA Pol II) promoters, yielding a primary transcript. These transcripts undergo sequential cleavage of Drosha and Dicer, inside and outside the cell nucleus, respectively. The mature miRNA generated by Dicer cleavage can target a protein complex (RNA-induced silencing complex, RISC) to various mRNA sequences harboring complementary sequences, which results in translational repression or mRNA destabilization [15].

facilitates apoptosis by targeting several anti-apoptotic genes. If miR-466h-5p activation is demonstrated to be a universal early response in apoptotic pathways in CHO, its targeted deletion or repression could result in stress-resistant CHO cells.

Based on these initial exciting proofs-of-principle of the functional importance of CHO miRNAs and the tools that are at hand now, including genomics (miRNA sequences), transcriptomics (profiling techniques), and proteomics (target identification), further breakthroughs are soon to be expected. Furthermore, because miRNAs are only one of many noncoding RNA species that do not add onto the translational burden that recombinant producer cells already have to bear, we are convinced that other noncoding RNAs will hold additional promises for engineering the 'perfect' host cell.

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Next generation stent coatings: convergence of biotechnology and nanotechnology

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The advent of percutaneous coronary intervention (PCI) as a less invasive method to coronary artery bypass graft (CABG) surgery has revolutionized the field of interventional cardiology. The use of metal stents as supporting structures, in addition to balloon angioplasty, for maintaining the patency of blocked coronary vessels was first pioneered in 1986 [1]. Before the introduction of drug-eluting stents (DES), bare-metal stents (BMS) were the mainstay in coronary stenting procedures. Indeed, coronary stenting accounted for 84.2% of all PCI procedures in 1999, and by 2005, 90% of stenting procedures were made using a DES [2]. DES had the added advantage of having anti-proliferative drugs on them, thus circumventing the problem of in-stent restenosis due to intimal hyperplasia

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Box 1. Convergence of biofunctionalized polymers, controlled drug release, and EPC capture

To address the problems seen in BMS and DES, the next generation cardiovascular stent could involve coating with special nanocomposite polymers such as POSS-PCU (Figure I). To enhance endothelialization, EPC-specific antibodies could be attached to the polymer. NO is essential for maintaining a healthy endothelium and preventing thrombosis, and NO-eluting polymers could also be developed. Multiple drugs could be incorporated using LbL coating technology (Table I). POSS-PCU is non-biodegradable, and so is used as the base coat to prevent bare metal from coming into contact with blood. POSS-PCL is biodegradable, and can be used together with drugs for controlled release. Abbreviations: EPC, endothelial progenitor cell; NO, nitric oxide; POSS-PCU, polyhedral oligomeric silsesquioxane poly (carbonate-urea) urethane; POSS-PCL, polyhedral oligomeric silsesquioxane poly caprolactone.

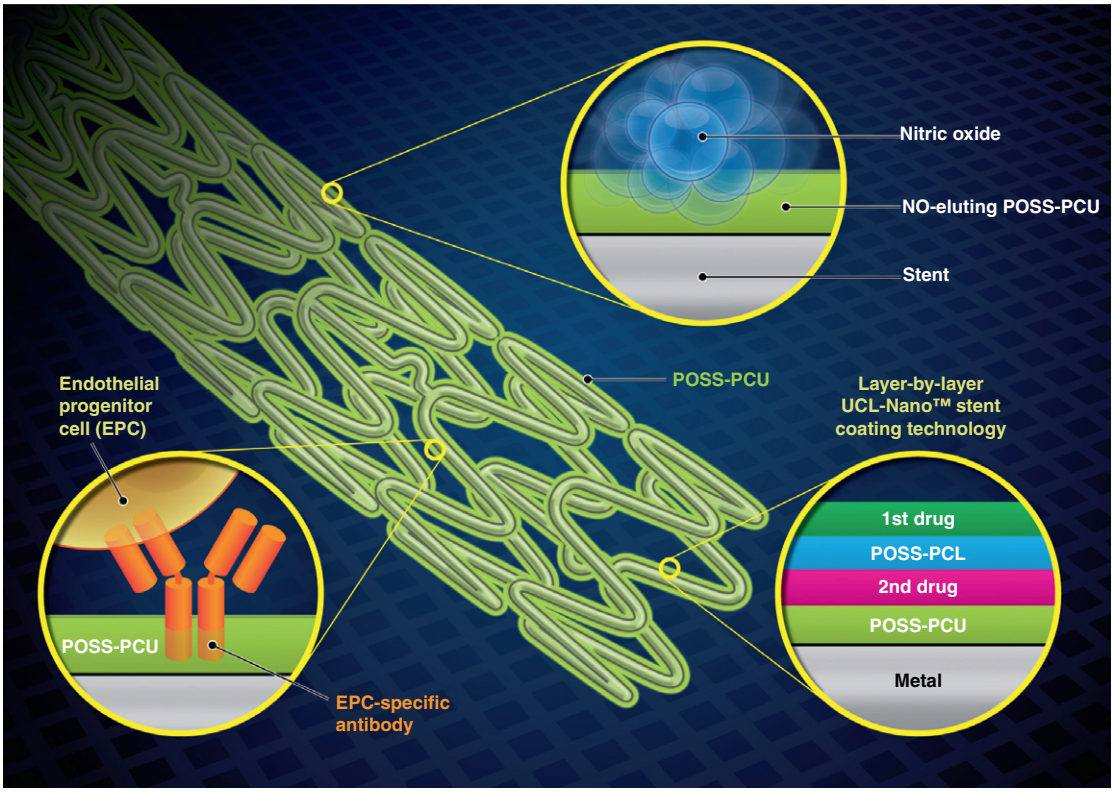

Figure I. A three-pronged approach for next generation coronary stents: biofunctionalized nanocomposite polymers, endothelial progenitor cell capture technology, layer-by-layer controlled drug release.

Table I. Stent coatings incorporating nanotechnology

Novel stent coatings	Overview[a]
LbL (for tunable and multiple drug release)	Ability to release multiple drug types in a controlled manner Potential to incorporate both anti-proliferative and anti-thrombogenic agents Can be fine-tuned to match vessel healing times Can be biofunctionalized with bioactive molecules such as NO, DNA, and antibodies
Antibody (for endothelialization)	Anti-CD34 antibodies for EPC capture (e.g., Genous™ stent by OrbusNeich) Other antibodies like anti-CD133 and VEGFR-2 are currently being explored
Peptide (for endothelialization and anti-thrombogenicity)	Synthetic functional peptides such as RGD and PA nanofibers can expedite rate of endothelialization GP IIb/IIIa integrin complex to prevent platelet aggregation and thrombosis
NO (for anti-proliferative and anti-thrombogenic effects)	NO precursors and donors (e.g., SNP and GSNO) can be integrated into polymer coatings NO would be released upon contact with physiological fluids conferring anti-proliferative and anti-thrombogenicity effects
Nanoparticle-eluting (for endothelialization and enhanced drug delivery)	Utilization of nanoparticles can increase therapeutic index Encapsulation of pharmacologic agents into liposomes can confer sustained intracytoplasmic release Magnetic nanoparticles can be guided to the stent area, increasing re-endothelialization and enhanced drug localization and delivery
Gene-eluting (for mitigating in-stent restenosis and augmenting re-endothelialization)	Plasmid DNA expressing eNOS and iNOS can inhibit smooth muscle cell proliferation and augment re-endothelialization Gene therapy can be delivered via adenoviruses or liposomes localized on stents

[a]Abbreviations: LbL, layer-by-layer; NO, nitric oxide; VEGFR-2, vascular endothelial growth factor receptor 2; RGD, arginine-glycine aspartic acid; PA, peptide amphiphile; GP, glycoprotein; SNP, sodium nitroprusside; GSNO, *S*-nitrosoglutathione; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase.

seen in BMS. However, the use of DES engendered yet another potentially life-threatening complication: late stent thrombosis (ST). Although not fully elucidated, polymer/drug coating hypersensitivity and lack of endothelialization are thought to contribute to ST. Hence, the advancement and convergence of nanotechnology and biotechnology has recently been explored for optimizing stent coatings (Box 1).

The properties of ideal stent coatings are: (i) biocompatibility, (ii) a non-biodegradable polymer basecoat to prevent blood from coming into contact with metal struts, (iii) anti-thrombogenicity, (iv) anti-calcification effects, (v) ability to interface with anti-proliferative drugs to prevent in-stent restenosis, (vi) superior mechanical properties to withstand stent apposition without polymer fracture, and (vii) the surface topography must be favorable for endothelialization to occur. We have developed and patented a proprietary nanocomposite polymer, polyhedral oligomeric silsesquioxane poly (carbonate-urea) urethane (POSS-PCU), which appears to fulfill the above-mentioned criteria. More significantly, POSS-PCU was recently used as the scaffold material in the world's first synthetic trachea transplant in a patient [3].

Sustained and controlled release of drugs is an important aspect in regenerative medicine. One way for achieving this is a technique called layer-by-layer (LbL) self assembly for tunable drug release in thin films [4]. It has been shown that LbL coatings on stents promote endothelialization and attenuate thrombosis. Having tunable release allows the drug delivery rate to be matched with healing time. Therefore, a plethora of drugs can be simultaneously incorporated onto stent coatings, including anti-proliferative, anti-thrombogenic, and anti-platelet agents to mitigate problems seen in the current range of BMS and DES.

Endothelialization is a process whereby endothelial progenitor cells (EPCs) adhere and proliferate on a surface to form a confluent layer of endothelium. It is imperative for endothelialization to occur on a stented vessel to prevent thrombosis. Promoting endothelialization can also be upregulated by conjugating antibodies on stent surfaces. This has been exemplified by the Genous™ stent (Orbus-Neich), which uses an EPC-specific anti-CD34 antibody [5]. Furthermore, the new Genous™ Combo stent, which also features drug release in addition to EPC capture technology, demonstrated favorable results in a clinical trial (see: http://www.prnewswire.com/news-releases/remedee-study-meets-primary-endpoint-orbusneichs-combo-dual-therapy-stent-is-non-inferior-to-des-133807003.html). Using the same concept, functional peptides sequences like RRETAWA [6] and peptide amphiphile nanofibers [7] can promote endothelial cell adhesion and proliferation.

Nitric oxide (NO) is widely recognized to play a significant role in vessel homeostasis. NO donors like sodium nitroprusside (SNP) can be incorporated into polymers, and it has the propensity to reduce neointimal hyperplasia [8]. The use of nanotechnology for nano-encapsulation of drug molecules into discrete particles is an exciting and rapidly advancing field. To increase therapeutic index and to facilitate sustained release of pharmacologic agents, nanoparticle-eluting stents can be developed to embody this [9]. Gene-eluting stents have also been proposed as a novel way of delivering genes encoding endothelial and inducible nitric oxide synthase (eNOS and iNOS) for the production of NO [10]. Nanoparticles such as liposomes can be used to encapsulate genetic material for localized gene delivery. These gene-eluting stents demonstrated the ability to inhibit smooth muscle cell proliferation, thereby limiting restenosis.

At the time of writing, there are 11 FDA-approved DES, with many of them sharing similar drug/polymer matrix platforms. The first DES (CYPHER®, Cordis) was approved in April 2003. Between 2011 and 2012, six DES were approved. This underscores the significance of DES in interventional cardiology, which also requires extensive R&D. Given the significant costs involved in R&D and gaining regulatory approval, the DES market is dominated by four major industry players: Boston Scientific, Abbott Vascular, Medtronic, and Cordis. A recent study by Global Industry Analysts Inc. values the stent market to reach US $9.8 billion by 2017 (see: http://www.prweb.com/releases/coronary_stents/bare_metal_drug_eluting/prweb8961534.htm).

The advent of nanotechnology for biomedical applications is advancing rapidly. From nanoscale drug formulations, lab-on-a-chip devices, to bioartificial human organ development [3,11], nanotechnology is poised to revolutionize biotechnology for medical applications. Considering that cardiovascular disease is one of the major killers in the 21st century, optimizing stent design parameters and coating technologies are of paramount importance. It is therefore pertinent to robustly ascertain the various polymer/drug matrices used in DES coatings, and the various experimental technologies being undertaken in developing the next generation stents.

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