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Nanomedicine: Addressing Cardiovascular Disease and Cardiovascular Tissue Regeneration

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Abstract

Cardiovascular disease is becoming an increasingly significant problem. In attempts to overcome many of the traditional hurdles of cardiovascular disease treatment, therapeutic approaches have been gradually moving beyond an exclusive focus on orally delivered drugs towards the development of nanoscale applications. These technologies exploit molecular scale events to improve drug and gene delivery applications, enhance preventative medicine and diagnostic strategies, and create biomimicking substrates for vascular tissue engineering. As nanoscale treatments enter the arena of clinical medicine, new ways of thinking about and routes for applying nanomedicine to cardiovascular health issues are emerging. With focuses on drug delivery, gene therapy, and biomimetics, this article will provide a comprehensive review of various nanomedicine applications for combating atherosclerosis and for improving upon current vascular tissue engineering designs.

BACKGROUND AND INTRODUCTION: WHAT IS NANOMEDICINE?

Recently, great interest has been generated in applications of nanomedicine for diagnostic and preventative purposes as well as for treatment of cardiovascular disease. ‘Nano’ structures, which refer to entities that are 100nm or less in size, exhibit many advantages over their macroscale counterparts. In fact, nanostructures possess many beneficial traits, such as the capacity for active targeting and delivery of genes and drugs, the ability to permeate the cell wall, a heightened surface area to volume ratio, attractiveness for drug presentation, and the aptitude for improving cell behaviors on a particular substrate as nanofibrous structures are capable of mimicking biological extracellular matrix (ECM). Furthermore, many current nanomedicine applications successfully exploit these unique properties for a wide variety of biomedical purposes.

Nanomedicine will first be discussed here in terms of gene therapy and drug delivery, as well as in terms of diagnostic applications. With a brief presentation of hybrid nano-macro

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materials, in addition to some of the potential drawbacks of therapies on a nanoscale, the discussion will conclude with tissue engineered approaches to cardiovascular therapies.

NANOSTRUCTURES IN GENE THERAPY

Though nanostructures may be engulfed by cells, it is important to investigate methods for preventing rapid clearance or removal of these agents by scavenging macrophages and by lysosomal degradation once transported across the cell membrane. For this reason, numerous research groups have dedicated their time and efforts to developing a vast array of nanodelivery vehicles which demonstrate a high capacity for the drug or gene of interest, a favorable loading/encapsulation efficiency, and tailorable stability both extra- and intracellularly (see Table 1).

For treatment purposes, several research groups have focused on identifying the genetic players in atherosclerosis. Seo *et al.*, in particular, harvested DNA from vascular segments of individuals diagnosed with atherosclerosis. These tissue samples were then evaluated using high throughput techniques such as microarray or gene chip technology to rapidly screen the expression of over 12,000 known genes. While deciphering which genes were upregulated or down-regulated in cases of atherosclerosis, these researchers pinpointed 208 gene predictors of severe disease in approximately 93% of samples. Several of the genes identified were, in some manner, linked to inflammation [1].

With knowledge of the gene clusters involved in cardiovascular disease, researchers and clinicians can begin to explore methods for preventing or halting atherosclerosis through gene therapy. Though a promising route, the effects of this gene therapy are often transient. Procedures for gene delivery have involved direct injection of plasmids into cardiac tissues of interest as well as catheter delivery of agents into the large coronary arteries. These strategies, however, are found to be both highly invasive and inefficient. In order to prolong the results of gene therapy, methods for sustaining gene stability and expression have been explored. An alternative to conventional invasive methods is to employ microbubbles, peptide or lipid self-assembled bubbles, which are often delivered by injection. Cavitation of gene-carrying microbubbles by ultrasound therapy allows for efficient intracellular drug and gene delivery. Furthermore, application of ultrasound to these microbubbles promotes the production of waves which enhance cellular permeability and simultaneously facilitates the release of encapsulated agents (e.g., anticoagulant, hirudin, tissue factor pathway inhibitor (TFPI), nitric oxide synthase, cyclooxygenase) (see Fig. 1). Several forms of gene-carrying microbubbles can be generated, all ranging in levels of complexity. For instance, a drug of interest may be incorporated through electrostatic or covalent interactions that permit binding to the microbubble membrane, or even embedding within the membrane itself. Microbubble formulations may also involve multiple layers or phases. Fig. (2) illustrates that these separate phases may consist of an inner core gas phase (i.e., perfluorocarbon gas for enhanced imaging), an interior oil phase, in which to hold more hydrophobic drugs, and a microbubble layer, to which moieties can be bound for specific targeting [2-5].

Traditionally, gene therapy systems have lacked the ability to efficiently deliver genes to the cell interior, compromising the effectiveness of the treatment. The half-life of exogenous

DNA is short due to rapid degradation by DNase enzymes within minutes of its presence in the blood stream [6]. However, delivery of DNA *via* calcium phosphate nanoparticle carriers has met with much success in ensuring intracellular delivery. By entrapping DNA within calcium phosphate nanoparticles as opposed to complexing DNA on the exterior, *via* the calcium precipitation method, Roy *et al.* demonstrated enhanced transfection efficacy. Exposure of the genetic material to DNase degrading enzymes was minimized by this encapsulation technique. Formation of these DNA-doped calcium phosphate nanoparticles (~80nm) relies on the phenomenon of complexing Ca^{2+} ions with negatively charged DNA molecules within an emulsion. This complex is then thought to be transferred across the cell membrane *via* ionic channel-mediated endocytosis [7].

Like ceramic-based nanoparticles, biodegradable polymeric nanoparticles have drawn attention for their potential as drug and gene delivery vehicles. In the case of polymer-based nanoparticles, degradation of these materials over time is expected to allow for controlled sustained release of therapeutic agents. Panyam, *et al.* evaluated poly(DL-lactide-co-glycolide) (PLGA) nanoparticles as a means for continuous and stable delivery [8,9]. One unique characteristic of the PLGA nanoparticles they developed is the ability to escape the lysosomal compartment, reducing the probability of lysosomal degradation of carried therapeutic agents. The postulated mechanism for escape from the lysosome involves reversal of the surface charge of the polymer nanoparticles once exposed to the acidic environment of the lysosome interior; this charge conversion forces nanoparticle extrusion through the negatively charged lysosome membrane. This transformation from an anionic particle to a cationic particle allows the nanoparticles to escape quickly and avoid degradation within minutes [8,9].

In addition to biodegradable polymers, calcium phosphate, and microbubbles, block copolymer micelles, formed by self assembly of amphiphilic copolymers in aqueous conditions, have been employed for drug delivery. The hydrophilic exterior with hydrophobic core allows for stabilization of water insoluble drugs [10]. Liposomes, which are also formed from amphiphilic lipid molecules are capable of forming a bilayer membrane encapsulating an aqueous drug-containing interior.

Although improved gene therapies are being developed and examined, the risks of adverse effects such as immune reactions or mutagenesis still exists and are strong deterrents from investigating gene therapy-based treatments [11]. Nevertheless, research groups are making successful headway in the creation of more efficient and less hazardous gene therapy applications.

NANOSTRUCTURES IN DRUG DELIVERY AND DIAGNOSTICS

To address the complex set of events that comprise cardiovascular disease, nanocarriers for treating atherosclerosis must demonstrate multiple functions. The ideal nano-carrier must possess the capacity for imaging, targeting, and delivery.

Several groups have successfully achieved two of the three ideal behaviors. For example, Lanza *et al.* developed nanocarriers capable of both cell targeting and delivery. One method

for preventing atherosclerotic plaque progression is to inhibit smooth muscle cell (SMC) propagation by delivering anti-proliferative agents such as paclitaxel. This group compared the efficacy of tissue factor (TF)-targeted nanoparticles loaded with paclitaxel to controls: TF-targeted drug free nanoparticles and untargeted nanoparticles loaded with paclitaxel. The results revealed a dramatic reduction in the replication of pig aortic smooth muscle cells, which constitutively express TF, in the presence of targeted paclitaxel-loaded nanoparticles compared to controls, underscoring the success of targeted nanostructures for drug delivery [12].

Another approach to confronting atherosclerosis is to target nanoparticles to adhesion molecules (i.e., I-CAM, V-CAM) that are upregulated during progression of the disease or by targeting molecules and enzymes (i.e., fibrin, fibrinogen, and tissue factor) involved in key atherosclerotic events [13,14]. For instance, $\alpha_v\beta_3$ integrin is a well known integrin expressed in vascular SMCs during migration and adhesion. This integrin, which is also expressed by activated endothelial cells, allows for targeting nanoparticles to developing atherosclerotic lesions. Moreover, inflammatory cells can serve as passive targets for nanoparticle drug delivery. Macrophages, which are abundantly present in these lesions, are expected to ingest foreign nanoparticles that have passively infiltrated the area of inflammation, permitting the uptake of any carried anti-proliferative or anti-inflammatory agents [15].

As mentioned earlier, one drawback with intracellular delivery of therapeutic agents is the likelihood that the drugs or genes of interest will be transported to and degraded within lysosomes. One study has investigated methods for evading the intracellular destruction of nanoparticle-carried treatments, with hopes of prolonging the action of targeted nanoparticles by reducing the rate of lysosomal degradation. As mentioned above, ICAM-1 is a promising target for nanoparticle treatment of atherosclerotic lesions. This adhesion molecule is shown to be upregulated during inflammation and thrombosis. While ICAM-1 alone is not internalized by endothelial cells (ECs), nanoparticles conjugated with anti-ICAM-1 antibodies were shown to enter HUVEC walls through endocytosis. These anti-ICAM-1 conjugated nanoparticles underwent lysosomal degradation following 1-2 hours. In this study, the investigators were able to effectively inhibit the rate of lysosomal degradation by delivering chloroquine, a drug that alters the lysosomal pH, and the microtubule disrupting agent, nocodazole, which inhibited the rate of nanoparticle transport to lysosomes [16].

Up to this point, we have only mentioned the use of loose or free nanoparticles for drug and gene delivery applications; however, nanotechnology for the treatment of atherosclerosis is not limited to these morphologies. Macroscale scaffolds and structures assembled from or combined with nanoscale components have also proved successful for drug delivery.

Hsieh *et al.* employed self-assembled peptide nanofibers (NFs) for the delivery and controlled release of platelet derived growth factor (PDGF) to myocardial tissue. PDGF induces a protective signaling cascade in cardiomyocytes and may also have adverse systemic effects. This supplies motivation for highly localized and targeted delivery. The self-assembled peptide nanofibers were essentially formed from peptide fragments which

assembled into nanofibers under physiological conditions and exhibited a diameter on the order of 10nm and an average pore range of 5-200nm. Furthermore, by incorporating controlled ratios of hydrophobic and hydrophilic amino acid residues, this nanofiber assembly process can be more finely tuned to create nanofibers with specific diameters and pore sizes [17,18].

HYBRID MACRO-NANOSTRUCTURES

During the latter stages of atherosclerosis, narrowing of the vessel can occur, threatening the supply of oxygen to downstream tissues. Following treatment of narrowed passages, stents may be employed to sustain a sufficient vessel diameter. However, in 10-60% of cases, restenosis may occur due to neointimal hyperplasia, scar tissue formation and expansion in response to the foreign stent [19-22].

It is apparent that in-stent restenosis presents a considerable hurdle to successful treatment of vessel occlusion. To combat this, much research is focused on developing drug-loaded stents, capable of minimizing inflammation and scar tissue proliferation through delivering agents that suppress smooth muscle cell proliferation. For example, ceramic nanoporous aluminum oxide stent coatings were produced to enhance drug delivery and eventually limit restenosis. The 500 nm thin nanoporous aluminum oxide layers were created by a dual mode procedure. First, an anodic arc discharge was used to deposit vaporized and ionized aluminum. Next, the resulting metallic layer was electrochemically converted to nanoporous ceramic (alumina/aluminum oxide/ Al_2O_3) with dilute oxalic acid in water treatment, creating a nanoporous structure that was expected to enhance the drug loading capacity. In the end, the nanoporous ceramic coating in combination with the anti-inflammatory drug tacrolimus reduced inflammatory cell accumulation at site of stent implantation [23].

Bhargava and other investigators were also able to formulate paclitaxel-eluting porous carbon-carbon nanoparticle coated stents for reducing neointimal propagation following stent implantation. The porous carbon-carbon coated cobalt-chromium paclitaxel-eluting stents showed reduced stenosis, inflammation, fibrin score, and internal elastic lamina area compared to cobalt-chromium stents coated with PLA/PLGA, which possesses a higher level of paclitaxel [24].

DRAWBACK OF NANOSTRUCTURE

Developing treatments based at the nanoscale offers several advantages with regards to drug and gene delivery, imaging and diagnostics, and manipulating cell behaviors. It is important to note, however, that though the area of nanomedicine for treatment of cardiovascular disease is promising, certain hurdles must be overcome in creating more efficient nanotechnologies.

For example, aluminum nanoparticles are the most abundant type of nanoparticles manufactured. Although these structures have been investigated for their imaging enhancement capabilities, alumina nanoparticles (10-20nm) have been proven to promote a proinflammatory response and increase the expression of endothelial cell adhesion molecules, VCAM-1, ICAM-1, and ELAM-1, events which are expected to exacerbate

plaque formation during atherosclerosis [25]. Research aimed to investigate the biocompatibility of aluminum nanoparticles also reveals the highly toxic effects of these nanoparticles in both humans and animals; and these findings have prompted stronger government regulation of these widely produced materials [26].

As mentioned earlier, one advantage to employing nanoporous materials in treatment devices is the presence of a high surface area to volume ratio. This characteristic provides the nanoporous structure with a high capacity for drug adsorption; and for this reason, nanoporous materials are often employed in drug-delivering stents. Studies by Kollum *et al.* revealed that stents with an average pore diameter of 5-15nm and a pore density on the order of 10^9 pores/cm² were capable of delivering the immunosuppressant, tacrolimus, in rabbit carotid arteries. However, degradation of the nanoporous aluminum oxide stent coatings resulted in the accumulation of erosion products that induced a proinflammatory reaction [27]. Another study by this same group further demonstrates the negative effects resulting from the ceramic aluminum oxide wear particles, revealing the presence of wear debris in the media and neointima [28].

PROSPECTIVE DIRECTIONS FOR NANOSTRUCTURE CARRIERS

Future investments in the nanotechnology arena are required as this area offers new hope for the development of more efficient treatments for atherosclerosis. In order to move forward, the issues that must first be addressed include but are not limited to: gaining a more comprehensive understanding of mechanisms underlying local extracellular and intracellular reactions in response to these nanoparticles, developing new and more compatible materials, and actively investing in formulating nanotechnologies that exhibit the trifecta of ideal properties: capacity for targeting, delivery, and imaging and diagnostics.

NEW MATERIALS AND TISSUE ENGINEERING APPLICATIONS TO CARDIOVASCULAR TISSUE REGENERATION

Nanomedicine is not limited to drug and gene delivery applications, rather its reach expands to cardiovascular tissue engineering. Normal cardiovascular tissues exhibit the mechanical strength, ductility, flexibility, and durability to withstand physiological blood pressures and flow. Synthetic materials, which should mimic these same characteristics, often prove too stiff or brittle for cardiovascular tissue engineering applications. Nevertheless, recent interests in elastomers are peaking and have generated much attention in the field. Elastomers are amorphous polymers which are often utilized above their glass transition temperature, accounting for their rubbery nature and elastic properties. One example is poly(glycerol-sebacate) (PGS); this polymer was introduced to the field in 2002 and has been proposed for a variety of tissue engineering treatment approaches [29]. In 2006, the hemocompatibility of PGS was investigated, specifically to assess its potential for use in engineered small diameter blood vessels, an area in which there is an immense need for alternatives to autologous grafts [30].

In a recent collaboration among MIT, Harvard, and several Boston-area hospitals, a new elastomer-based adhesive has just been reported with properties that mimic those of the

sticky feet of geckos. Using the acrylated form of PGS (PGSA), this group has created a tape-like adhesive with the nano-topography of an array of pillars, mimicking gecko foot hair [31]. The nanoscale patterning approach has allowed the group of clinicians, engineers, and scientists to formulate a wet tissue adhesive with successful biocompatibility in both *in vitro* and *in vivo* models. The applications to cardiovascular tissue engineering are obvious as PGSA possesses the ability to flex with heart muscle or expand and contract with blood vessels, and down the road, behave potentially in a drug delivery capacity. Interest in this material is expected to continue to grow, especially in light of its inherent elasticity and hemocompatibility, and its ability to achieve nanoscale topology.

Another set of new materials gaining interest in cardiovascular tissue engineering are composite materials or blends, as described in Table 2. These materials are advantageous as they combine the favorable properties of two different materials to create a blend. For example, a polymer with appropriate mechanical properties for cardiovascular tissue may be blended with a material having superior biocompatibility and hemocompatibility to form a blend or composite material displaying all three favorable properties: mechanical strength, biocompatibility, and hemocompatibility. Millon and Wan have investigated a nanocomposite material containing poly(vinyl alcohol) (PVA), a common hydrophilic, biocompatible polymer which can be formed into a hydrogel yielding mechanical properties appropriate for cardiovascular applications. This material can be reinforced with nanofibers of bacterial cellulose to form a nanocomposite, providing a broader range of mechanical properties and more closely mimicking the native stress-strain properties of porcine aorta [32].

Another group has investigated nanocomposites for a slightly different purpose. Alobaid *et al.* have evaluated nanocomposite sheets of polyhedral oligomeric silsesquioxane (POSS) nanocages containing bioactive peptides, specifically the RGD sequence, to encourage the endothelialization of grafts by circulating cells [33]. Endothelialization is the formation of an endothelial cell layer on the inner surface of the graft, stent, or other device, which helps improve and prolong the usefulness of the graft. The ability to promote endothelialization during any cardiovascular procedure could dramatically reduce the frequency of restenosis or loss of vessel patency.

Moreover, some vascular engineering approaches have involved the controlled release of proangiogenic factors for driving neovascularization. VEGF, for instance, has been well characterized as a selective mitogen for vascular endothelial cells. Additionally numerous studies also support the proliferative role of VEGF in vascular SMCs, the cells responsible for the dynamic vessel contraction and dilation as well as support [34-36].

In attempts to encourage vascularization, the Laurencin laboratory developed and evaluated the growth factor encapsulating and releasing capabilities of hybrid polymer ceramic scaffolds. The investigators applied novel techniques for incorporating proteins in composite polyphosphazene-nanocrystalline hydroxyapatite (Pphos-HAp) scaffolds (see Fig. 3). For their composite nanocrystalline hydroxyapatite-Pphos microsphere scaffolds, BSA was successfully released at a steady rate over a 21 day time period, following a zero order release profile. It is speculated that the nanocrystalline HAp particles in the composite

scaffolds served to improve the release pattern by interacting electrostatically with the protein of interest, as well as by providing an increased surface area for protein adsorption [37,38].

In shifting the focus to biomimetic substrates, Li *et al.* of Drexel University have succeeded in forming polymer blends using a nanofabrication technique called electrospinning [39]. Because an excellent review of electrospinning for engineering soft tissues has been published by Kumbar *et al.*, the following discussion will focus solely on blends for cardiovascular applications [40]. Electrospinning has drawn significant interest in recent years due to its ability to produce nanoscale diameter fibers from virtually any polymer, including natural polymers such as collagen, gelatin, and elastin, among others. Nanofibers are an ideal building block for blood vessel grafts as they can recapitulate the natural extracellular matrix environment in geometry and size scale as well as aid nutrient flow and endothelialization. This particular group fabricated nanofiber blends of PLAGA with elastin and gelatin. For this application, the presence of PLAGA allows the nanofibers to retain stability and resist swelling in an aqueous environment, a stability which cannot be accomplished with the use of either pure gelatin or pure elastin fibers. The addition of a synthetic polymer to natural extracellular matrix-based proteins in electrospun meshes is often used to overcome the necessity for harsh chemical crosslinkers normally required to retain the durability of electrospun proteins. Another group found PCL to be an ideal synthetic polymer for use with extracellular matrix proteins. Heydarkhan-Hagvall *et al.* examined the tensile properties as well as *in vitro* cell attachment and migration on composite PCL-protein scaffolds and found favorable properties for use as a tissue engineered vascular substitute [41]. Our laboratory and other groups have electrospun other extracellular matrix components as shown in Fig. (4) such as collagen, laminin, and fibrinogen with the ultimate thought that mimicking the extracellular matrix and providing proteins for cell attachment will help achieve better healing in any application [42-45]. Many of these natural polymers are currently being investigated as scaffolds alone or as part of a bio-artificial composite scaffold for cardiovascular engineering purposes.

One particular benefit of electrospinning as a technique for fabricating vascular grafts or scaffolds is the ease by which one can form tubular structures. With other processing methods, intricate molding and leaching techniques are often required to generate tubular scaffolds. During electrospinning, however, tubular structures can be formed by simply employing a rotating mandrel collector, allowing this technique to translate quickly and easily to *in vivo* models and ultimately clinical use. He *et al.* had recent success applying tubular constructs of composite PLAGA and poly (ϵ -caprolactone) (PCL) to replace the inferior superficial epigastric veins in rabbits [46]. They found that their synthetic nanofiber conduits maintained their structural integrity and patency over 7 weeks *in vivo*, signifying potential for clinical application. Another group explored the bio-artificial approach, combining the synthetic polymer polyglyconate (Maxon) with elastin or gelatin to form their bio-artificial electrospun tubular scaffolds [47]. As discussed earlier, the mechanical properties for vascular tissue engineered devices are extremely relevant, as the blood vessel graft must successfully withstand blood pressure and flow over time, especially in cases of large artery or vein replacement. When Thomas *et al.* evaluated their scaffolds for

appropriate tensile properties under dry and wet conditions, they found this particular combination scaffold could successfully mimic the tensile mechanical properties of the native femoral artery [47].

Electrospinning has been combined with other techniques as well to create scaffolds for tissue engineering applications. For example, Erisken *et al.* have combined electrospinning with a twin-screw extrusion process to form hybrid scaffolds of PCL nanofibers and tricalcium phosphate nanoparticles. The novel process allows the researcher to tailor the concentration of nanoparticles between two nanofibrous meshes [48]. Their particular goal was to form mineralized matrices for bone tissue engineering purposes, but one could imagine, instead of tricalcium phosphate, the incorporation of growth factors such as vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF) within the nanoparticles to enhance blood vessel formation or maturation within the graft area.

The logical next step, once bio-artificial, biodegradable vascular grafts have been successfully fabricated, is the tailoring of these grafts on a patient-by-patient basis. Uchida *et al.* have begun this process by using a multitude of fabrication methods, including rapid prototyping, dip coating, selective dissolution, and salt leaching, to achieve the naturally occurring range of mechanical strength in human blood vessels [49]. This would allow surgeons to specifically tune vascular graft properties to match the mechanical properties of the patient's own vessels, improving the integrative and healing capacity.

Though the beginning of this review discusses nanomedicine and drug delivery matters, and the latter half focuses on new materials and processing methods for cardiovascular tissue engineering, these ideas and approaches are interdependent and further combinatorial approaches are encouraged in order to develop smart, more efficient nano-structures for cardiovascular treatment and repair applications. Ultimately, the field is moving toward the convergence of these two foci, with the end point being nanomedicine and drug delivery from biocompatible, biodegradable scaffolds for improvement upon and replacement of autologous materials.

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Fig. (1). Effective gene delivery *via* cavitation of a DNA-loaded microbubble vehicle

The gas and gene filled microbubble is ruptured in response to application of ultrasound.

The wave energy is also responsible for enhancing cell permeability and allowing for intracellular delivery of genetic material. Modified from: Blomley MJ, Cooke JC, Unger EC, Monaghan MJ, Cosgrove DO. Microbubble contrast agents: a new area in ultrasound. *BMJ*. 322: 122-1225, 2001.

Fig. (2). Schematic of multilayered drug-loaded microbubble

In the case of hydrophobic drugs, water insoluble agents may be incorporated in an oil phase within the microbubble system. Water soluble agents may also be incorporated in aqueous layer as opposed to an oil (hydrophobic) phase. The perfluorocarbon gas phase provides the microbubbles with MRI imaging capacity, allowing for simultaneous drug delivery and detection. Modified from: Blomley MJ, Cooke JC, Unger EC, Monaghan MJ, Cosgrove DO. Microbubble contrast agents: a new area in ultrasound. *BMJ*. 322: 122-1225, 2001.

Fig. (3). Composite polyphosphazene nanocrystalline hydroxapatite microsphere scaffolds
Scanning electron micrographs of solvent sintered PNPhGly and PNPhGly-HAp microsphere scaffolds. Images **A** (45um scale bar) and **B** (1585 um scale bar), depict two magnifications of the PNPhGly-based scaffold. Images **C** (45um scale bar) and **D** (1585 um scale bar) depict two magnifications of composite PNPhGly-nanocrystalline HAp-based scaffold. Note the increased surface roughness resulting from the incorporation of nanosized HAp particles.

Fig. (4). Extracellular matrix protein nanofibers

Representative scanning electron micrographs of electrospun (A) laminin isolated from murine Englebreth-Holme-Swarm (EHS) tumor, (B) collagen isolated from rat tail, and (C) blended laminin and PCL.

Table 1

General Classification of Nanostructures for Drug and Gene Delivery

Material	Nanocarrier Type	Functions
Peptide	Microbubbles, Nanofibers	Cavitation and intracellular delivery capable with ultrasound application; extracellular matrix (ECM) mimic [2-5,17,18]
Lipid	Microbubbles, Micelles, Liposomes	Cavitation and intracellular delivery capable with ultrasound application; capacity for hydrophobic; capacity for hydrophilic drugs [2-5,10]
Polymer	Nanospheres, Nanocoatings, Nanofibers	Intracellular delivery; prolonged effects due to endosomal escaping capabilities; sustained drug release though gradual polymer degradation; heightened surface area to volume ratio [8,9,24,38]
Ceramic	Calcium Phosphate Nanospheres, Nanocoatings	Intracellular delivery; encapsulation provides DNA protection from degrading enzymes; heightened surface area to volume ratio [7,23]

Table 2

Bio-Artificial Blends Currently Under Study

Synthetic Polymer	Natural Polymer	Scaffold Form	Ref.
Poly(vinyl alcohol) (PVA)	cellulose	Hydrogel	[33]
Polyhedral oligomeric silsesquioxane (POSS)	RGD peptide	Polymer sheets	[34]
Polyphosphazene	hydroxyapatite	Microspheres	[38]
Poly(lactide-co-glycolide) (PLAGA)	gelatin, elastin	Nanofibers	[40]
Poly(ϵ -caprolactone) (PCL)	collagen, laminin, fibrinogen	Nanofibers	[41]