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Nanotechnology Advances in Drug Delivery

Diana Velluto[1,2] and Camillo Ricordi[1-3]

[1]*Diabetes Research Institute and Cell Transplant Center, University of Miami, FL, USA*
[2]*Diabetes Research Institute Federation, Miami, FL, USA*
[3]*Division of Cellular Transplantation, Department of Surgery, University of Miami School of Medicine, Miami, FL, USA*

***Correspondence to:**
Camillo Ricordi
Division of Cellular Transplantation
Department of Surgery
University of Miami School of Medicine
Miami, FL, USA
Tel: 305-243-6913
E-mail: CRicordi@med.miami.edu

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Abstract

Drug delivery emerged as a discipline to solve problems associated with the majority of the current drugs, such as poor water solubility, poor physical stability, poor absorption and side effects. Large pharmaceutical companies are investing in drug delivery technologies to find better ways to administer existing drugs rather than designing new products. The secret to a successful drug delivery system, one that allows controlled release over a prolonged period of time, is in the carrier. An ideal drug carrier must be biocompatible, biodegradable, water friendly, selective, easy to prepare, stable, cheap, and finally, ultra-small. Therefore, the interdisciplinary field of nanotechnology and nanomaterials is playing a big role in drug delivery by providing new tools to develop ideal nanocarriers and find appropriate solutions for medical problems. In this review, we briefly recapitulate the history of nanomaterials in drug delivery, explore their unique properties, and report an example of the design and development of polymer-based nanomaterials. We also revisit the most challenging applications of drug delivery for cancer treatment, cell and tissue transplantations and stem cell therapies. Overall, nanotechnology-based drug delivery systems administered by different routes can be considered promising tools to improve patient compliance and achieve better therapeutic outcomes in critical illnesses.

Keywords

Nanomedicine, Polymers, Self-assembly, Tumor treatment, Cell transplantation

Introduction

Drugs have been always used to improve or extend the life of patients, but in certain clinical situations, such as cancer, patients have experienced debilitating side effects from the treatment, or in other cases, such as organ and cell transplantations, patients must be on life-long systemic immunosuppression. In those and similar situations, the ability to deliver drugs and pharmaceutical compounds safely in the body specifically to their sites of action, could radically improve the life of the patients. "Drug delivery" began as the science and technology that modify drug pharmacokinetics to improve efficacy, eliminate or reduce side effects, and meet patient needs. Currently, it is the results of a cross-disciplinary research that involves engineers, chemists, biologists, and physicians with the aim to create personalized medicines.

The first potential drug delivery system was reported in a paper published in 1964 by Bangham and Horne [1]. They showed electron micrographs

of multilamellar phospholipid vesicles named, with a generic term, "banghasomes" (after the author's name, Alec Bangham). In 1965, Bangham and coworkers demonstrated and published that lipid bilayers of the vesicles could maintain concentration gradients of ions such as potassium and sodium, but when natural or synthetic surfactants perturbed the bilayer, the gradient was disrupted [2-4]. A few years later (1968), Gerald Weissmann, after visiting Bangham's lab, renamed those closed phospholipid bilayers with a more descriptive term: "liposomes" (lipid nanoparticles), and he published about their use as a model for biological membranes [5]. The name still survives and has been counted in almost 40,000 publications in PubMed. The pioneering work of liposome researchers (Alec Bangham, Gregory Gregoriadis, Vladimir Torchilin, Demetrios Papahadjopoulos), at the early 1970s, established the concept of liposomes as drug delivery systems by demonstrating their ability to entrap drugs [6, 7]. Their work, although is often forgotten, led to important technical advances for the development of liposome delivery systems, such as self-assembling, homogenization via extrusion, PEGylation for stability enhancement, active targeting by conjugating receptor antibodies, peptides or small cell ligands to the drug carrier. Currently, a number of liposomes are on the market covering different therapeutics: for cancer therapy (i.e. Doxil® [8] DaunoXome®), for fungal diseases (i.e. Amphotec® and Ambisome®), for viral vaccines (i.e. Epaxal®, Inflexal®) and many more are under different phases of clinical trial [9]. The strongest impulse to the drug delivery field was given by the '*nanoscopic era*', which began in the mid to late 1970s when the concept of "nano-therapeutics" arose around the world [10]. Since the 1970s, nanotechnologies have been applied in medicine to improve the different clinical treatments and to provide new and more personalized medical solutions for patients [11]. Drug delivery today is based on the use of biocompatible and biodegradable *nanocarriers* that medicinal chemists design to be always more efficient and transport available drugs more precisely to their pharmacological target, away from sites of toxicity, and/or to maintain drugs at a therapeutic concentration over long periods of time. Liposomes, quantum dots, gold nanoparticles, carbon nanotubes, dendrimers, nanogels, and biodegradable polymers found valuable applications as nanoscale drug delivery systems. Among those, highly promising are biodegradable polymers, some of which are already FDA approved for clinical use [12]. Our research team is actively involved in the investigation of novel polymeric nanomaterials for drug delivery and regenerative medicine, some of which are described in this review.

Nanomedicine…Why?

The main reason why nanomaterials have seen such an exploitation in medicine is discussed in this paragraph. The most recurrent question today is not about what nanomaterials are, but rather *what the real breaking property of nanomaterials is.* The answer is their dimension (comparable to the dimension of a virus). At the nanoscale level, the essential structure of the material can be arranged to achieve specific and unique physical and chemical properties, such as high surface area to bind, absorb and carry molecules, high permeability and retention, ability to reach areas difficult to access, biocompatibility and biodegradability. The recent advances in techniques such as microscopy, including cryo-electron microscopy (cryo-EM) that has undergone an incredible revolution in the last few years [13], have given scientists new tools to observe and understand the material when is organized at a nanoscale level. Particularly exciting are the advances that nanomaterials are seeing in the pharmaceutical field, such as drug delivery vehicles for new nanomedicines, and other modern medical techniques including bio-sensing, stem cell modulation and tissue engineering. The evolution of nanotechnology in medicine, known as *nanomedicine*, during the last two decades is demonstrated by the large amount of literature (research articles and reviews) published in peer-reviewed journals. Furthermore, the analysis conducted by the Center for Drug Evaluation and Research (CDER) within the US Food and Drug Administration (FDA) identified several trends in the development of drug products containing nanomaterials [14]. For example, before the nano-era (the early 1970s), it was impossible to administer pharmaceutical suspensions (dispersion of solid particles, namely the drug, in water) by intravenous injections due to the risks of embolism. The development of nanoparticles enabled to solubilizing any kind of active agents, allowing the use of these medicines intravenously, improving their absorption when administered orally, and giving rise to new therapeutic and diagnostic tools.

A single and fully comprehensive definition of *nanomaterial* or *nanoparticle doesn't exist*, but it is generally accepted that nanoscale materials must have at least one dimension that is less than approximately 100 nanometers. A large variety of nanomaterials can in theory be engineered and chemically synthesized from all kinds of macromolecules, such as polymers, lipids, peptides and proteins, sugars, surfactants, and composed of almost any chemical, organic and inorganic (carbon or minerals based). As a result, they can differ with respect not only to chemical composition, but also particle size, shape, surface functionalization and strength of particle bonds.

There are many potential applications for nanomaterials in drug delivery. First, they enable stable aqueous dispersions of poorly water-soluble therapeutic agents for delivery in the biological fluids. Their composition, size, shape, and surface properties can be perfectly designed so that, when introduced in the body, they can protect the encapsulated agents from degradation caused by various endogenous mechanisms such as enzymatic degradation, immune degradation, sequestration by the reticuloendothelial system (RES) in the bloodstream, acid hydrolysis in the stomach, etc. Therefore, the control of the nanomaterial properties allows one the enhancement of the circulation time and stability of the drugs, but also to target or localize drugs to specific cells or tissues, and to trigger or delay their release. Finally, the nanomaterial should exhibit no toxicity and be safely excreted by the body. Some of the advantages of nanomedicines in respect to conventional medicines are listed in table 1.

Another important matter is *how to engineer and develop nanomaterials for drug delivery.* Is it a process based on rigorous

Table 1: Summary of common drug limitations versus challenges of nanomaterials drug delivery systems.

Conventional Drug Systems	Nanomaterial based Drug Delivery Systems (nDDS)
Limited solubility	Increase of drug solubility
Poor distribution within the body	Effective targeted delivery
Lack of selectivity	Reducing the amount of drug needed
Unfavorable pharmacokinetic	Decreasing drug toxicity and side effects
Unintentional damages to healthy tissues (side effects)	Obtained continuous regulation of drug levels within the therapeutic range

design or inspiration? For anyone approaching the field of nanomedicine, the method to develop nanomaterials must be based on:

• experimentation (synthesis and characterization of nanomaterials of different size, shape, surface, aggregation behavior, and their exposure to biological systems),

• scientific inspiration (rather than on rigorous engineering design), such as the observation of the nature and its mechanism,

• knowledge of the state of the art,

and is always the result of the interactions of all other scientific fields, such as chemistry, biology, physics, materials science and engineering. Throughout the years, the advances in any of those fields also affected the extremely small world of nanosciences and nanotechnology and the production of nanoscale drug delivery systems. In such an active developing process, at least three generations of nanocarriers can be identified, where the latter ones are more sophisticated than the former. Namely, the 1st generation nanocarriers consists of nanoparticles made through bioinspiration from nature, and characterized by basic surface chemistry, only assessing biocompatibility and toxicity, and of which the drug release mechanism is based on simple dissolution, diffusion, osmosis and ionic exchange. Examples in this category are nanoparticles made of building blocks found in our body, such as phospholipids and albumin, or biodegradable polymers (i.e. polylactate-co-glycolate). The 2nd generation of nanocarriers are those with optimized surface chemistries for improved stability and targeting in biological systems (i.e. PEGylation, conjugation of antibodies, etc.). Stealth liposomes are those nanoparticles made with PEGylated phospholipids to confer them higher stability in the body fluids and to avoid the opsonization process. Recent advances in material chemistry led to the 3rd generation of nanocarriers, also considered as "smart nanocarriers". They are able to respond to exogenous and endogenous stimuli (change in temperature, pH, light, ultrasounds or the presence of specific enzymes), and deliver drugs in spatial-, temporal- and dosage-controlled manner. The most advanced thermoresponsive nanocarriers are the liposomes where the phase transition of the lipids is associated with change in the bilayer conformation; among the others, the poly(*N*-isopropyl acrylamide) (PNIPAM) is the preferred building block for thermoresponsive polymeric nanocarriers because exhibits a low transition temperature allowing controlled drug release at low hyperthermia (40 °C). The advantage of polymeric nanocarriers is that tuning the nature and the composition of the polymers allows to obtain transition temperatures close to body temperature. pH responsive nanocarriers are made of materials very sensitive to even slight difference in pH (such as between healthy tissues and tumors). Examples are chitosan where the protonation of the amino groups causes swelling and drug release, or PEG-(β-amino esters). Detailed examples of stimuli responsive nanocarriers for drug delivery are reported in the review published by Mura et al. [15]. A large variety of smart nanocarriers are currently under developing and investigation by many researchers. Once fully established and applicable, they will provide treatments for the most severe diseases of our age (e.g. targeted delivery of anticancer drugs, long term delivery systems, delivery of combination therapies etc.) [16], allowing targeted and personalized therapies [17] and aiming to a better quality of life. An example of nanomaterial development and evolution is reported in following section.

Polymeric Nanomaterials

Among the different type of nanomaterials for drug delivery, polymeric nanocarriers are some of the most used because of the flexibility in tailoring their chemical composition, size, biodegradability, morphology, and surface functionality. As a result, they are excellent drug carriers for a range of applications in sensing, imaging, and therapeutics [18]. The first type of polymers employed for the construction of nanoparticles were non-biodegradable, such as polyacrylamide or polymethylmethacrylate [19], and not applicable for administration in humans. The discovery of novel biocompatible polymers such as albumin [20] (a polyamide used to carry paclitaxel, marketed as Abraxane®), polyalkylcianoacrylate [21] (a polyanhydride), polylactate-co-glycolate (PLG, a polyester that is FDA approved), solid lipid nanoparticles (SLN, produced using high-melting lipids) [22], chitosan (a hydrophilic and cationic polysaccharide obtained from crab shell) etc., opened the way for the clinical application, mostly in cancer treatment, of polymeric nanomaterials. Usually biodegradable polymers are organic macromolecules (where carbon is the main component) that contain etheroatomes (-C-O-; -C-N-; -C-S-) to facilitate degradation via hydrolysis, oxidation and bond cleavage [23]. Such type of polymers can be included in what we previously classified as 1st generation of nanomaterial. Another challenge for material and polymer chemists, was targeting polymeric nanomaterials towards specific tissues and organs: this was first achieved by decorating the nanoparticles with hydrophilic and flexible polymers like PEG (PEGylation). This simple physical-chemical strategy allowed the nanocarries to avoid opsonization enabling them to passively target diseased

tissues due to the enhanced permeability and retention effect (i.e. tumor tissues). New efficient bio-conjugation strategies, such as 1,3-dipolar cycloaddition, commonly known as click-chemistry, offer many possibilities for nanocarrier functionalization with targeted ligands [24] (i.e. antibodies) in order to deliver drugs into a specific cell population, or with tracking molecules (i.e. fluorescent probes) for *in vitro* and *in vivo* imaging. Advances in synthetic polymer chemistry (including control of 3D architecture to form hydrogels), the introduction of biodegradable materials, and advances in understanding of endocytosis and intracellular pathways in health and disease opened new opportunities for design and clinical use of polymer based therapeutics. Readers can find a comprehensive list of polymer therapeutics and other nanomedicine products in clinical use and clinical development in the references [25] and [26]. Here we describe a specific group of nanomaterials made from amphiphilic self-assembling block copolymers, as we are actively involved in the design, development and functionalization of such a class of macromolecules for their application as a nanoscale drug delivery systems.

Amphiphilic self-assembling block copolymers

Block copolymers are polymer chains covalently linked as a series of two or more blocks. They can be designed to have the same amphiphilic character as lipids and to self-assemble in water in a wide range of morphologies based on their block composition.

Several block copolymers have been investigated by pioneering scientists (among the others Kazunori Kataoka, Dennis Disher, Alexander Kabanov and Jeffrey Hubbell) in the past few decades [27-30], including Pluronics®, PLG, PEG-PCL, PEG-PLL, PEG-PAsp, PEG-PLys etc. The synthesis of these materials was inspired by engineering principles developed by nature itself, specifically, the intrinsic ability of biological proteins and peptides to self-assemble and form solid nanofibrils or hollow nanotubes. Based on previous achievements and inspired by the same principles, over the past 10 years, we have developed block copolymer-based nanomaterials applicable as nanoscale drug delivery systems within the fast-growing fields of drug delivery and tissue engineering.

Design and development of bioinspired polymeric nanomaterials for drug and gene delivery

Here we report the achievements of a relatively new family of block copolymers based nanomaterials developed and optimized by Dr. Hubbell (University of Chicago) and Dr. Velluto. This family can be divided in three main generations (Scheme 1A-1C), the design of which was based on focused experimentation, inspiration driven by observing natural phenomena, and state of the art:

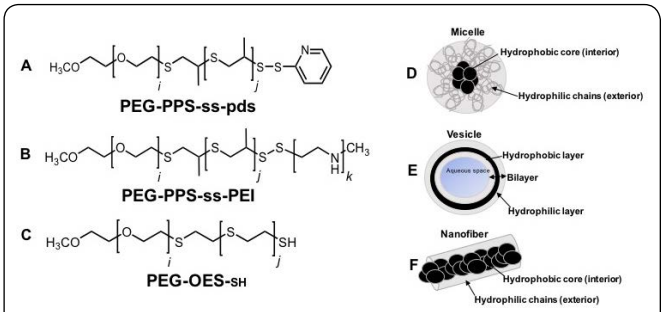
1) PEG-PPS diblock copolymers

2) PEG-PPS-ss-PEI triblock-copolymers

3) PEG-OES diblock copolymers.

PEG-PPS vesicles, filaments and micelles

The first generation of bioinspired nanomaterial that was developed is a highly customizable, yet low cost nanomaterial-based drug delivery system made of poly(ethylene glycol)-poly(propylene sulfide) (PEG-PPS) block copolymers (Scheme 1A). PEG-PPS block-copolymers are macromolecular amphiphiles prepared by anionic living polymerization, where the PEG block was selected because it is hydrophilic, already used in the pharmaceutical industry (e.g. stealth liposomes) and capable of material passivation and of delaying the protein clearance of the circulating material. The PPS is the hydrophobic polymer characterized by a very low glass transition temperature that permits easy manipulation of the monomer during the synthesis. Due to the presence of the sulfur, the PPS block is sensitive to oxidation [31]: in an oxidative environment (e.g. in the lysosome compartment) the polymer becomes more water soluble and, as such, non-toxic. Because PEG is water soluble and PPS is strongly water insoluble, these block copolymers can self-assemble into a variety of nanostructures (Scheme 1D-1F), including vesicles, solid core micelles, and filaments, all exhibiting tunable drug encapsulation capacity and allow rapid and stable incorporation of therapeutics without the need for chemical conjugation [32, 33]. These block copolymers offer considerable advantages compared to classical amphiphiles such as low molecular weight surfactants and phospholipids, or more common block-copolymers such as Pluronics®. The main advantages include:



Scheme 1: Generic chemical structure of **A**) poly(ethylene glycol) – poly(propylene sulfide) (PEG-PPS) diblock copolymers [32]; **B**) poly(ethylene glycol) – poly(propylene sulfide) – (polyethylene imine) (PEG-PPS-ss-PEI) triblock copolymers [39]; **C**) poly(ethylene glycol) – oligo(ethylene sulfide) (PEG-OES) diblock copolymer [40]; **D-F**) schematic representation of a micelle, a vesicle and a nanofiber respectively. Micelles are designed for loading hydrophobic drugs in their core; vesicles can host hydrophilic drugs in their aqueous space and amphiphilic molecules in their polymeric membrane (bilayer); nanofibers are designed to be more stable than micelles and carry hydrophobic drugs for a prolonged period of time in the blood circulation.

(i) The chemical synthesis is performed in mild conditions; therefore biological species can be incorporated in the polymer structure. The 'one pot' character of the synthesis [34] allows the use of the method by operators with limited skill in organic synthesis. No traces of toxic reagents or solvents are left after the purification.

(ii) They can self-assemble into nanostructures at very low critical aggregation concentration (CAC) [35]. A very low

CAC confers great stability to the drug delivery system and overcomes an important drawback of conventional amphiphiles and macroamphiphiles such as liposomes and surfactants, which limits their *in vivo* circulation times.

(iii) The aggregation morphology is predictable and controllable by chemically changing the ratio of hydrophilic to hydrophobic components. By changing the composition ratio, the copolymers can self-assemble into a variety of nanostructures, including vesicles, solid core micelles, filaments, and hydrogel networks.

(iv) The unique chemistry of these block copolymers allows rapid and stable incorporation of therapeutics with diverse properties without the need for chemical conjugation. For example, in the case of cyclosporine A, a notoriously hydrophobic calcineurin inhibitor, the use of the PEG-PPS micelles enables more than 100-fold increase of drug solubility in aqueous solution [32]. Moreover, these block copolymers can be further functionalized for additional applications such as the delivery of nucleotides, peptides or proteins and for targeted delivery via disulfide exchange at the end of the PPS chain. A disulfide bond can also be introduced between the hydrophilic and hydrophobic blocks (i.e. PEG-ss-PPS) to make the nanostructures sensitive to a reductive environment. This sensitivity triggers the nanoparticle disruption and their content release within the endosome, the interior of which becomes more reductive due, for example, to the influx of cysteine [33].

(v) They are non-immunogenic and non-toxic to mammalian cells and have revealed no detectable side effects when administered to mice [33, 36].

(vi) Finally, due to their adequate size (in the 20 to 100 nm range), these systems preferentially traffic to lymph nodes (LNs) and target specific immune cell populations that reside there and are responsible for modulating the response to allografts [36]. It has been previously shown that particles smaller than 100 nm are efficiently transported through lymphatic vessels to LNs draining an injection site [36-38].

PEG-PPS-ss-PEI cationic micelles as non-viral gene delivery system

With PEG-PPS block-copolymers as a starting template, triblock copolymers of PEG-PPS-ss-PEI (PEI is a polyethylene imine cationic polymer) (Scheme 1B) can be prepared and used to construct micelle–DNA and micelle–mRNA complexes of controllable diameter (~30 nm). Such micelle-DNA complexes demonstrated higher transfection efficiency and reduced cytotoxicity compared to standard gene-delivery systems *in vitro* and *in vivo* in a tumor immunotoxicity model in mice. Velluto et al. investigated the transfection with an antigen *in vitro* and *in vivo*, first looking just at the expression level of the protein (using real-time PCR) in B16-F10 melanoma cells and in the tumor tissues, then the effect of the antigen transfection on the tumor growth in mice that had already developed an immune response against the antigen. *In vivo*, micelle-pDNA complex-based antigen led to consistent reduction of tumor growth relative to control-transfected tumors that received the micelle-empty pDNA and also relative to the tumors receiving only the plasmid [39]. Therefore, PEG-PPS-ss-PEI triblock copolymers are currently being investigated for the delivery and sustained release of oligonucleotides, including aptamers.

PEG-OES nanofibers and hydrogel networks

The most recent block copolymers of this family are made of poly(ethylene glycol)–oligo(ethylene sulfide) (PEG-OES) (Scheme 1C) and were designed for higher nanomaterial stability upon oxidation [40]. In these block-copolymers the PPS block is replaced by the OES, selected based on his physical-chemical properties similar to PPS but characterized by higher crystallinity. The combination of its hydrophobicity and crystallinity leads the self-assembling into long linear-fibrils (nano-fibers, Scheme 1F). Further, these nanofibers can physically cross-link to form three-dimensional networks in water, i.e. hydrogels. These assemblies exhibit robust aqueous stability and capability of mechanical deformation, in addition to their utility as drug delivery systems [40]. The chemistry of the hydrogels makes possible easy manipulation of their viscoelastic properties and of their gelation kinetics, permitting their applications for cell encapsulation, local and sustained drug delivery, and as scaffolds for cell transplantation and tissue regeneration.

In summary, the preparation of sulfide-containing block copolymers and their role in supramolecular assemblies as nanoscale drug delivery systems have been revisited. Given the ease and efficiency with which the block copolymer nanostructures may be loaded, the high load of bona fide soluble drug that they contain, and their oxidative mechanism of degradation, they can be employed in a number of applications. These include cancer chemotherapy, as well as situations of surgery, when the sustained release characteristics of the materials may be particularly useful. Although these systems show significant promises, this relatively new family of block copolymers has still a long way to go before reaching the clinic: deeper biodistribution, pharmacokinetic and toxicology studies *in vivo* are required to prove their safety and efficacy and to get acceptance from the FDA.

Novel Clinical Applications of Nanomaterial-based Drug Delivery

Multiple drugs tumor treatment

Most nanomedicine studies concentrate on cancer treatment [41]. It is well known that due to the presence of leaky vasculature and defective lymphatic drainage in solid tumors, nanoparticles can selectively accumulate in the tumor via the mechanism of the enhanced permeability and retention (EPR) effect. In addition, the combination of multiple drugs has been recently demonstrated to be more effective than single treatment. However, the different physicochemical and pharmacokinetic (PK) profiles of each drug make the optimal administration and delivery very challenging. Nanomaterial systems have the potential and efficacy to combine delivery

of multiple drugs, thereby unifying their PK profiles. For example, co-encapsulation of a pro-apoptotic drug and an antiangiogenic agent in pH-sensitive nanoparticles provides a promising strategy to effectively inhibit malignant neoplasm progression in a synergistic manner, reducing the drug toxicity [42].

Thus, nanoparticles are uniquely suitable for delivering diagnostic and/or imaging agents, chemo and gene drugs, and agents for immunotherapies in tumors. Here we refer to some of the very recent works and reviews reported in literature [43-46].

Targeted and local immunomodulation in cell transplantation

Nanomaterials also have enormous potential in the treatment of other medical conditions that could benefit from targeted and local drug delivery, including cellular therapies for treatment of type 1 diabetes, an autoimmune disease that affects over three million Americans. Current approaches under evaluation in our laboratory integrate new technologies to optimize pancreatic cell engraftment using nanomaterials designed for local and targeted delivery of immunomodulatory drugs.

In current clinical practice, the therapeutic promise of organ, as well as cell transplantation, is considerably limited by the need of lifelong immunosuppression. Despite all advances, systemic and long-term immunosuppression is unavoidably accompanied by serious side effects including organ toxicity and increased susceptibility to infections and malignancies. As largely recognized, targeted delivery of drugs or other therapeutics to the organ, tissue, or cell where it is required may address some of these problems. Indeed, transplant-based approaches could become more widely applicable, if a successful localized immunosuppression and anti-inflammatory regimen could be established.

These problems are worsen in the case of pancreatic islet transplantation [47], as all existing systemic immunosuppression regimens have serious negative effects on the engraftment, function, and survival of transplanted islets, thereby limiting the success of islet implantation [48, 49]. In addition, most therapeutic candidates for immunosuppression and anti-inflammatory regimen, including most clinically approved immunosuppressive drugs, have poor water solubility and poor stability. Therefore, delivering and maintaining therapeutically relevant levels of immunomodulating agents is challenging. To overcome these challenges, the use of nanomaterial-based drug delivery system looks promising and valuable for effective immune-therapies that are lymph node targeting or localized to the vicinity of the transplant graft [36]. Reddy et al., reported the potential of ultra-small nanoparticles (Pluronic-PPS nanoparticles of 25 nm in diameter) to be efficiently transported by the interstitial flow into lymphatic capillaries and their draining lymph nodes when delivered intradermal or subcutaneously, targeting half (or more) of the lymph node–residing dendritic cells [50, 51]. If delivered from the transplant site, such nanoparticles would likely target the draining lymph nodes. Those nanoparticles have been used for vaccines, but the potential exists to adapt the technology for the blockade rather than induction of T cell activation.

Biodegradable polymers have been used extensively to deliver everything from small molecule drugs to large bioactive proteins and the genes encoding these proteins [52]. Multiple agents can be delivered simultaneously, and the polymers can be engineered in order to make the delivery persisting for hours, days or weeks [48]. For example, PLG (poly(d,l-lactide-co-glycolide)) microspheres are an established (FDA approved) platform for localized factor delivery to a target site [28]. The PEG-PPS and PEG-OES block copolymer families, described above, have shown great potentiality as local drug delivery systems, even though more experimental investigation must be carried out to obtain FDA approval for clinical uses. Alternatively, drugs and other therapeutic agents may be delivered directly from the scaffold used for cell transplantation [48], with the scaffold functioning as a support for growth and a vehicle for sustained release. For example, localized production or pre-treatment with angiogenic factors, such as VEGF and platelet-derived growth factor have been employed to improve vascularization and subsequent islet function and engraftment.

The current goal in cell transplantation is to develop a clinically applicable local immunomodulation platform using biodegradable polymeric nanomaterials, that improves drug solubility, stability, and site targeting and that can be combined with the novel approaches for cell transplantation [53].

Nanomaterial-incorporated stem cell therapy

Nanotechnology based drug delivery is also being investigated in the field of stem cell therapy mostly for tissue and organ regeneration. Here we describe the link between those modern technologies.

Although many researchers are adopting the strategy of stem cell transplantation, or their differentiated derivatives, for tissue engineering and repair, this technology does have a number of limitations, including cost, safety issues and immune rejection [54]. Nanotechnology represents a strong frontier to overcome those drawbacks. One of the strategies adopted to promote stem cell differentiation into various lineages, and their possible therapeutic applications, such as transplantation, include the use of nanomaterials and nanoparticles. Nanomaterials can provide a better control of the adhesion and differentiation of stem cells *in vitro* and *in vivo* in the transplantation site. The advantage is that nanomaterials can be engineered to form biomimetic scaffolds tailored in order to reproduce the biological and mechanical properties of the original extracellular matrix (ECM), and this is of tremendous importance for tissue regeneration. On the other hand, the extremely small size and large surface area of nanoparticles, allow them to readily enter the cell and enhance various molecular changes, or deliver drugs, genes and proteins. Therefore, has been proved that nanoparticles of different kind and composition can strongly stimulate stem cell differentiation into osteogenic, adipogenic, chondrogenic, neuronal, myogenic, and hematopoietic cell lines [55].

An alternative, or adjunct, to transplantation of cells, is to manipulate the stem cells directly *in vivo* [56], for example, by stimulating their proliferation or differentiation into the cell type needed by administration of therapeutic molecules, (genes, proteins and drugs). However, this approach requires therapeutically targeting progenitor cells and their niche *in vivo*, but there are several challenges therein, not least that of achieving specificity of delivery and responses. To maximize the therapeutic effect of those drugs and biomolecules, their sustained and controlled release is crucial and can be achieved by using tailored nanoparticles. Given the properties of biocompatibility and biodegradability of the nanoparticles, many studies now exist on the use of nanomaterials to prolong the expression of proteins for improved angiogenesis and recruitment of stem cells for tissue and organ regeneration (i.e., cardiac and vascular tissues [57, 58]). Among the others, carbon nanotubes (CNTs), PEGylated multi walled carbon nanotubes (MWCNTs) [59], chitosan nanoparticles (CSNPs) [60], poly(lactic-co-glycolic acid) (PLGA) scaffolds [61], polycaprolactones (PCL) scaffolds [62], poly-L-lactic acid (PLLA) [63], polyethyleneimine (PEI) have been already explored [64].

The cellular uptake of nanoparticles by endocytosis can be modulated by varying their size, shape, surface chemistry and concentration. Modulation of nanoparticle endocytosis and cellular delivery of their content can strongly influence the process of cellular differentiation and proliferation, revealing their utility in tissue engineering and regenerative medicine.

Conclusions

Although great progress has been achieved in the field of nanomedicine, and in particular, in the nanoscale drug delivery systems, several challenges remain before nanomaterials can become a largely accepted platform for drug administration and site specific targeting. Only a few of them are currently on the market (e.g. the already mentioned Abraxane®[20] and Endorem®[65]). A few reasons are that many nanomaterials have poor drug loading efficiency (< 5%, weight of the drug respect to the nanocarrier) or too rapid a release profile after administration ("burst release"). Therefore, the design of ideal, multifunctional nanomaterials for drug and gene delivery is still a challenge. In this review, only a few examples of nanomaterials were discussed while the field and its applications are still in development. In particular, synthetic polymers offer the ability to modify their chemistries to control their structural and therapeutic properties, their stability and biocompatibility, in order to develop efficient nanomaterials for innovative nanomedicine strategies. Therefore, chemists with physicists, biologists and clinicians should exploit their creativity, to find "nano-solutions" to the complex, multidisciplinary medical problems.

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