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Three-Dimensional Porous Scaffolds at the Crossroads of Tissue Engineering and Cell-Based Gene Therapy

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ABSTRACT

In the last 20 years, more than 1,500 gene therapy clinical trials have been approved worldwide targeting a variety of indications, from inherited monogenic diseases to acquired conditions such as cancer, cardiovascular and infectious diseases. However, concerns about the safety and efficacy of gene therapy pharmaceuticals justify the development of alternative strategies to ensure the clinical translation of this still promising field. In particular, ex vivo gene therapy strategies using autologous adult stem cells coupled to three-dimensional (3D) porous scaffolds show great promises in preclinical studies. Developments in the fields of biomaterial sciences and tissue engineering have already helped understanding how we can harness to regenerative potential of many cell types to create artificial tissues and organs and vastly improve the engraftment of ex vivo manipulated adult stem cells. In this article, we will review the current state of the art in tissue engineering by exploring the various types of clinically available biomaterials and the methods used to process them into complex 3D scaffolds. We will then review how these technologies are applied in cell-based gene therapy and identify novel avenues of research that may benefit patients in the near future. J. Cell. Biochem. 108: 537–546, 2009.

KEY WORDS: GENE THERAPY; CELL THERAPY; 3D POROUS SCAFFOLDS; BIOMATERIALS; TISSUE ENGINEERING; ADULT STEM CELLS

Gene therapy is defined by the use of recombinant genetic material therapeutically to correct the genotypic defect causing a disease or to modulate a pathological response. Since the first human treated by gene therapy in 1989, 1,537 clinical trials had been approved worldwide as of March 2009 (see http://www.wiley.co.uk/genmed/clinical/). Despite the fact that gene therapy was first conceptualized as a treatment option for inherited monogenic diseases, a large fraction of those studies targeted acquired conditions such as cardiovascular, neurological, and infectious diseases, as well as cancer. As a result of this broad application of gene therapy, we now have 20 years of clinical experience with gene therapy targeting various types of cells or tissues with different vectors (viral and non-viral) and different strategies (ex vivo and in vivo gene transfer) [Edelstein et al., 2007]. The implementation of standard clinical gene therapy protocols in routine clinical practice has, however, been slower than first expected due to the very few clinically meaningful gene therapy successes and the risks associated with platform-specific immunogenicity and genotoxicity. Nevertheless, this clinical experience allows us to draw certain conclusions about how gene therapists should direct future research to facilitate clinical translation of this still extremely promising field.

In its simplest form, gene therapy consists of either local or systemic delivery of a gene transfer vector. This approach has a number of inherent limitations that include: (1) inadequate gene transfer efficiency and gene expression, (2) inefficient targeting of appropriate cells or tissues, and (3) poor overall safety profile of the vectors (fatal immune responses, insertional mutagenesis, detection of viral vectors in semen) [Thomas et al., 2003; Nathwani et al., 2005; Porteus et al., 2006]. Another approach consists of using ex vivo modified cells, autologous or allogeneic, to be grafted into the patient. Although this avoids systemic dissemination vectors in subjects, it is also limited by the safety, low engraftment, and immunogenicity of ex vivo gene-engineered cells. The lack of safety and efficiency of these approaches to gene therapy justifies on-going development of alternative cell and gene delivery strategies addressing these specific issues.

The integration of biomaterial engineering, tissue engineering, and stem cell research provides us with innovative tools for developing delivery platforms maximizing safety and efficacy.

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Biomaterials have been used as gene and protein delivery vectors, to sustain long-term engraftment and differentiation of cells, to produce immuno-isolation devices and for ectopic transplantation of cells. Novel biomaterial processing techniques allow us to produce custom-designed three-dimensional (3D) porous scaffolds that can be used in combination with genetically modified, autologous somatic stem cells. However, because of the multi-disciplinary nature of this field, the enormous therapeutic potential of 3D porous scaffold-based gene therapy remains highly experimental to date.

We will here review the use of 3D biocompatible porous scaffolds in cell-based gene therapy. We will first describe the main classes of clinically useful biomaterials and the techniques used to process them into 3D porous scaffolds. The last section will cover the main indications targeted by gene therapy and explore how these could benefit from the use of 3D scaffolds and tissue engineering technologies. We will refer the reader to recent reviews and apologize for not being able to cite individual work for lack of space.

BIOMATERIALS USED FOR 3D POROUS SCAFFOLDS: THE BUILDING BLOCKS

Successful transplantation and long-term survival of a tissue-engineered construct require hierarchical design of the scaffold at different levels of complexity (Fig. 1), the first of which being the biochemical composition of the scaffold itself. The ideal biomaterial should be biocompatible, degrade (with non-toxic degradation products) at a predictable rate to be replaced by extracellular matrix (ECM), and possess a mechanical modulus similar to that of the tissue it is re-creating (this ensures integration with adjacent tissue but also influence cell fate, differentiation, and survival) [McBeath et al., 2004; Engler et al., 2006]. Modifications of traditional biomaterials to increase their biological activity (such as incorporation of cytokines, growth factors, peptide chains containing RGD moieties, etc.) have been largely explored, but this exceeds the scope of this review. We will here describe the main categories of biomaterials as well as their respective uses and limitations. These are also summarized in Table I.

NATURAL POLYMERS

Natural polymer-based biomaterials can be classified into two categories: protein-based and polysaccharide-based [Mano et al., 2007]. They are usually processed as gels or thin films for transplantation and are typically short lived in vivo (1–4 weeks) unless highly cross-linked by chemical agents. Protein-based biomaterials have been widely used because they are made of bioactive molecules mimicking the extracellular environment and contain motifs for cell adhesion. The most commonly used are collagen, fibronectin and fibrin. Polysaccharide-based materials are obtained from animal sources (hyaluronic acid, chitin, and its derivative chitosan) from algae (agar, alginate), or microbial sources (dextran). Alginate microcapsules can be used as immuno-isolation devices for the implantation of xeno/allogeneic cells, allowing diffusion of small molecules (oxygen, glucose, waste products) while avoiding immune rejection [Chang, 1999]. In addition to their short persistence in vivo, limitations to natural polymer-based materials are their poor processability into complex 3D structures, low mechanical modulus, and inconsistency due to their organic origin. However, they are useful when a localized and short-term response is sufficient and they can be delivered non-invasively by percutaneous injection.

SYNTHETIC POLYMERS

To address the aforementioned limitations of natural polymers, synthetic polymers have been created and already extensively used. The most important class for tissue engineering are poly (α-hydroxyesters) and copolyesters of lactic and glycolic acid [Yang et al., 2001]. The most common forms of these polymers are polylactic acid (PLA), polyglycolic acid (PGA), and copolymers thereof [poly(lactic-*co*-glycolic acid), or PLGA]. These materials were first developed as materials for sutures and have been used clinically for over 20 years. They owe their broad use on their good biocompatibility and non-toxic degradation products (lactic acid and glycolic acid), which are produced by simple chemical hydrolysis (i.e., non-enzymatically; making their degradation rate highly consistent and predictable) and eliminated through normal metabolic pathways. The degradation rate and mechanical modulus of these polymers can easily be modulated by varying the lactide/glycolide ratio and polymerization conditions. As a general rule, PLGA degrades most rapidly, followed by PGA and PLA, respectively. Poly(ε-caprolactone) (PCL) is another similar polymer (and suture material) that has received recent attention in the field of tissue engineering. All of these synthetic polymers can be processed into porous 3D scaffolds using a variety of techniques. Their main limitations are the release of acidic degradation products (affecting cell behavior and survival) as well as their lack of chemically reactive side chains for attachment of peptides, growth factors, or other biological signals. However, coating with collagen or serum is usually sufficient to allow initial cell adhesion and ECM deposition.

CERAMICS

Biomimetic ceramics were initially developed as substitutes to metal-based implants (stainless steel, cobalt, titanium) in orthopedic and maxillofacial surgery [Ohgushi and Caplan, 1999; Yang et al., 2001]. Metals possess good mechanical modulus but are not biodegradable, do not integrate into adjacent tissues (no bone-bonding), and have a finite lifespan. The most commonly used bioceramics are made of calcium phosphate (mimicking the mineral phase of bone) and are bioactive and biodegradable. These include non-sintered hydroxyapatite (HA), α- and β-tricalcium phosphate (TCP), tetracalcium phosphate, and octacalcium phosphate. Non-resorbable bioceramics such as Bioglass, sintered HA, and alumina (Al_2O_3) are also used clinically but have limited use in tissue engineering as they are non-resorbable and non-bioactive. Bioceramics not only mimic the structure and composition of bone tissue but are also osteoconductive (they induce osteoprogenitor cells differentiation) [Ohgushi and Caplan, 1999]. Furthermore, as each bioceramic has specific osteoconductive properties and degradation rate, the speed of bone formation and scaffold degradation can be modulated by mixing ceramics at various ratios (HA/β-TCP composites, for instance). In general, HA promotes

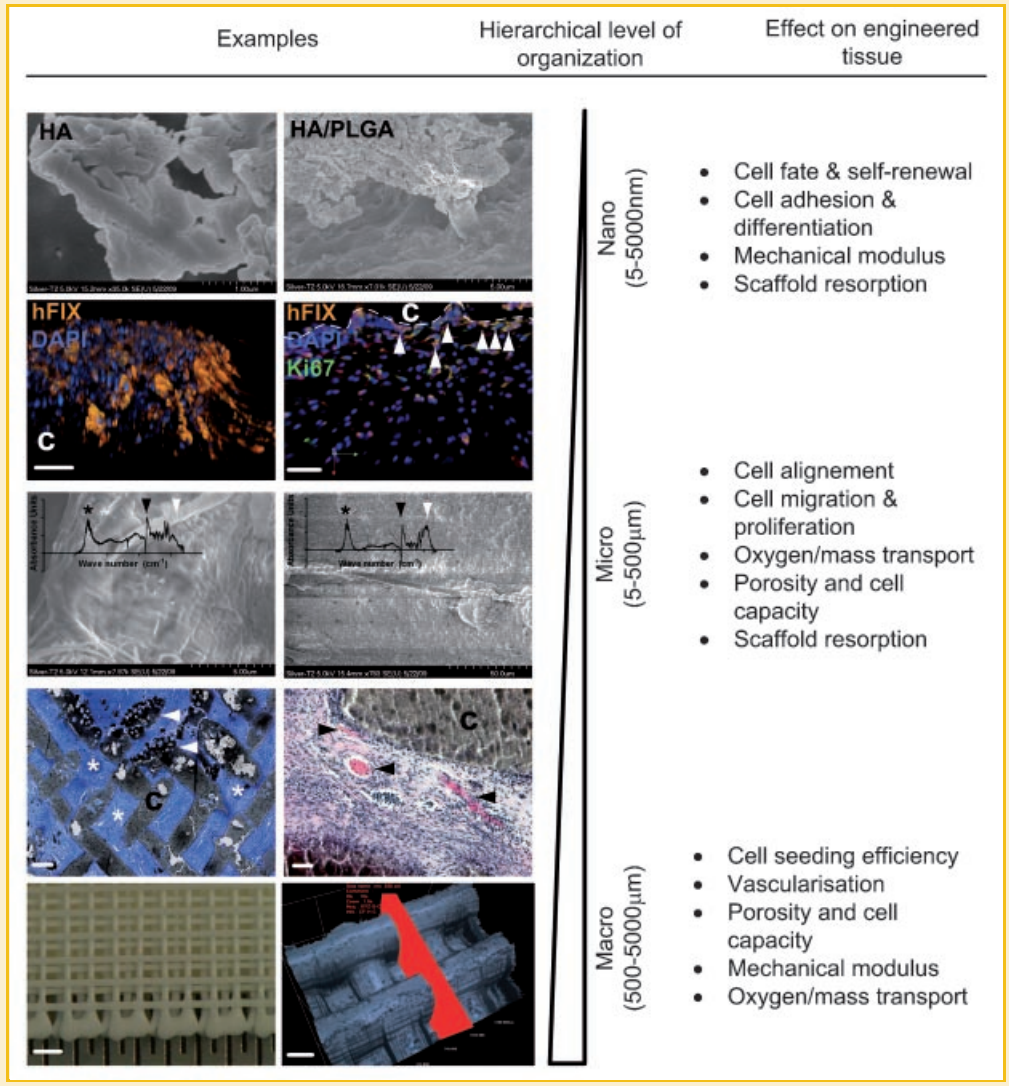

Fig. 1. Hierarchical organization of porous scaffolds and its effect on engineered tissue. From top to bottom and left to right: Hydroxyapatite (HA) crystals seen under scanning electron microscope (SEM). SEM of HA/PLGA composite scaffolds showing HA aggregates embedded in the PLGA polymer. Adhesion and spreading of human coagulation factor IX (hFIX) expressing MSC on HA/PLGA carrier (c) scaffolds seen by confocal microscopy (scale bar = 50 μm). Confocal microscopy also shows the persistence of hFIX + MSC co-expressing the proliferation marker Ki67 (white arrowheads) at the biomaterial/tissue interface (dotted line) 12 weeks post-implantation in mice, suggesting HA maintains MSC self-renewal in vivo (scale bar = 50 μm). Scaffold microtopology modified by coating with collagen (left) of mineralized collagen (right); the efficiency of the coating is determined by SEM and its quality by FTIR spectroscopy (insets); a relative increased signal for phosphate (white arrowheads) is seen in mineralized collagen compared with amine (black arrowheads) and hydroxyl signals (black asterisks). Micro- and macrotopology affect cell capacity (white asterisks), fate, and differentiation (black von Kossa staining indicating osteogenesis) as well as scaffold resorption (white arrowheads; scale bar = 200 μm). Adequate pore size and interconnectivity are required for efficient vascularization (black arrowheads; scale bar = 50 μm). HA/PLGA composite scaffold with a simple architecture created using a Bioplotter to maximize cell capacity and oxygen transport (scale bar = 500 μm). Scaffold topology and pore size as seen with confocal microscopy reveal a pore size of 300 μm, adequate for bone tissue formation (scale bar 200 μm).

fast bone formation but degrades very slowly whereas TCP is less osteoconductive but is replaced more rapidly by ECM. Other factors that can be manipulated to modulate biodegradation rate and osteoconductivity include total surface area of the particles, crystallinity, crystal size, and crystal perfection. The main limitation in the use of bioceramics resides in their poor processability; however, ceramic micro-/nanoparticles can be incorporated into synthetic polymers such as PLA or PGA to create complex 3D architectures.

PROCESSING OF BIOMATERIALS: CREATING 3D POROUS SCAFFOLDS

While scaffolds biochemical composition affects mechanical modulus and resorption as well as cell fate and behavior, proper engineered tissue function, engraftment, and survival require an appropriate 3D architecture and topology at the macro-, micro-, and nanoscale levels [Fig. 1 and Griffith, 2002; Muschler et al., 2004]. Macrotopology dictates cell capacity and distribution within the

TABLE I. Properties of Commonly Used Biomaterials

Biomaterial	Resorption rate	Mechanical modulus	Processability	Application	Comments
Natural polymers					
Collagen	Fast (1–2 weeks)	Low	Low	Local injectable delivery	Cross-linking can improve persistence and strength
Fibronectin					
Fibrin					
Synthetic polymers					
PLA	Long (months)	High	High	Surgical local delivery	Adjusting PLA:PGA ratio in PLGA affects persistence and strength
PGA	Intermediate	Intermediate			
PLGA	Adjustable	Adjustable			
PCL	Intermediate	Intermediate			
Ceramics					
Alumina	Long to intermediate (months to years)	High to intermediate	Low	Injectable (particles), surgical (3D scaffolds)	Processing facilitated by emulsion in synthetic polymers
Hydroxyapatite					
Tri-, tetra-, octacalcium phosphate					

scaffold. Pore size, interconnectivity, and alignment also affect oxygen and mass transport (influx of nutrients, wastes elimination), cell seeding efficiency, the capacity to support vascularization, and the mechanical modulus of the scaffold. At the microscale level, the biochemical composition and topology of the cell–material interface directly influence cell morphology, differentiation, and migration. The incorporation of micropores can also dramatically increase oxygen and mass transport through the implant. The nanoscale topology of a substrate also dramatically influences various cell types fate and differentiation [Martinez et al., 2009]. This section will review the main techniques that have been used to process biomaterials into well-defined 3D porous scaffolds incorporating these various levels of complexity.

ISOTROPIC METHODS

A number of simple techniques have been used to create isotropically distributed pores of desired size into a wide variety of biomaterials [Sachlos and Czernuszka, 2003]. Porogen leaching, freeze drying, phase separation, and gas foaming all use the incorporation of particles, solvent, or gas bubbles into a material, which are then removed by dissolution (leaching) or evaporation. These techniques have been widely used because of their versatility but have intrinsic limitations, including the difficulty of obtaining interconnected pores with a desired orientation, of modulating the mechanical properties of the scaffolds, and the relatively simple architecture of the scaffolds produced. Electrospinning techniques use an electric field to deposit biomaterials strands in the desired orientation and have been used to create fiber meshes or arrays with strand diameters typically between 200 and 2,000 nm [Arumuganathar and Jayasinghe, 2008]. Electrospinning has been used with synthetic polymers to create thin meshes of various shapes that support cell adhesion and proliferation. This technique is thus promising to create simple, well-defined topologies at the microscale, but its use is limited for creation of larger, more complex 3D structures.

COMPUTER-ASSISTED METHODS

To allow greater control over scaffold architecture than isotropic methods, sophisticated techniques have been recently developed [Yang et al., 2002; Hollister, 2005]. These techniques use computer-assisted design to create customized 3D structures with well-defined internal architecture. Importantly, these techniques (often referred to as solid free-form fabrication or rapid prototyping) can be coupled to imaging data to approximate the anatomical defect to be repaired. There are three main types of solid free-form processing (Fig. 2), all of which use fused-deposition modeling to create a structure layer by layer, the fabrication platform being lowered for the next layer (which may or may not have the same topology). Laser-based systems are used to photopolymerize liquid monomers or to sinter powdered materials. 3D printing systems typically deposit a chemical binder or glue in a defined pattern on a powdered material layer. The most promising systems are probably 3D plotters, also known as *XYZ* plotters or nozzle-based systems. These systems are exemplified by the Bioplotter, the first commercially available system for solid free-form fabrication. The Bioplotter processes biomaterials thermally and/or chemically as it is extruded from a mobile and computer-controlled nozzle. This allows movements in two dimensions, whereas the mobile fabrication platform provides movement in the third dimension. Importantly, the Bioplotter is compatible with a wide variety of biomaterials and is also designed to allow plotting of biomaterials incorporating live cells. Moreover, its relatively small size allows the fabrication of scaffolds in laminar flow hoods.

CELL-BASED GENE THERAPY: INTEGRATING THERAPEUTIC GENES, CELLS, AND SCAFFOLDS

The final level of complexity in 3D porous scaffold-based gene therapy relates to the biological components of the tissue-engineered construct. Successful gene therapy requires good knowledge of the pathology to be treated as well as identification of the appropriate gene to be delivered and target cells or tissue. All of these will dictate whether an ex vivo strategy is feasible (for increased safety), the delivery vector to be used, and the level of transcriptional regulation needed. Whether the genetic defect has a cell-autonomous nature (when the protein product requires cell-specific expression) is also an important consideration. Indeed, in cell non-autonomous indications, the therapeutic gene can be introduced into potentially any cells or tissues (including tissue-engineered constructs implanted ectopically) to reverse the deficiency in a paracrine manner or in *trans*. This approach is particularly attractive for a wide range of monogenic diseases (Fig. 3 provides an example of this strategy). Alternatively, when tissue-

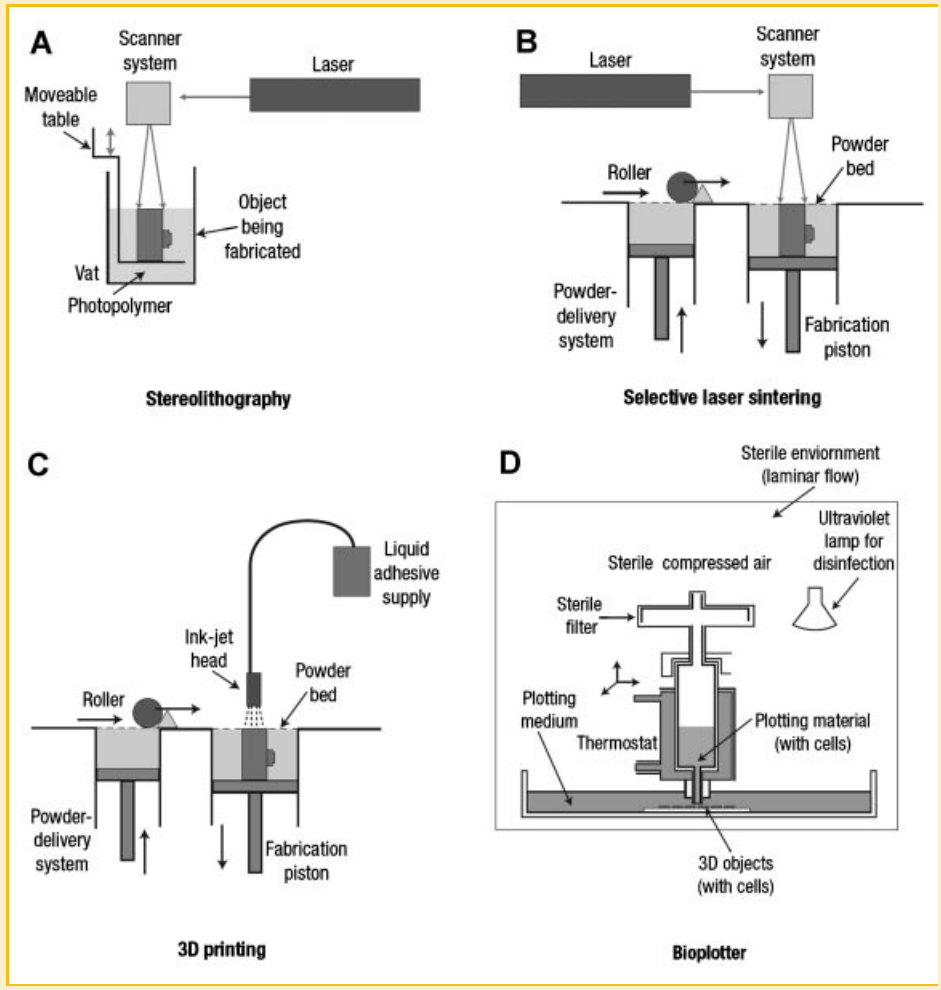

Fig. 2. Processing of 3D porous scaffolds using computer-assisted design and fused-deposition modeling. A: Stereolithographic platforms use a laser to photopolymerize a liquid or viscous biomaterial, layer by layer, into a 3D object. B: Other laser-based systems use sintering of a powdered biomaterial to create a 3D object. C: In 3D printing, an adhesive (glue) or a chemical binder is directly printed on a layer of powdered material. D: 3D plotter such as the Bioplotter extrudes materials through a mobile nozzle while simultaneously processing them either chemically or thermally. Depending on the material used and processing required, living cells can be incorporated in the material prior to the plotting, allowing good control over cell distribution. [All panels adapted and reproduced with permission from Hollister, 2005.] [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

specific expression is required, scaffolds can be used to facilitate delivery and engraftment of cells into a specific anatomic location for the treatment of cardiovascular, neurological, and orthopedic conditions for instance. In this section, we will review the main categories of diseases targeted by gene therapy and identify those that can benefit from the use of 3D porous scaffold technology. We will also review the pre-clinical data available relating to the use of 3D scaffolds for treating these conditions.

INHERITED MONOGENIC DISEASES

Liver protein deficiencies such as the hemophilias and metabolic liver diseases are a major class of monogenic diseases that could benefit from 3D scaffold-based gene therapy. The hemophilias are great candidates for gene therapy because they are well characterized, have a broad therapeutic index (levels as low as 2% of the normal plasmatic levels are considered therapeutic but levels at 150% are frequent), they are caused by deficiencies in plasmatic proteins circulating systemically (coagulation factors VIII and IX for hemophilias A and B, respectively) and neither protein requires tissue-specific expression and can thus be delivered ectopically [Lillicrap et al., 2006]. Moreover, large animal models are available for both diseases. Limitations to gene therapy of the hemophilias include the large size of the factor VIII gene (important for gene delivery vector design) and the high plasmatic levels required for factor IX (100 ng/ml are needed to achieve the 2% therapeutic threshold). Clinical studies have so far focused on in vivo delivery of adeno- or adeno-associated virus either to the liver or skeletal muscle and have demonstrated low efficiency (only short-term expression of the protein) as well as immune and toxicity problems associated with the viral vectors [Murphy and High, 2008]. One clinical trial using transfected autologous fibroblasts injected in the omentum [Roth et al., 2001] also demonstrated only marginal responses and no efforts were made to assess cell survival post-implantation.

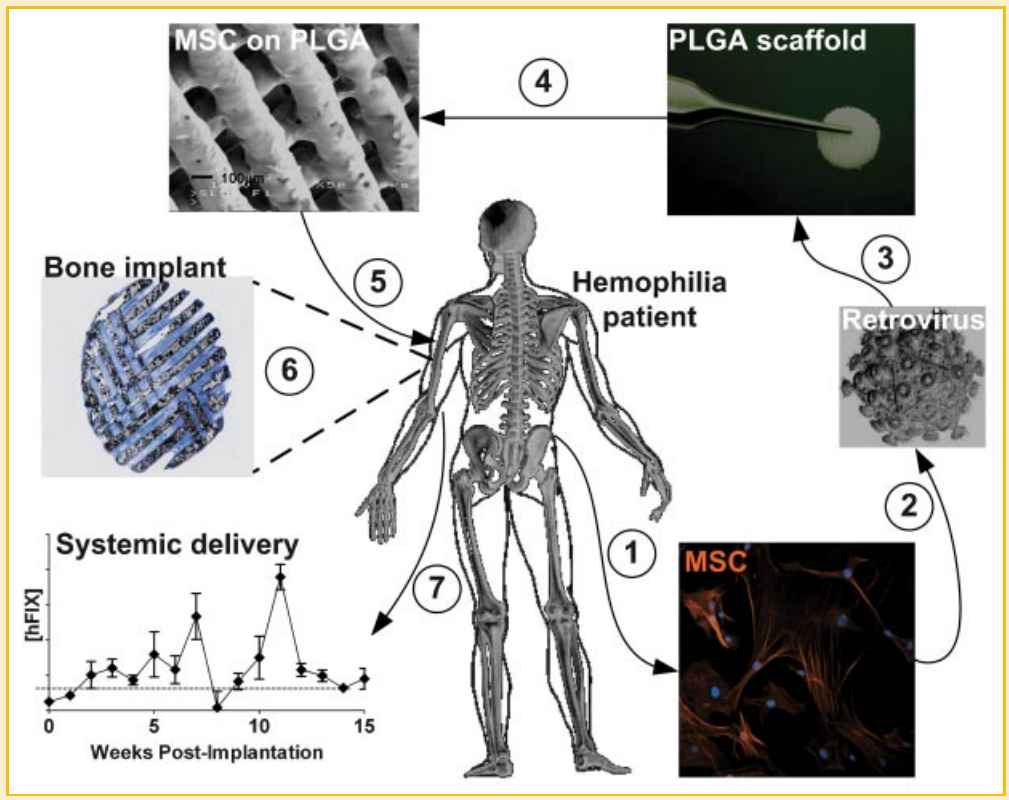

Fig. 3. Example of a simple 3D porous scaffold-based gene therapy platform for hemophilia B. Hemophilia B is a monogenic disease that can be treated by systemic delivery of coagulation factor IX (hFIX). In this platform autologous, patient-specific stem cells (here MSC) are isolated from the iliac crest (1) and characterized in vitro. The MSC are genetically engineered using a retrovirus to produce hFIX (2). The osteogenic stem cells are then seeded onto a biocompatible, osteoconductive porous scaffold (3) and allowed to colonize the material (4). The artificial bone can then be implanted ectopically (subcutaneously, for instance) allowing their easy removal for safety (5). The efficiency of bone formation can be assessed by imaging methods, but precise cell tracking and analysis of cell fate in vivo typically require histological methods (6). The efficacy of gene therapy for hemophilia is then measured by plasmatic hFIX levels over time (7). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

To increase the safety and efficacy of gene therapy for hemophilia, the use of retrievable, synthetic tissues or organs implanted subcutaneously or intraperitoneally has been suggested. In preclinical trials, encapsulated immortalized cell lines, xeno-/allogeneic cells as well as bioengineered muscles and Matrigel-embedded autologous endothelial cells have been tested in hemophilic animals [Hortelano et al., 2001; Garcia-Martin et al., 2002; Krebsbach et al., 2003; Thorrez et al., 2006; Matsui et al., 2007]. Most studies reported short-term plasmatic detection of the protein (averaging about 10 days) with very low levels achieved (below the therapeutic threshold). This reflects the low cell capacity of microcapsules and/or low engraftment of transplanted primary cells. Only autologous endothelial progenitor cells implanted subcutaneously in Matrigel demonstrated long-term correction of hemophilia A in mice (although the use of Matrigel for which no human equivalent is available is a clear limitation to the study). Work from our laboratory demonstrated that ectopic implantation of gene-engineered mesenchymal stromal cells (MSC) to create artificial endocrine devices can deliver various cytokines in vivo (erythropoietin, IGF-1, IL-2, IL-12, and chimeric fusion proteins). More recently, we showed that gene-enhanced MSCs implanted subcutaneously in an osteoconductive 3D porous scaffold provided long-term protein delivery in rodents (manuscript submitted). Our study demonstrated that optimization of the scaffolds at the macro-, micro-, and nanoscale levels was necessary to provide maximal cell delivery and survival, recombinant protein delivery, and osteogenesis by MSC, respectively. We used a 3D plotter (Bioplotter) to create porous scaffolds made of a HA/PLGA composite and modified the surface texture and biochemical composition with coating procedures. We showed that HA allowed long-term MSC self-renewal in vivo and thus long-term correction of hemophilia B. Taken together, these pioneering studies provided the proof of principle that ectopically implanted gene-engineered cells seeded onto a 3D porous scaffold can provide long-term systemic protein delivery in a safe and potentially reversible manner. However, these studies also highlight the complexity of designing efficient and clinically relevant systems for relatively simple diseases. The main considerations remain the choice of an appropriate cell source and careful design of the scaffold to allow maximal cell delivery and survival. Although any cell type could be used in theory, cell types which biology is well understood should be preferred as it greatly facilitates intelligent design of the scaffolds and analysis of cell fate post-implantation. Moreover, the use of solid free-form fabrication allows good control over total surface area (cell capacity) and pore interconnectivity (cell seeding, vascularization). It is also apparent that the composition of the scaffolds should reflect the cells

used and allow their self-renewal when long-term correction is sought.

Metabolic liver diseases may also be targeted by 3D scaffold-based gene therapy. Although not applicable to diseases having a cell-autonomous nature or requiring expression of the protein in most or all hepatocytes (ornithine transcarbamylase deficiency and other urea cycle disorders, α1-antitrypsin deficiency), diseases such as familial hypercholesterolemia, Crigler–Najjar syndrome, and others could potentially benefit from such technologies. There has been a few studies, clinical and preclinical, exploring the use of ex vivo gene-engineered hepatocytes for the treatment of those diseases, most showing only marginal responses [Brunetti-Pierri and Lee, 2005]. Poor cell engraftment may be identified as a cause. It is worth noting that physical interactions between hepatocytes and endothelial have been demonstrated to be necessary for proper hepatocyte differentiation and function [Pryor and Vacanti, 2008]. Thus, co-implantation of both cell types may be required. This important cross-talk between different cell types has long been recognized in developmental biology and is also required in other systems, such as during endochondral ossification. Nevertheless, the recent development of highly sophisticated 3D porous scaffolds for liver tissue engineering promise to circumvent problems associated with low hepatocyte engraftment [Borenstein et al., 2007; Fiegel et al., 2008; Hoganson et al., 2008]. These scaffolds have not yet been thoroughly tested in vivo, but the relatively simple tissue architecture and good regenerative properties of the liver should facilitate the development of bioartificial livers for partial liver replacement or ectopic implantation. However, an appropriate cell source will need to be identified, as hepatocytes from patients suffering from liver diseases may not be readily harvested or maintain their functionality ex vivo. The relatively high regenerative potential of the liver suggests the existence of adult liver stem cells and work is on-going to identify, isolate, and characterize them [Duncan et al., 2009]. Alternatively cell types with potential liver plasticity such as MSC or hematopoietic stem cells (HSC) may be considered.

Another important class of monogenic diseases (comprising more than 40 different entities) that can benefit from systemic delivery of a protein is lysosomal storage disorders (LSD) [Sands and Davidson, 2006]. These deficiencies in lysosomal enzymes are characterized by the progressive accumulation of their catabolic substrate and enlargement of the lysosomes, leading to multisystem anomalies. Many LSD are candidates for enzyme replacement therapy (ERT) since the discovery that many lysosomal enzymes can be delivered in *trans*, endocytosed by the mannose-6-phosphate-receptor-mediated pathway and correctly targeted to the lysosome [Desnick and Schuchman, 2002]. As such, they are also great candidates in gene therapy providing systemic protein delivery. LSD usually affect cells of reticuloendothelial systems (in the spleen, liver, and bone marrow) but some also have a central nervous system (CNS) involvement. Because circulating lysosomal enzymes cannot cross the blood–brain barrier, LSD with CNS involvement are usually not candidates for ERT or gene therapy providing systemic protein delivery. Of the LSD that are candidates for this type of therapies we note Fabry disease, Gaucher disease type I, Pompe disease, and mucopolysaccharidosis types I, II, and VI. Clinical trials for ERT of those LSD are on-going and have already helped identify obstacles that may also affect efficient gene therapy. For instance, only certain glycoforms of the lysosomal enzymes can be properly endocytosed by cells, some affected cells types have a low exogenous protein intake (such as chondrocytes in mucopolysaccharidoses) and animal models of LSD are scarce. Current gene therapy strategies for LSD have focused on the use of systemically delivered gene-engineered autologous cells, including HSC, MSC, and macrophages and as such still raise safety concerns. Alginate-microencapsulated cells have also been suggested as a potential strategy for LSD but experimental evidence of feasibility and efficiency in small animal models is still preliminary [Chang, 1999]. Systems such as those described above for hemophilia could be considered to ensure maximal cell delivery and survival while providing a safe and long-term correction of the disease.

CARDIOVASCULAR DISEASES

Gene therapy for cardiovascular diseases has mainly been considered to enhance the efficiency of cell-based therapies, either for myocardial regeneration after infarction or for peripheral arterial diseases. Cellular therapy has shown promising results in animal experiments but have yet to be replicated in humans [Laflamme and Murry, 2005; Segers and Lee, 2008]. It is difficult to draw any conclusions or make comparisons from the clinical trials conducted so far owing to interstudy variations in design, cell type used, outcome measures, delivery routes, and timing of therapy [Prockop and Olson, 2007]. Most trials have used intracardiac or intracoronary delivery of poorly characterized mononuclear cells derived from marrow or blood (cardiomyocytes, skeletal myoblasts, and endothelial progenitor-like cells have also been tested). One consistent fact emerges from the clinical and experimental data available: survival of cells transplanted in the heart is at best very low, independently of the cell type used. The use of genetically modified cells coupled to appropriate scaffolds may thus be necessary to achieve therapeutic efficacy. The standardization of study designs would also help the interpretation of the clinical data.

Strategies for myocardial repair include reconstruction of the cardiac muscle itself, modulation of angiogenesis to restore blood flow to the infarct zone, and modulation of the inflammatory response to limit scar tissue formation. The choice of cell type used, gene delivered, and scaffold design should reflect the desired therapeutic effect. Myocardial muscle regeneration will occur depending on the cell type and scaffold material used. The scaffold should allow long-term survival, migration, and proliferation of cardiomyogenic cells, but also support functional (electrical, mechanical, tissular) integration with adjacent tissue and sustain the mechanical stress in the heart. Angiogenesis and inflammation can be modulated by specific cell types or through genetic modification of the cells (with VEGF or FGF-2, for instance). In these cases, when short-term response is sought, injectable gels or thin films (cellular patches) may be sufficient. There is an extensive body of literature available on preclinical testing of various cell/biomaterial combinations and reviewing all of them exceeds the scope of this review [Davis et al., 2005; Jawad et al., 2007]. The use of porous scaffolds has generally improved cell engraftment in infracted myocardium, but cardiac tissue formation and functional

electrical coupling of the engineered construct with adjacent tissue has never been unequivocally demonstrated. Scaffolds based on PLGA or other synthetic polymers and processed using solid free-form fabrication should here be preferred as they provide better mechanical properties, oxygen/mass transport (of great importance because of the high metabolic demand of cardiac tissue) and control over input cell number, distribution, and density. The main obstacle to clinical translational of cardiac cell and gene therapy relates to the choice of an appropriate cell source. The recent identification of stem-like cells within adult cardiac tissue may help resolve this issue, although these cells have not been extensively characterized yet [Beltrami et al., 2003]. Their availability from autologous source in older patients suffering from cardiac insufficiency may also be problematic. Until an appropriate cell source is identified, the systematic testing, intelligent design, and optimization of scaffold materials remain difficult. As our knowledge about cardiac stem cells increases, a potentially interesting avenue would be to use genetically modified cells to activate and direct endogenous, resident stem cells to colonize the scaffold and promote myocardial repair, eliminating the need to isolate, expand, characterize, and differentiate those cardiac stem cells.

MUSCULOSKELETAL DISEASES

Cell-based gene therapies also show great promise for the regeneration of skeletal muscle, bone, and articular cartilage. Bone graft is often required to repair lesions caused by cancer, trauma (non-union fractures), for spine fusion, revision total joint arthroplasty, maxillofacial reconstruction, and segmental bone defect. The current gold standard is autogenous bone graft, where the patient's own bone (usually from the iliac crest) is used to repair the lesion. However, this technique is associated with severe donor site morbidity including pain, infection, and nerve damage. Because of the strong clinical need for bone tissue, osteomimetic biomaterials have been developed and already have a long history of clinical use. This combined with the identification of osteogenic stem cells (MSC) in adult bone marrow almost 40 years ago has made bone regeneration one of the most striving and well-understood fields of tissue engineering [Ohgushi and Caplan, 1999; Caplan, 2009]. The biology and fate of MSC seeded on calcium phosphate ceramics has been thoroughly studied both in vitro and in vivo. MSC seeded on these materials and implanted in bone defects or subcutaneously can recapitulate both developmental processes of bone formation: endochondral ossification and intramembranous ossification. Four major factors have been shown to be involved in bone formation; the osteoprogenitor cells themselves, an appropriate osteoconductive scaffold or matrix, osteogenic signals (morphogens and other cytokines), and importantly vascular invasion (for endochondral ossification).

Early trials for bone regeneration used biomaterials (mostly collagen gels) to deliver recombinant proteins [Gamradt and Lieberman, 2004]. The osteogenic proteins have been shown in clinical and preclinical trials to induce bone formation are: bone morphogenic proteins (BMP) 2, 4, and 9, IGF-1, TGFβ, FGF-1, PDGF, OP-1, and LMP-1. Clinically, the results observed were variable but overall disappointing. The studies demonstrated that a large fraction of the protein delivered was inactive, probably because of low bioavailability and inefficient presentation to the cells. In fact, most of these osteogenic proteins need to be incorporated in the ECM to have a biological activity. Because of this, a very high protein load was necessary to obtain minimal activity and this raised concerns about costs and the systemic effects of supraphysiological protein concentrations.

More recently, MSC seeded onto porous ceramic scaffolds have been tested in large animal models and humans and proved to be more efficient than scaffolds alone [Srouji et al., 2006]. Thus, the feasibility of this approach has been demonstrated and has shown no adverse effects. 3D porous scaffolds seeded with MSC engineered to produce osteogenic proteins have also been tested in large animal models and showed promising results [Cancedda et al., 2007]. However, bone reconstruction using genetically modified cells still requires some optimization before being implemented in the clinic. First, in most cases the mechanical properties of the regenerated bone do not match that of load-bearing bones. Because resorbable ceramics are favored but have lower mechanical modulus than non-resorbable ceramics, a compromise needs to be made between mechanical stability and material degradation rate. Second, bone formation on the scaffolds usually is restricted to the periphery of the implants, with central necrosis being observed. To address this issue, scaffolds with well-defined internal structure allowing oxygen and mass transport through the tissue as well as efficient vascularization are required. Finally, the macro-, micro-, and nanoscale topology of the material has been shown to have a profound effect on MSC behavior and differentiation. Recent applications of solid free-form fabrication techniques to bioceramic processing have successfully created highly porous scaffolds of defined architecture with very high mechanical modulus and will undoubtedly address most of these issues [Hollister, 2005].

Bioartificial muscle tissues are also needed for the treatment of various myopathies caused by trauma or muscular dystrophies. Most preclinical and clinical trials have so far focused on myoblasts transplantation therapy, using skeletal muscle satellite cells (muscle stem cells) injected without scaffold materials [Scime et al., 2009]. Limitations to this approach have been very low engraftment of the transplanted cells (below 10%) and the strong immune and inflammatory response induced by the multiple injections required (injections every millimeter are required to obtain relevant engraftment). Genetic modification of myoblasts with IGF-I or VEGF has been shown to increase their engraftment in vivo. Furthermore, a number of different biomaterials have been shown to increase muscle progenitor cells engraftment in skeletal muscle [Thorrez et al., 2008; Scime et al., 2009]. These include Matrigel, collagen gels, fibrin gels, PLA, and PGA. Synthetic polymers are probably the best candidate so far and have been the most extensively tested. They have good mechanical properties and have been shown to support myoblast fusion and myofiber alignment along the material strands. Because of the very high metabolic activity and oxygen demand of muscle tissue, special attention should be directed toward the careful processing of the material. As with cardiac and bone tissue engineering, the mechanical properties of the scaffolds are also of great importance for successful skeletal muscle regeneration. Alternative cell sources are currently tested in preclinical trials for muscle regeneration and include

muscle-derived side-population cells, muscle-derived CD34+ cells, and MSC.

NEUROLOGICAL DISEASES

The discovery of neural progenitor cells (NPC) in the adult and fetal brain in 1992 triggered a vast enthusiasm because it opened the possibility of repairing the brain and peripheral nervous tissues which were first thought to have very limited regenerative properties. Experimental work soon followed for the treatment of various pathologies including CNS and spinal cord injuries as well as neurodegenerative disorders. NPC are typically isolated from two distinct areas of the brain: the subventricular zone (in the anterior lateral wall of the ventricles) and the subgranular zone of the hippocampal dentate gyrus. However, experimental evidence suggests that they may be present in other, if not all areas of the brain. The isolation of NPC is a highly invasive procedure and as such, the use of autologous NPC for the treatment of neurological diseases remains difficult and particularly challenging to translate to the clinical setting. Nevertheless, in animal models of CNS injury or neurodegenerative diseases, NPC transplantation has been shown to promote some functional recovery although low cell survival and poor integration of the cells with adjacent tissue was generally observed [Potter et al., 2008].

As with other organ or tissue systems described above, a number of biomaterials have been tested to promote NPC survival and integration with adjacent tissue post-implantation [Teixeira et al., 2007; Potter et al., 2008]. PLA and PGA-based materials stand out again as the most promising scaffold materials because of their versatility, easy processing, and biocompatibility. Furthermore, they have been shown to promote NPC survival, migration, and differentiation and to allow neurite extension in vivo. The composition and 3D architecture of the scaffolds also seem to have a profound influence on NPC behavior and fate, but very little experimental data are yet available on this subject [Hsu et al., 2009]. NPC genetically engineered to secrete FGF-1 and 2, NGF, BDNF, GDNF, and neurotrophins also showed promising results (increased engraftment and/or regeneration, reduced glial scar formation) in animal models of diseases.

The field of cell and gene therapy for neurological disorders is still very young but already shows great promises. Obviously, the main challenge remains the identification of an appropriate cell source that could be used clinically. Data from preclinical trials using ex vivo expanded NPC mean little since it cannot be directly applied in humans. However, they do provide important insights into NPC biology. As suggested for cardiac tissue engineering, an interesting avenue would be the use of non-NPC cells to deliver signals to activate and recruit endogenous NPC to the injured area. These cells coupled to an appropriate scaffold could direct NPC differentiation and fate toward an appropriate phenotype leading to regeneration of the injured tissue. To support this idea, MSC have been tested for their neural regenerative properties in small animal models, where their therapeutic effects were mainly attributed to paracrine recruitment of endogenous cells [Prockop, 2007]. Identification of the signals required for NPC recruitment, migration, and differentiation is needed for efficient gene therapy using this strategy.

CONCLUDING REMARKS

After nearly 20 years of clinical experience with various gene therapy strategies, it is apparent that safe and efficient correction of genetic defects or modulation of pathological responses is more complex than first envisioned. Ex vivo approaches are now preferred owing to their increased safety. There is still a strong need to identify appropriate cell sources, preferably autologous, for many indications targeted by ex vivo gene therapy. Moreover, a better understanding of stem cell behavior, differentiation, and fate as well as molecular characterization of some pathologies is still required. The use of 3D porous scaffolds to deliver cells and genes has already shown promises for a wide variety of applications but the intelligent design of the scaffolds to address specific requirements of cells or tissues is still in its infancy. The clinical development of those technologies will undoubtedly require the close collaboration of multidisciplinary teams composed of stem cell biologists, biomaterial engineers, and physicians. The challenges are still immense but the rewards should match the efforts taken toward this goal.

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