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Integration of Drug, Protein, and Gene Delivery Systems with Regenerative Medicine

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Abstract

Regenerative medicine has the potential to drastically change the field of health care from reactive to preventative and restorative. Exciting advances in stem cell biology and cellular reprogramming have fueled the progress of this field. Biochemical cues in the form of small molecule drugs, growth factors, zinc finger protein transcription factors and nucleases, transcription activator-like effector nucleases, monoclonal antibodies, plasmid DNA, aptamers, or RNA interference agents can play an important role to influence stem cell differentiation and the outcome of tissue regeneration. Many of these biochemical factors are fragile and must act intracellularly at the molecular level. They require an effective delivery system, which can take the form of a scaffold (e.g. hydrogels and electrospun fibers), carrier (viral and nonviral), nano- and micro-particle, or genetically modified cell. In this review, we will discuss the history and current technologies of drug, protein and gene delivery in the context of regenerative medicine. Next we will present case examples of how delivery technologies are being applied to promote angiogenesis in non-healing wounds or prevent angiogenesis in age related macular degeneration. Finally, we will conclude with a brief discussion of the regulatory pathway from bench-to-bedside for the clinical translation of these novel therapeutics.

Keywords

Regeneration; drug delivery; gene delivery; protein delivery; biomedical engineering; angiogenesis

A. Introduction

The term “regenerative medicine” was coined in 1999 by William Hasetine, and was predated by the term of tissue engineering, which creates organs or tissues in vitro [1]. Regenerative medicine employs aspects of tissue engineering, stem cell therapy, genetic engineering, materials science, drug delivery and biomedical engineering to develop

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therapies that maintain and restore the normal function of damaged, diseased, or deficient tissues or organs. This field has increasingly captured the imagination of scientists and laymen alike because of the promise of restoring the functions of damaged tissues or organs *in vivo*. However, the excessive cost of commercialization and difficulties in the regulatory approval of complex therapeutic systems have delayed the translation of these therapies from bench-to-bedside [2]. Two of the oldest and most successful regenerative medicine companies are Organogenesis (specializing in wound healing and regeneration) and Medtronic (specializing in cardiac and vascular diseases, diabetes, and neurological and musculoskeletal conditions). However, many companies have failed on the path to clinical translation, possibly due to the difficulty of developing a business model that can maximize the commercial impact of cell-based therapies [3].

Despite the difficulties surrounding commercialization of cell-based therapies, bone-marrow derived stem cells have been used successfully in the clinic for bone, cartilage, spinal cord, cardiac, and bladder regeneration [2]. This field has been fueled by exciting advances in stem cell biology, particularly the recent discovery that adult cells can be reprogrammed into pluripotent stem cells [4] or directly into cells of another lineage [5]. Although various forms of stem cells (embryonic, progenitor, induced, or transdifferentiated) often play a central role in regenerative medicine, biochemical cues in the form of drug, protein, or nucleic acid can provide a supportive or even decisive role in determining the fate of the stem cells, and the eventual outcome of the tissue regeneration. These soluble therapeutics alone can also in some cases achieve a regenerative outcome, by acting on the resident cells at the tissue site. For example: heparan sulfate is a form of regenerative therapeutic that can be administered to recruit endogenous growth factors at the site of injury to initiate repair due to the specific interactions of heparan sulfate with many growth factors [6]. Readers are referred to recent excellent reviews on stem cell based-regenerative medicine [7–10]. This review will focus only on the role of soluble therapeutics, and their effective delivery, in advancing regenerative medicine.

Many therapeutics relevant to regenerative medicine are delicate growth factors and nucleic acids, often with short half-lives and requiring intracellular delivery. Effective drug delivery systems (DDS) are needed to realize their potential. Fortunately, needs for other therapies have already stimulated the development of drug delivery technologies for decades. One of the pivotal discoveries that stimulated protein delivery development was the characterization of restriction endonucleases. This allowed for mapping of DNA and the invention of recombinant technology, where a foreign protein could be expressed in bacterial cells [11]. This led to the commercial production of proteins in 1982, when the Food and Drug Administration (FDA) approved the first recombinantly generated protein: insulin. The approval of insulin brought with it a need for delivery systems that would increase the half-life, and sustain the release of proteins [12]. At the time that the methodology for protein delivery was being established, investigators began to work on gene delivery. In 1989 the first human gene transfer was achieved [13], and shortly thereafter the first gene therapy for Severe Combined Immunodeficiency (SCID) was developed in 1990 [14]. The development of nonviral gene delivery systems has been stimulated by the realizations that (1) viral gene transfer will hinder eventual clinical translation, and (2) gene transfer relying on naked

plasmid DNA is woefully inefficient. The sophistication of drug delivery has since progressed from macroscopic (1960–80), to microscopic (1980–90), and finally nanoscale (1990-present) delivery systems (Fig. 1).

Delivery is considered the single toughest barrier to clinical translation of protein- and gene-based therapeutics. In this article, we will discuss the current state of small molecule drug delivery for regenerative medicine, as well as the most promising peptidic and nucleic acid based drugs that we expect to be at the forefront of regenerative therapeutics translating into clinical use. From there we will examine delivery vehicles commonly used to extend the lifetime, circulation, and specificity of these soluble factors. As a case example, we will consider arguably one of the most important issues in regenerative medicine: the clinical progress being made by delivery of soluble factors to promote or inhibit angiogenesis. Delivery of soluble factors using synthetic or biologic DDS presents a new wave in regenerative therapeutics moving towards clinical translation. As an understanding of the regulatory pathway between developmental research and the clinic is critical for clinical translation, we will conclude with a brief discussion of the pathway to the clinic for regenerative therapeutics.

B. Soluble Factors as Regenerative Therapeutics: Low Molecular Weight Drugs, Polypeptides, and Nucleic Acids

Cells respond to soluble cues present in their microenviroment. Soluble factors such as synthetic small molecule drugs (or low molecular weight drugs), proteins (polypeptides), and nucleic acids (genes), are being developed to mimic these cues and drive regeneration. Polypeptides have been delivered to drive regeneration in the form of growth factors [15], zinc finger protein transcription factors [16], zinc finger nucleases, transcription activator-like effector nucleases, and monoclonal antibodies [17]. Nucleic acids have had success when delivered in the form of cDNA [18], RNAi [19], and aptamers [20]. The definitions, uses, positive, and negative attributes of these soluble factors as tools for regenerative medicine will be examined in this section.

B.1 Small Molecule Drugs

Regenerative therapeutics involving small molecule drugs (SMDs) is a recent area of research, with more than 5 times as many publications on their use since 2008 than in the 10 years prior. SMDs that influence cell behavior are being studied from existing drug libraries, as well as in novel formulations [21]. Select start-up companies like ChemRegen are focusing developing novel SMDs for cardiac muscle regeneration therapies [21]. Current research of SMDs targeting regenerative medicine focuses primarily on compounds that stimulate stem cell differentiation [22] and somatic cell behavior [23] such as proliferation, differentiation, and intra-cellular signaling, to drive and direct tissue regeneration. SMDs for regenerative medicine could have a massive translational impact because they are well understood by the FDA and pharmaceutical drug companies, and the machinery to safely manufacture and distribute SMDs is already in place around the world. SMDs are typically far less expensive for consumers, have a longer shelf life, and are less complicated than protein, nucleic acid, or cellular based therapies. However, the identification and

optimization of new SMDs is a slow, laborious, and costly process averaging $1.8 billion in company spending to bring a single new drug to market [24].

Because of the cost associated with drug development, previously discovered drugs are being studied for their regenerative effects. For example, rolipram, an anti-inflammatory and phosphodiesterase 4 inhibiting SMD, had been previously shown to improve spinal cord regeneration in small doses but had adverse effects when delivered in larger doses [25]. The incoporation of this SMD into a microfibrous patch for continuous local delivery generated greater functional and anatomical recovery in rats following spinal cord injury in low dose groups, and resulted in reduced survival rates in high dose groups [26]. Another previously developed SMD, valproaric acid (VPA), had been shown to promote cortical neuronal growth *in vitro* [27], and was recently shown to enhance sciatic nerve regeneration in rats when delivered locally within silicone tubes [28]. Rats treated with VPA showed a significant decrease in sciatic nerve index as well as increased motor-nerve conduct velocity, amplitude of activity potential, regenerated axon number, and thickness of mylin sheath compared with controls which received saline injection [28]. These works highlight the need for reliable, local DDS in the use of SMDs for regenerative applications.

B.2 Protein-based Therapeutics

Recombinant DNA technology has enabled protein-based molecules such as growth factors, zinc finger protein transcription factors, zinc finger nucleases, TALENs, and monoclonal antibodies, to be developed and used as drugs [29]. Recombinant DNA techniques are used to clone, express and purify any protein with a known DNA sequence *in vitro* using cost effective methods [30]. Since the first FDA-approved pharmaceutical recombinant protein entered the market in 1980, the biotechnology industry has grown substantially [31]. Currently 25% of commercial pharmaceutical sales are biopharmaceuticals, with sales in 2010 US exceeding $100 billion USD [32]. Protein-based therapeutics are successful in regenerative applications because they can mimic, activate, or inhibit endogenous pathways, helping the body to heal. Bioengineers can mimic biochemical cues in nature by developing recombinant proteins and engineering their delivery. However, the use of protein-based drugs for regenerative therapeutics is limited by their propensity for instability *in vitro* and *in vivo*, presenting a challenge for handling, and implying a need for repeated doses over time and the possibility for unwanted side effects. Cost of development is also high, the estimated research and development cost for a single biopharmaceutical approved molecule is estimated around $1.3 billion [33].

B.2.1 Growth Factors—The term ‘growth factor’ encompasses a range of signaling proteins that influence cellular processes including migration, proliferation, and differentiation (Fig. 2a). Growth factors exist in the body as soluble molecules secreted by cells, bound to the extracellular matrix (ECM) or cleaved and released from the ECM by enzymes. Growth factors interact with cells through transmembrane receptors to naturally regulate tissue regeneration. Vascular endotheial growth factor (VEGF) has been shown to be a key regulator of blood vessel formation, and as such been widely studied for angiogenesis. However, bolus administration of naked VEFGA leads to leaky blood vessels that can be toxic to cells, and result in hypotension *in vivo* [34]. Intracoronary infusion of

naked VEGF for treatment of ischemia for vascular angiogenesis (VIVA) showed success in animal trials and went through phase II clinical trials, but failed to show success compared to control [35]. This could have occurred because VEGF has a short half-life of 90 minutes *in vivo* [36], and is cleared from the body rapidly (within 8 hours) [37]. Unfortunately, the field has a lot of progress to make before GF therapeutics reach clinical use since their failure in phase II and III clinical trials has become more common than their success. Growth factors typically demonstrate efficacy above certain concentrations, can be toxic in excess, are subject to enzymatic cleavage, and have short half-lives. As such, controlled release technologies that can achieve local and sustained delivery are needed facilitate their clinical success.

B.2.2 Zinc Finger Protein Transcription Factors & TALEN's—Zinc Finger Proteins (ZFPs) are named because of their shape, which is created by a short stretch of amino acid residues wrapped around a zinc atom in the shape of a finger (Fig. 2b). ZFPs are the most common DNA-binding proteins in eukaryotes; their main role is in the recognition of specific DNA sequences. ZFPs are modular constructs with each binding domain recognizing 3–4 DNA base pairs. Several binding domains can be stitched together to recognize a unique sequence of DNA. Artificial ZFPs are commonly designed with 6 binding domains to recognize a unique sequence in the human genome of 18–19 base pairs. However, similar to other protein cues, the *in vivo* half-life of ZFPs is on the order of hours [38].

ZFPs can bind to a specific DNA stretch and physically block transcription from occurring. The addition of an effector domain to the ZFP construct gives another level of modularity for transcription activation or repression of any endogenous gene, hence the term Zinc Finger Protein-Transcription Factor (ZFP-TF). ZFP-TFs have a unique design advantage over most other DNA-binding motifs in that they do not have to bind to target DNA as dimers. Because of their small size, multiple ZFP-TFs can be included in one gene transfer vector and their encoding DNA delivered using viral or non-viral vectors. ZFP-TFs have been mainly studied for the regulation of genes involved in cancer [39]. Perhaps the most exciting use of ZFP-TFs is their ability to activate or repress any endogenous gene, theoretically expressing all of its splice variants [40, 41]. ZFP-TFs have been shown to preserve hindlimb grip strength and improve functional outcomes for treatment of ALS in a rat model [16], and to increase VEGF mRNA, capillary density, and proliferating cells in ischemic tissue in rabbits [42]. ZFP-TFs are typically generated in random libraries and selected for specificity using phage display. Despite the well described steps for protein production and purification, ZFP-TF production remains a high-cost, time-consuming process that makes their translation into clinical medicine difficult.

Zinc Finger Nucleases (ZFNs) are similar to ZFP-TFs in their structure but work as restriction enzymes for genome editing, rather than as transcription factors. ZFNs are generated by fusing a ZFP DNA-binding domain to a nonspecific DNA-cleavage domain [43]. ZFNs have been used to genetically modify patient derived iPSCs (see section C.4.1) for the treatment of Sickle Cell Anemia and Parkinson's disease [44, 45]. ZFN modified autologous T-cells are currently in clinical trials for HIV treatment [46]. ZFNs face the same barriers to translation as ZFP-TFs, specifically high cost and difficulty of generation.

Transcription activator-like effector nucleases (TALENs) have recently emerged as an alternative to ZFNs for genome editing. Although in their infancy, TALENS have been suggested to cleave DNA with similar efficiency as zinc finger nucleases, but boast simplistic design methods [47]. Because of their ease and low cost production TALENs are likely to join, or even surpass, ZFP-TFs as regenerative therapeutics over the next decade. Both of these classes of nucleases are being used as novel methods to generate cell populations for drug screening [48]; and offer hope of modified cellular therapies for patients with genetic disorders that are resultant of small genetic abnormalities, such as sickle cell anemia. However, both ZFNs and TALENs can cause unwanted mutation in the genome, limiting their use to applications outside the body such as modified cellular therapeutics and generation of disease modeling methods [49, 50].

B.2.3 Monoclonal Antibodies—Monoclonal antibodies (mAB) are large proteins naturally produced by B-cells in the immune system for the recognition of specific antigens in the body. Each mAB binds to a specific epitope on an antigen and either physically inactivates it, or recruits immune cells to destroy it (Fig. 2c). Humanized mABs are a rapidly growing category of targeted, protein based therapeutics. Due to their mechanism of action, therapeutic mABs are used to inactivate pathways associated with disease states. Humanized mABs are in clinical trials for treatment of arthritis, cancer, immunological diseases and infectious diseases [51]. Ranibizumab is a recombinant mAB that neutralizes all active forms of VEGF-A. It is used clinically for treatment of the excessive neovascularization of the macula associated with Age-related Macular Degeneration (AMD) [17]. Since its FDA approval in 2006, Ranibizumab has shown success in slowing the vision loss associated with AMD in up to 96% of patients [52]. *In vivo* mABs bind to protective receptors on cells, elongating their clinical half-life up to 4 weeks; however, their large size causes them to distribute slowly into tissue [53]. Clinically, mABs are administered intravenously, intramuscularly, or subcutaneously every 3–4 months [54]. Unfortunately, complications such as cytokine release syndrome (CRS) and immunogenicity are still concerns for these mAB therapeutics [55].

B.3 Nucleic Acid Therapeutics

There are several forms of nucleic acids which are delivered for regenerative medicine, the three most prominent are plasmid complementary DNA (cDNA), small RNA, and aptamer. cDNA encoding for a therapeutic gene is delivered directly to cells *in vivo* or *in vitro* so that they will express that gene or protein of interest. Methods of DNA delivery to cells will be discussed in the carriers section. Small RNAs are used to regulate gene expression *in vivo* by controlling protein transcription and translation at the mRNA level, this process is known as RNA interference (RNAi). Aptamers are similar to mABs in that they bind and inhibit a specific biological target, such as an enzyme or receptor, but they are generated by chemical methods. Nucleic acid therapeutics act at the molecular level, however their intracellular delivery is inefficient because they will not passively cross the plasma membrane of cells due to their size and negative charge.

B.3.1 Gene Delivery - cDNA—cDNA are DNA sequences that are complementary to mRNAs encoding for a specific gene, and are used to introduce genes to cells. cDNA

delivery is a highly versatile and widely used method for regenerative therapeutics as cells can be modified to express any gene of interest. cDNA delivery encoding for Insulin-like growth factor-I and keratinocyte growth factor has successfully accelerated endogenous VEGF and collagen type IV expression, neovascularization, and epidermal regeneration of dermal wounds in a rat model [18]. The greatest setbacks to the therapeutic use of cDNA are the delivery method and the risk of accidental DNA insertion into an important region in the human genome. Rather than delivering cDNA to regulate mRNA, direct delivery of mRNA has been explored as a gene delivery tool for cell modification [56]. Since mRNA will not integrate into the genome as DNA can, even when delivered non-virally, it offers a gene delivery option free of the threat of insertional mutagenesis.

B.3.2 RNAi—RNA interference (RNAi) takes advantage of the ability of small RNA to regulate gene expression. Small RNAs are endogenous single or double stranded nucleic acid sequences between 21–24 nucleotides in length that do not code for proteins, but rather inhibit mRNA that do. Small RNAs present a unique opportunity to control protein transcription and translation in the body. Many small silencing RNAs have been discovered recently. Those used in regenerative therapeutics include small interfering RNA (siRNA) and microRNAs (miRNA), both mediate the down regulation of gene expression. siRNAs bind to specific mRNAs and label them for nuclease destruction, while miRNA attach to the encoding mRNAs and physically prevent them from being translated to proteins. SiRNA-mediated endogenous gene silencing in mammalian cells was first demonstrated in 2001 [57]. Current research directs siRNA against mRNA encoding proteins involved in degenerative diseases, and vascularization of cancerous tumors [58]. siRNA is currently in clinical trials for use against VEGF and its receptor (VEGFR-1) to treat AMD [59]. These trials involve direct injection of siRNA targeted at genes for VEGF and VEGFR-1 into the macula and have shown some therapeutic treatment potential in their inhibition of the excessive vascularizton of the eye that leads to AMD. siRNA has also been used to induce therapeutic angiogenesis in a murine diabetic wound model by inhibiting the hypoxia-inducible factor (HIF)-l inhibitor Prolyl hydroxylase domain 2 (PHD2) [19]. In this study, the inactivation of PHD2 led to the stabilization of HIF-1 and subsequent production of VEGF and fibroblast growth factor 2 (FGF-2) in the wound bed, leading to improved closure time in diabetic wounds. The main barrier against the use of RNAi therapeutics in regenerative medicine is delivery because they are rapidly cleared *in vivo*, are not tissue specific, and their negative charge and size prevents passive endocytosis across the cellular membrane. While RNAi is heralded for its myriad of clinical uses, most of those fall outside the realm of regenerative medicine, focusing rather on disease remediation [60].

B.3.4 Aptamers—DNA and RNA aptamers are non-biological oligonucleotides that bind to specific protein targets. Aptamers bind to their target with high affinity and specificity, and work by inhibiting its action. Aptamers for a specific target are generated by *in vitro* by a selection process called Systemic Evolution of Ligands by Exponential Enrichment (SELEX) where a random library of sequences (20–100 residues in length) is screened for aptamer-target conjugation [61]. Using the SELEX process, aptamers can be selected that only work in specific physiological conditions such as pH, salt concentration and temperature. Therapeutic aptamers are primarily delivered as systemic anticoagulants and

cancer therapeutics, [62]. However, aptamers have also had regenerative success in the treatment of AMD [20]. Pegaptanib, an anti-VEGF RNA aptamer marketed under the drug name Macugen, was approved by the FDA in 2004 and has been used clinically for the treatment of all types of AMD [63]. Aptamers are generated by chemical processes resulting in little batch-to-batch variation, and are essentially non-immunogenic even when delivered in excess of therapeutic dose [64]. However, aptamers are rapidly degraded *in vivo* and can be costly to generate, making commercialization and clinical translation difficult.

B.4 Summary and Comparison of Soluble Cues

The induction and control of regenerative therapies can be influenced by a variety of soluble factors including small molecule drugs, polypeptides and nucleic acids (Table 1). Small molecule, chemical drugs poses great potential for affordable regenerative therapies following research, development and regulatory approval. Protein therapeutics, including growth factors, ZFP-TFs, ZFNs, TALENs, and mABs, recently gained the opportunity for clinical use because of the advances in the industry of biotechnology and recombinant DNA technologies. Nucleic acid based therapeutics can be generated using chemical methods and offer another avenue for control of endogenous pathways and protein production. Delivery of cDNA encoding for *in vivo* expression of a specific gene or protein therapeutic can cause cells to express any protein or gene, including growth factors. However, all of the soluble factors listed suffer from rapid degradation and clearance from the body if they are not delivered directly to their site of action, hinging their efficacy on the success of their carrier.

Selection of the appropriate soluble factor will be highly dependent on the pathogenesis of the disease state being studied. First one must ask if the need is chronic or acute. Chronic disease states may benefit most from the genome editing provided by nucleic acid based drugs; whereas acute disease states may be best treated with more temporary soluble factors, such as protein-based drugs or SMDs. If the disease state necessitates increased expression of certain genes or proteins, the administration of SMDs, growth factors, ZFP-TFs, or cDNA should be considered. Alternatively, if inactivation of a gene or repression of the translation of a specific protein is needed, SMDs, ZFPs, TALENs, mABs, RNAi, or aptamers could all be considered. Table 1 highlights the pro's and con's associated with each soluble factor, as well as its most common use for comparative analysis and selection.

C Methods of Delivery

Once the appropriate soluble factor has been identified for the pathogenesis being studied, the appropriate delivery vehicle must be chosen. Soluble factors may need to be delivered in many ways, from systemic administration to intracellular delivery. Systemic delivery is appropriate for systemic diseases, but is rarely the most attractive or effective option for regenerative medicine applications because soluble factors degrade rapidly without an efficient carrier. For localized tissue regeneration, the ideal therapeutic would have a controlled, local delivery, to limit toxicity and minimize the amount of drug needed to achieve a therapeutic effect. There are several approaches for controlled local delivery (Fig. 3). Suspension in an implantable or injectable scaffold, and immobilization on or inside of biomaterial constructs give the most common examples. For example, the drug can be

chemically linked to the network of a hydrogel or covalently immobilized to the surface of a scaffold, such as an electrospun fibrous matrix [65, 66]. Scaffolding systems such as these provide the option of controlled release by varying porosity and degradation rate. Paniculate delivery systems such as micro- and nano-particles have also emerged as useful delivery vehicles since their size can be tailored to deliver the cargo of interest extra- or intra-cellularly. Viral vectors can be used to deliver and promote the expression of DNA based therapeutics *in vitro* by the generation of genetically modified cells or, less frequently, *in vivo*. Genetically modified cells can be used as carriers, typically following transduction or transfection with a gene encoding the protein of interest. These carrier systems are often combined to create an optimal release profile in the tissue of interest.

C.1 Scaffolds for Delivery

Biomaterial scaffolds have received a great deal of attention in tissue engineering for local and sustained release of soluble factors. Scaffolds can be constructed from a variety of materials, the most common of which are hydrogels [67], and electrospun fibers [68]. These scaffolds can be loaded with soluble factors and rationally designed to control release kinetics by altering construction material, topography, porosity, and degradation characteristics [69]. Scaffolds are unique carriers because their material composition, stiffness, and size, can be designed to fill a 3-dimensional defect space to further assist in regeneration. Scaffolds can also be modified to provide physical and chemical cues to the surrounding tissue by mimicking the ECM in construction, stiffness and protein coatings [70]. Injectable hydrogels for spinal cord regeneration, resorbable nerve conduits and electrospun fibers are common examples of drug eluting scaffolds [71, 70, 72, 73].

C.1.1 Hydrogels—Hydrogel is a network of hydrophilic polymers rendered insoluble in water but swollen due to physically or chemically interacting crosslinks (Fig. 3a). Hydrogels are often used as drug delivery scaffolds in regenerative therapies because their mechanical properties and drug release kinetics can be easily tailored [74, 75]. Hydrogels can be constructed from biocompatible synthetic or natural polymers. Synthetic monomers include poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG), poly(N-isopropylacrylamide) (pNIPAAm), and polyacrylates such as poly(2-hydroxyethyl methacrylate) (PHEMA), among others [76]. Biological materials include, but are not limited to, chitosan, agarose, collagen, alginate, fibrin, and hyaluronan [76]. Hydrogels are often designed to release soluble factors by undergoing conformational changes in response to small molecule drugs [77], pathological metabolites [78, 79], or physiological conditions including body temperature [80] and high or low pH environments [81]. Such stimuli sensing systems are referred to as “smart” hydrogels and are commonly used as drug delivery vehicles in regenerative medicine. For example, a hydrogel of poly(N-isopropylacrylamide-co-acrylic acid), a copolymer of acrylic acid and thermosensitive pNIPAAm, was used to deliver EGF and VEGF directly to the wound bed via pH sensitive release. Treated mice showed improved wound healing compared to those given the same growth factors loaded in PLGA microspheres that were delivered within a collagen hydrogel. These data suggest that a wound-pH-responsive hydrogel can evoke a better healing response than a pH-insensitive delivery systems. Similarly, in tissue engineering applications, injectable smart or

photopolymerizable hydrogels with a controllable sol-gel transition can be delivered to fill a defect and excrete pro-regenerative signals to cells [70].

Hydrogels can also be used to maintain smaller DDS at the site of injury that would otherwise rapidly disperse throughout the body due to their size, such as micro- or nano-particles. For example, to treat retinal blinding diseases that result from an inadequate supply of retinol, an alginate hydrogel was loaded with poly(D,L-lactide-co-glycolide) (PLGA) microparticles carrying retinoids and injected intravitreally in murine model [82]. This bi-phasic system achieved sustained release of 9-cis-retinol, a chemically synthesized SMD, causing improved visual function and retinal structure. Hydrogels are also used to encapsulate and deliver therapeutic cells [83]. A chitosan hydrogel scaffold containing a proangiogenic small molecule (DFO) was generated and loaded with hMSCs for delivery to ischemic tissues to facilitate neoangiogenesis [84]. Multimodal constructs such as these can better imitate natural processes and will likely continue to be at the forefront of this field. However, the body's response to the hydrogel, as well as induced structural variations undergone during any "smart" transitions can alter porosity and delivery kinetics and must be considered when designing hydrogel delivery systems [85].

C.1.2 Electrospun Fibrous Scaffolds—Over the past two decades, electrospinning has become a popular fabrication technique for generation of nano- and micro-fiber scaffolds due to its simplicity, and versatility to fine-tune the mechanical and mass transport properties of the scaffolds. Fibrous scaffolds have been hallmarked as biomaterial implants due to their high surface to volume ratio and tunable biomimicry [68]. Electrospun fibrous scaffolds have been applied towards a broad range of regenerative medicine applications including dermal wound healing, skeletal muscle regeneration, nerve regeneration, and spinal cord regeneration [86, 26, 72, 87]. Soluble factors can be immobilized on the surface of fibers to for local delivery to the site of interest (Fig. 3b) [65]. Further control of release kinetics can be achieved by encapsulation of the soluble factor within the fibers. There are two methods by which substances can be encapsulated inside electrospun fibers. The first is blending the substance with the polymer materials prior to spinning. In this process the substance is dissolved in organic solvent along with the polymer, during the spinning process the organic solvent rapidly evaporates leaving the substance immobilized on the surface of the fibers [88]. Bone morphogenic protein-2 (BMP-2) dissolved in PLGA/hydroxylapatite prior to spinning generated homogenously loaded, electrospun scaffolds with a sustained release of BMP-2 *in vivo* for bone regeneration [89]. However, this method often results in uneven distribution of the proteins within the fibers and is generally characterized by a burst release response [88, 90]. The second method is the generation of core-sheath fibers by emulsion electrospinning or core-shell electrospinning. Emulsion electrospinning is a recently developed, facile technique for the generation of core-sheath fibers in which the spinning solution is replaced by a water-in-oil emulsion [91]. This method is commonly used to encapsulate a hydrophilic drug into a hydrophobic polymer shell [92]. Since the encapsulated factor may contact organic solvents in the emulsion, co-axial electrospinning is safer for protein delivery [93]. Coaxial electrospinning employs a dual needle apparatus where a soluble inner core solution is spun within a polymer solution shell [94]. Using this method delicate proteins and even live cells, that are sensitive to the

organic solvents commonly used in electrospinning, can be suspended within the inner aqueous phase of hollow polymer fibers [95]. The release of these bioactive agents can be modulated by increasing the flow rate of the inner solution so that the core is larger and the shell thinner, or by including porogens in the shell such as PEG [96]. In a combinatorial study, coaxial electrospun polyurethane scaffolds including PEG as a porogen, were loaded with VEGF and PDGF then seeded with Factor VIII expressing skeletal myoblasts to treat hemophelia A in mice [87]. Induction of angiogenesis with VEGF and PDGF facilitated improved delivery of FVIII throughout the bloodstream *in vivo* and improved clotting time to clinically relevant levels.

C.2 Particulates

Micro- and nano-particles are highly used for protein and SMD delivery as well as the non-viral delivery of nucleic acids (see Section 3.2.2) [97]. Micro- and nano-particle carriers are separated based on their size; micro-particles have a diameter in the micron range and nano-particles in the sub-micron range (Fig. 3c). A variety of materials can be used to generate these carriers. Liposomes, micelles, dendrimers, and hydrogels are common paniculate systems used to carry soluble factors that can be generated in the nano- to micron size range. Liposomes are lipid carriers that have an outer lipid bilayer and an inner aqueous space for soluble factors (Fig. 3**c.1-upper**). Micelles are self-assembling lipid monolayers with a hydrophobic core and hydrophilic shell, making them useful for insoluble cargo such as some SMDs (Fig. 3**c.1-lower**). Dendrimers have branched monomer units that soluble factors can be conjugated to for targeted and controlled delivery (Fig. 3**c.2**). Hydrogels are both hydrophilic and tunable in chemical composition, making them a suitable micro-vehicle for soluble factor delivery (Fig 3**c.3**). However, precise size control when generating these particulates remains an issue in the field [98].

Carrier size has a large impact on drug delivery kinetics because the surface to volume ratio is inversely proportional to the radius of the particle. Microparticles do not cross most biological barriers, but particles less than 10μm can be taken into cells by phagocytocis [99]. Microparticles are most successful when injected at the site of interest and have a tendency to stay in the general area where they are injected for weeks. For example, local injection of VEGF loaded microspheres has been used to induce angiogenesis *in vivo* in murine hind limb ischemia [100]. On the contrary, nanoparticles are capable of traversing biological barriers and cellular membranes, but can be cleared by the body in a matter of days [99]. For example, when vascular smooth muscle cell (VSMC) targeted liposomes, with diameters between 100–400nm, were delivered intravenously to prevent restenosis in injured blood vessels, those not successfully delivered to VSMCs were found accumulated in the liver and spleen, suggesting clearance by the reticuloendothelial system [101]. Nano- and micro-carriers can encapsulate a number of biomolecular cues and be tailored to release their cargo in response to certain stimuli such as temperature, light, pH change, or small molecule administration.

C.3 Carriers for Nucleic Acids

The delivery of intact nucleic acids *in vivo* is difficult because naked nucleic acids are rapidly cleared and degraded in the extracellular environment. Nucleic acids can be

delivered to the cell nuclei *in vivo* or *in vitro* using viral transduction and non-viral transfection methodologies. *In vivo* DNA delivery is the most complex as researchers have the least control over which cells are transfected/transduced. Because of this lack of control, *in vivo* delivery can be associated with high health risks, especially with the risk of insertional mutagenesis using viral delivery. Viral DNA delivery boasts high transfection efficiency, but poses safety concerns stemming from immunogenicity, toxicity and untargeted DNA insertion. Non-viral vectors offer transgene expression without immunogenic, integrating carriers; but the efficiency of non-viral delivery must be increased before it can be successful in regenerative therapies. Although non-viral DNA delivery boasts a decreased risk for insertional mutagenesis when compared to integrating viral DNA delivery, the risk still exists. Even a small risk for insertional mutagenesis will make DNA delivery difficult to translate into clinical use.

C.3.1 Viral Vectors for Nucleic Acid Delivery—Viral gene delivery has been studied using three main viruses: Adenovirus (AV), Adeno-associated virus (AAV), and Retro/Lentivirus (RV/LV) (Fig 3d). Both AV and AAV lead to transient expression while RV/LV give permanent gene expression. AV is highly immunogenic when used in multiple doses *in vivo*, as such their primary application lies in transient therapeutic application, or *ex vivo* modification of T cells for immunotherapy. AAV does not integrate its DNA cargo into the genome, but can achieve prolonged expression as an episomal vector. AAV has recently had success in treatment of blindness, hemophilia and muscular dystrophy [102, 103]. Lenti- and retroviruses are unique in that they integrate their cargo into the host genome for long term expression. In some cases, this insertion takes place within important endogenous genes and can result in gene activation. The random insertion of viral DNA into the host genome resulting in unwanted mutation is referred to as insertional mutagenesis. In 2003, a clinical trial was conducted to treat patients with severe immunodeficiency using gene therapy with retroviruses. Four years after success had been proclaimed, insertional mutagenesis caused accidental gene activation in five of the seventeen cured patients resulting in leukemia [104]. Because of the dangers associated with viral gene delivery, non-viral delivery has become a major focus of research in the fields of drug delivery and regenerative medicine.

C.3.2 Non-Viral Methods for Nucleic Acid Delivery—Non-viral gene transfer does not integrate the exogenous gene into the host genome, and the risk of insertional mutagenesis drops drastically. Non-viral gene delivery can be conducted *in* vitro via direct injection of a plasmid (circular DNA construct) into a cell, membrane poration, or complexation of plasmid DNA (pDNA) with carriers such as lipids (lipofection) (Fig.3**f.2**) [105], particulates (Section C.2) [106], or cationic polymers (Fig.3**f.3**) [107, 108]. Non-viral DNA delivery can be carried out *in vivo* when using the appropriate carrier to prevent degradation and enhance endosomal escape once endocytosed into the cell [109]. To date, muscle is the only tissue where direct injection of naked pDNA has been show to successfully lead to gene transfer, although the level of transgene expression is generally deemed too low to be therapeutic [110].

Nonviral vectors, although achieving only transient and low gene expression levels, compensate with their ease of synthesis, low immunogenicity, and unrestricted plasmid size

[111, 112]. They have the potential to be administered repeatedly with minimal host immune response. Nonviral vectors also face less of a challenge than viral vectors in addressing pharmaceutical issues such as scale-up, storage, stability, and quality control. Numerous studies have investigated the use of cationic polymers or cationic lipids to form nanocomplexes with pDNA, known as polyplexes and lipoplexes, respectively (Fig. 3**f.l, 3f. 2**). Lipopolyplexes, the product of lipid-polymer-DNA complexation, leverage on the advantages of a cationic polymer and a lipid to formulate a DNA-polymer core in a lipid shell (Fig. 3**f.3**) [113].

Polyplexes, lipoplexes, and lipopolyplexes all serve to protect the DNA from enzymatic degradation during transit from the extracellular space to the nucleus of the cell. However, the DNA must be released from these nanocomplexes for transcription to take place. Finding the balance between tight packing extracellularly and efficient unpacking intracellularly has been a challenge to effective nonviral gene delivery [114, 115]. Polymeric gene carriers enjoy the advantage of versatility to address this balance. Rigidity, hydrophobicity/ hydrophilicity, charge density, biodegradability, and the molecular weight of the polymer chain are all parameters that can be adjusted to achieve an optimal complexation with DNA. Versatility is important in view of the broad range of regenerative medicine applications and their unique demands. It is likely that for different tissues, or different routes of administration *in vivo*, the desirable characteristics of the DNA nanocomplexes would differ. A powerful approach to identify the optimal nonviral carrier for different applications is to use combinatorial synthesis to generate a large number of candidates for high-throughput screening against the cell type of interest. Both polyplex and lipoplex development have greatly benefited from this approach [116–118]. The caveat is that *in vitro* and *in vivo* correlation remains imperfect; the best carriers identified *in vitro* by the combinatorial approach may not be optimal for *in vivo* applications.

C.4 Genetically Modified Cells as Carriers

Cells that have been genetically modified to express a gene or protein of interest can be used to produce protein and nucleic acid based soluble molecules *in vivo* (Fig. 3e). Any cell type can be modified in this way, including stem cells and adult differentiated cells from either allogenic (from the same species) or autogenic (from the same person) sources. Allogenic cell sources risk immune response from the patient, but have an advantage in regenerative medicine because they can be shelf-ready for use in the operation room. In an autologous system, a patient's own cells are removed, modified *ex vivo*, and re-administered to regenerate tissues in the diseased site, obviating the risk of an immune response. Genetic modification of a patient's own cells allows for autologous therapeutics, but the cost involved with harvesting, purifying, genetically modifying, and implanting each individual patient's cells is a concern when considering commercialization. Genetically modified cells (GMC) are commonly implanted on a scaffolding system or injected in a hydrogel suspension because freely injected cells typically diffuse away from the injection site prior to integration with the host tissue [119]. Delivering cells as carriers of therapeutic soluble factors offers a unique two-pronged technique for treatment of degenerative diseases by increasing cell number at the defect with cells that secrete pro-regenerative soluble factors

[120]. However, cellular replacement therapies such as these rely on the sourcing of correct cell type, as well as the retention of delivered cells at the site of the defect.

Within the last decade, gene delivery has generated two new genetically modified cell types that have shown great promise as cellular carriers for regenerative therapeutics: induced pluripotent stem cells (iPSC) and transdifferentiated cells. With the advent of induced pluripotent stem cells (iPSCs) and transdifferentiation techniques, new methods of cellular therapy using patient-specific cells may become a reality. Since iPSCs display pluripotency, the ability to differentiate into any cell type within the body, and can be derived from any tissue at any age, the ethical, sourcing and political issues amongst researchers and the public that arose when dealing with human embryonic stem cells (hESCs) can be avoided. iPSCs and transdifferentiated cell types provide the option for *ex vivo* reprogramming and generation of patient-specific cellular therapeutics for regenerative medicine.

C.4.1 Induced-Pluripotent Stem Cells (iPSCs)—iPSCs are fully differentiated somatic cells that have been reprogrammed to a pluripotent state by over-expression of specific transcription factors (TFs). This method was recently reported in 2006 when adult murine fibroblasts were de-differentiated to pluripotent stem cells using viral delivery of genes encoding for specific TFs [4]. As such, the cellular processes that mediate this conversion are not yet fully understood. iPSCs are currently being used for disease modeling and drug discovery [9]. Because of their pluripotency, iPSCs have great therapeutic potential. However with pluripotency comes the risk for teratoma formation which gives reason for concern when considering iPSCs as cellular therapies in humans. iPSC could soon be used in clinical trials in Japan; the success or failure of these trials, the first to be performed in humans, will have a massive impact on the future of iPSCs as cellular therapeutics [121].

C.4.2 Transdifferentiation—Transdifferentiation, where an adult cell is directly converted into another adult cell type without passing through a pluripotent stage, presents a novel approach to generating cells for patient-specific implantation. The process for generating transdifferentiated cell types is very similar to that of generating iPSCs. It is typically carried out by DNA-transfection or protein-based procedures. Researchers have successfully converted fibroblasts into cardiomyocytes [122] and neurons [123] with the long term goal of generating functional cells for cellular therapies. Transdifferentiation differs from reprogramming and iPSCs because it offers an opportunity to convert cells directly across distinct linage barriers. Since cells do not have to spend time in a pluripotent state during the conversion process across linages, transdifferentiation avoids the possibility of teratoma formation and offers a one-step conversion to the final cell type of interest. However, transdifferentiation efficiency remains a barrier to this technology, with efficiency of reprogramming often hovering only a few percent above the starting number of cells [124].

C.5 Carrier Selection

In this section a variety of delivery systems used in regenerative medicine, each with unique properties and drawbacks have been described. It is important to select the correct delivery

system based on the soluble factor and disease pathogenesis (Fig. 4). One must consider where the soluble factor is active- does it need to be delivered extracellularly (SMD), intracellularly (siRNA, mRNA), or to the nucleus (pDNA)? Size and specificity of the carrier are important factors in this consideration and for biodistribution. Nanoparticles and viral delivery often have the most success for intracellular delivery, while 3D scaffolds, microparticles and GMCs are primarily used for extracellular delivery. The nature of disease pathogenesis, what biological tissue or cell population the soluble factor must reach, will also influence the carrier selection. In the case of regenerating a tissue defect, an implantable or injectable 3-D scaffold system with or without GMCs may be optimal. Intravenous or direct injection of targeted particulates carrying protein or nucleic acid cues would be ideal for a systemic or localized pathogenesis.

When selecting a carrier, one should pose the question- do the physical and chemical traits of the carrier alone have therapeutic effects in absence of the delivery of soluble factors? Environmental cues from the ECM and soluble factors directly affect cell morphology, proliferation, and differentiation [125]. Carriers can mimic the material composition of the ECM by using naturally derived biomaterials such as collagen, and constructing the carrier as a porous micro- or nano-scale scaffolding structure to promote cellular ingrowth [126]. For example, collagen sponges with pore sizes 100μm±50 μm filled with collagen hydrogel aided in bone reconstruction and cellular infiltration when implanted into periodontal defects in dogs [127]. Also, hollow collagen nerve conduits have had clinical success and are manufactured for peripheral nerve defects less than 3 cm in length by companies such as Stryker and AxoGenic [128]. These results show that the cues cells received from scaffold composition and physical characteristics aided in tissue regeneration. Although tubular nerve guides can promote axonal elongation over short gaps, the inclusion of cells, drugs, electrospun fibers, or hydrogel fillers within the guide can significantly improve regeneration [129]. Polymer scaffold conduit permeability has been shown to alter nerve regrowth [130]. Physical characteristics including diameter and 3-D orientation of electrospun fibers within nerve guides has also been shown to affect nerve regrowth [131, 132, 72]. The fibrous nature of electrospun scaffolds may provide topographical cues to the adherent cells that mimic the structure of the native ECM.

One should also consider carrier charge when designing carriers for negatively charged soluble factors, such as nucleic acids; but does charge alone have an effect? The use of charged nerve guides, such as carbon nanotubes (CNT), has recently shown promise in improving neural regeneration. CNTs have been shown to improve the responsiveness of neurons by forming tight contacts with the cell membranes, this interaction is thought to favor electrical shortcuts between the proximal and distal compartments of the neuron [133]. Since cells glean cues from the carrier as well as the soluble factor being delivered, the characteristics of the carrier should be chosen logically to enhance the success of the regenerative therapy. With a combinatorial approach using soluble factors delivered by rationally designed carriers and scaffolds, regenerative technologies will become a more prominent feature in clinical medicine.

D. Modulating Angiogenesis in Regenerative Medicine

Blood vessel growth is a complex process and presents one of the largest barriers to tissue engineering. However the controlled delivery of soluble factors can be used to stimulate this process *in vivo*, or enhance it in tissue engineered implants [134]. Blood vessels deliver oxygen, nutrients, and metabolites to tissues, and clear waste away from sites of inflammation. As such a lack of vasculature will lead to tissue necrosis. Directed formation of microvasculature requires precise control and coordination of cells with their environment. Because of this complexity, the largest clinical successes in tissue engineering have been achieved in tissues that do not require intricate vasculature, such as the bladder and non-loadbearing cartilage [135–137].

Blood vessel growth is characterized by two processes: angiogenesis, the formation of blood vessels from pre-existing blood vessel populations, and vasculogenesis, the formation of blood vessels without the presence of existing vasculature [138]. Vasculogenesis primarily occurs during fetal development of the circulatory system, but is also seen during tumor growth. During vasculogenesis, endothelial precursor cells migrate and differentiate in response to cues, such as growth factors and the surrounding ECM, to form new blood vessels. In contrast, during angiogenesis existing blood vessels split or sprout to form new blood vessels. There are three major types of blood vessels: arteries, capillaries and veins. Arteries carry blood away from the heart and are multilayered structures consisting of an outer layer of connective tissue, intermediate layer of fibroblasts, and inner layer of endothelial cells. Arteries carry oxygen and nutrients through the body to reach capillaries that will facilitate their delivery to surrounding cells and tissues. Capillaries are responsible for the exchange of water and chemicals between blood vessels and tissues. Finally, veins carry blood from capillaries back to the heart.

Successful angiogenesis requires a complex cascade of events including endothelial cell (EC) activation, migration and proliferation, followed by EC arrangement into immature vessels, addition of pericytes and smooth muscle cells (SMCs), and deposition of ECM as the vessels mature [139]. Vascular endothelial growth factor (VEGF) and Fibroblast Growth Factor (FGF) are heparin-binding growth factors that are involved in the initiation of angiogenesis, and induction of EC proliferation and migration. Platelet derived growth factor B (PDGF-B) recruits pericytes and SMCs, while transforming growth factor beta (TGF-β) causes ECM deposition of laminin and collagen types I & IV to give new vessels stability [140]. In some diseases such as cancer, diabetic angiopathy, and AMD, leaky blood vessels contribute to disease pathogenesis and anti-angiogenic therapeutics must be developed. Pigment epithelium-derived factor (PEDF) is a multifunctional, secreted protein that has been shown to be a potent inhibitor of neoangiogenesis [141].

There are many groups working to modulate angiogenesis *in vivo* by the delivery of small molecule drugs [142], growth factors [143], or nucleic acids [144, 145]. Readers are directed to recent and more detailed reviews on the use of protein [146, 147] and nucleic acid [148, 149] delivery systems for the modulation of angiogenesis [150–152]. Delivery of these soluble factors using synthetic or biologic DDS presents a new wave in regenerative therapeutics moving towards clinical translation. This is evidenced by the FDA opening a

new regulatory division, the Office of Combination Products, in 2002 to analyze complex drug and cell delivery systems for regenerative medicine. Angiogenesis of non-healing wounds and anti-angiogenesis in the treatment of AMD are examples of clinical applications in which soluble factor delivery can be used to control blood vessel growth.

D.1 Promoting Angiogenesis in Non Healing Wounds by Nucleic Acid Delivery

Chronic non-healing wounds are a major medical problem that affect millions of Americans annually and cost billions of dollars per year [153]. There are several different causes of non-healing wounds including decubitus ulcers, venous stasis ulcers, arterial insufficiency ulcers, and diabetic foot ulcers (DFU). DFU develop in 15% of people with diabetes, and 14% to 20% of them require amputation [153]. A leading cause of DFU is vascular insufficiency [154, 155]; thus, application of pro-vasculogenic scaffolds, introduction of GMCs, or delivery of pro-angiogenic factors is likely to improve DFU care. At the time of this writing, there is only one registered study on clinicaltrials.gov (a completed Phase I study) where a drug delivery matrix has been applied to DFU. In the study, an adenoviral vector carrying the DNA encoding for PDGF-B was incorporated into a collagen matrix for application to DFU [156]. The study revealed that the bovine type I collagen matrix, termed Gene Activated Matrix (GAM), could deliver the PDGF-B gene to invading cells to produce the protein at the wound site for a sufficient period of time [157]. The data leading to this clinical trial came from pre-clinical *in vivo* studies where a PDGF-B encoding adenovirus delivered via the GAM was found to enhance granulation tissue deposition and epithelialization in a rabbit model. The vector DNA and transgene mRNA were found within wound beds as late as 28 days post-treatment, and a single application of the biologic scaffold was equivalent to repeated applications of the PDGF-B protein [158]. Cardium Therapeutics, which owns the technology, is using GAM as a new product platform to deliver advanced wound care and therapeutic products including anti-infectives, antibiotics, peptides, proteins, small molecules, DNA, and GMCs.

D.2 Inhibiting Angiogenesis to Prevent Age Related Macular Degeneration (AMD)

In contrast to DFU, where there is a lack of angiogenesis, age related macular degeneration is caused by excessive blood vessel formation. AMD is the leading cause of legal blindness in people older than 55 years in the United States, affecting more than 1.75 million Americans. In the wet form, newly formed abnormal blood vessels grow under the center of the retina. These faulty vessels leak blood and scar the retina, eventually destroying central vision of the patient. As of the writing of this manuscript, there are six studies listed on clinicaltrials.gov under the search terms “macular degeneration” AND “drug delivery” AND “angiogenesis”. In one Phase I study, AdGVPEDF.11D was given as an open-label, single administration, dose to 28 patients with wet AMD to determine safety [159]. AdGVPEDF.11D, a replication deficient AV vector containing the gene for the anti-angiogenic PEDF protein, was delivered once via intravitreal injection into one eye. Investigators observed positive changes in vision and retinal appearance at the higher dose cohorts in some patients, no dose limiting toxicities or drug-related severe adverse events were reported [160]. The study sponsor GenVec believes that early intervention therapy with AdGVPEDF.11D holds the promise to stabilize or improve vision in patients with AMD. Their hypothesis is supported by pre-clinical investigations where they found that AdPEDF rapidly elevates

intraocular PEDF protein levels, inhibits abnormal blood vessel growth, and causes abnormal blood vessel regression.

E. Future of Regenerative Medicine and Pathway to the Clinic

The first-steps to translating a regenerative medicine technology are: identifying an unmet medical need and developing a hypothesis explaining how new technology might alter disease pathogenesis (Fig. 5 Step 1: Disease Pathogenesis). To be successful in this first step, it is critical to understand normal biology, have a perspective of what models are used to study the disease, and lastly, to understand the mechanism of action as it relates to the disease process. The next step in the process is to analyze the competitive commercial landscape and consider how the disease is currently managed in the clinic. Consider how and why the technology would be better than existing therapies for the specific disease (Fig. 5 Step 2: Current Treatments). Examples of how the technology could improve patient care are enhanced effectiveness, cost benefits, or reduced toxicity. Translating, the technology should also be patentable. Without patent protection, commercialization will be difficult, if not impossible. Patent protection ensures the investor(s) will have financial security and enables them to assume financial risks inherent in running clinical trials. The next step (Fig. 5 Step 3: Proof-of-Concept Testing) is to test the technology in the appropriate pre-clinical *in vitro*, small animal, and large animal models capable of predicting success in humans. Animal models of human disease vary in their accuracy in predicting human response. Upon successful completion of animal studies, meetings with the FDA will guide the subsequent steps in development, including standardized testing for safety, toxicity, and effectiveness (Fig. 5 Step 4: FDA Pre-Clinical Testing). Once the FDA is satisfied that appropriate pre-clinical studies have been completed, human clinical trials may commence (Fig. 5 Step 5: Clinical Testing in Humans). There are four phases of clinical trials. Phase I trials are the first stage of testing carried out in human subjects and emphasize safety. They are designed to assess the drug's most frequent and serious adverse events, as well as how the drug is metabolized and excreted. Phase II trials evaluate the effectiveness, short-term side effects, and common risks associated with the drug.

Phase III trials obtain information on the effectiveness of the drug across different populations, dosages, and in combination with other drugs. Upon successful completion of phase III clinical trials, technologies can be commercialized for use in humans. Phase IV is often referred to as post-marketing surveillance. In this period, the ongoing safety and efficacy of the drug is monitored in large populations, and additional uses of the drug are identified that might be approved by the FDA. In summary, following this simplified 6 step process, researchers and clinicians can develop therapies that translate into improved patient care (Fig. 5 Step 6: Clinical outcomes).

Conclusion

With a combinatorial approach using soluble factors delivered by rationally designed carriers and scaffolds, regenerative technologies will become a more prominent feature in clinical practice and regenerative medicine. SMDs have only recently been developed for applications in regenerative medicine, however the identification and optimization of new

SMDs is a slow, laborious, and costly process limiting their development. Polypeptide and nucleic acid-based therapeutics likely represent the future of this field. Clinical success has already been seen with mABs and apatmers, and there are ongoing clinical trials using growth factors, cDNA and RNAi technologies for regenerative medicine. Genome editing using ZFP-TFs, ZFNs and TALENs represents an exciting direction in regenerative therapeutics that is still in its infancy.

Development of these technologies for regenerative medicine will require continued, multidisciplinary research and an understanding of the regulatory pathways that will lead to clinical success. Recent progress in this field suggests that bioengineered delivery of soluble factors for regenerative medicine will have expanded clinical applicability in the future. This field represents an exciting collaborative effort between basic scientists developing therapeutic soluble factors, biomedical engineers improving their delivery, and clinicians driving and translating the technologies in the clinic. Regenerative medicine has the potential to drastically change the field of health care from reactive, to preventative and restorative.

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References

1. Hasetine W. A brave new medicine. A conversation with William Haseltine. Interview by Joe Flower. Health Forum journal. 1999; 42(4):28–30. 61–65. [PubMed: 10539016]
2. Chen FM, Zhao YM, Jin Y, Shi S. Prospects for translational regenerative medicine. Biotechnology advances. 2012; 30(3):658–672. [PubMed: 22138411]
3. Couto DS, Perez-Breva L, Cooney CL. Regenerative medicine: learning from past examples. Tissue engineering Part A. 2012; 18(21–22):2386–2393. [PubMed: 22697402]
4. Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell. 2006; 126(4):663–676. doi:http://dx.doi.org/10.1016/j.cell.2006.07.024. [PubMed: 16904174]
5. Davis RLWH, Lassar AB. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell. 1987; 51(6):987–1000. [PubMed: 3690668]
6. Rai BNV, Cool SM. Heparan sulfate-based treatments for regenerative medicine. Crit Rev Eukaryot Gene Expr. 2011; 21(1):1–12. [PubMed: 21967329]
7. Cherry ABC, Daley GQ. Reprogramming Cellular Identity for Regenerative Medicine. Cell. 2012; 148(6):1110–1122. [PubMed: 22424223]
8. Robinton DA, Daley GQ. The promise of induced pluripotent stem cells in research and therapy. Nat. 2012; 481(7381):295–305.
9. Cohen DE, Melton D. Turning straw into gold: directing cell fate for regenerative medicine. Nat Rev Genet. 2011; 12(4):243–252. [PubMed: 21386864]
10. Jopling C, Boue S, Belmonte JCI. Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. Nat Rev. 2011; 12:79–89.
11. Smith HO, Wilcox KW. A restriction enzyme from Hemophilus influenzae. I. Purification and general properties. J Mol Biol. 1970; 51(2):379–391. [PubMed: 5312500]
12. Brown LR. Commercial challenges of protein drug delivery. Expert Opin Drug Deliv. 2005; 2(1): 29–42. [PubMed: 16296733]

13. Rosenberg SA, Aebersold P, Cornetta K, Kasid A, Morgan RA, Moen R, Anderson WF, et al. Gene transfer into humans--immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med. 1990; 323(9): 570–578. [PubMed: 2381442]
14. Blaese RM, Culver KW, Miller AD, Carter CS, Fleisher T, Clerici M, et al. T Lymphocyte-Directed Gene Therapy for ADA− SCID: Initial Trial Results After 4 Years. Sci. 1995; 270(5235): 475–480.
15. Egermann M, Baltzer AW, Adamaszek S, Evans C, Robbins P, Schneider E, et al. Direct adenoviral transfer of bone morphogenetic protein-2 cDNA enhances fracture healing in osteoporotic sheep. Human Gene Therapy. 2006; 17(5):507–517. [PubMed: 16716108]
16. Kliem MA, Heeke BL, Franz CK, Radovitskiy I, Raore B, Barrow E, et al. Intramuscular administration of a VEGF zinc finger transcription factor activator (VEGF-ZFP-TF) improves functional outcomes in SOD1 rats. Amyotroph Lateral Scler. 2011; 12(5):331–339. [PubMed: 21864053]
17. Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY, et al. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006; 355(14):1419–1431. [PubMed: 17021318]
18. Jeschke MG, Herndon DN. The combination of IGF-I and KGF cDNA improves dermal and epidermal regeneration by increased VEGF expression and neovascularization. Gene Ther. 2007; 14(16):1235–1242. [PubMed: 17538636]
19. Wetterau M, George F, Weinstein A, Nguyen PD, Tutela JP, Knobel D, et al. Topical prolyl hydroxylase domain-2 silencing improves diabetic murine wound closure. Wound Repair Regen. 2011; 19(4):481–486. [PubMed: 21627711]
20. Gragoudas ES, Adamis AP, Cunningham ET, Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med. 2004; 351(27):2805–2816. [PubMed: 15625332]
21. Sooriakumaran P, Coley HM, Fox SB, Macanas-Pirard P, Lovell DP, Henderson A, et al. A Randomized Controlled Trial Investigating the Effects of Celecoxib in Patients with Localized Prostate Cancer. Anticancer Res. 2009; 29(5):1483–1488. [PubMed: 19443354]
22. Blumenthal GM, Cortazar P, Zhang JJ, Tang SH, Sridhara R, Murgo A, et al. FDA Approval Summary: Sunitinib for the Treatment of Progressive Well-Differentiated Locally Advanced or Metastatic Pancreatic Neuroendocrine Tumors. Oncol. 2012; 17(8):1108–1113.
23. Russell JL, Goetsch SC, Aguilar HR, Frantz DE, Schneider JW. Targeting Native Adult Heart Progenitors with Cardiogenic Small Molecules. ACS Chem Biol. 2012; 7(6):1067–1076. [PubMed: 22413910]
24. Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov. 2010; 9(3):203–214. [PubMed: 20168317]
25. Nikulina E, Tidwell JL, Dai HN, Bregman BS, Filbin MT. The phosphodiesterase inhibitor rolipram delivered after a spinal cord lesion promotes axonal regeneration and functional recovery. Proc Natl Acad Sci U S A. 2004; 101(23):8786–8790. [PubMed: 15173585]
26. Downing TL, Wang AJ, Yan ZQ, Nout Y, Lee AL, Beattie MS, et al. Drug-eluting microfibrous patches for the local delivery of rolipram in spinal cord repair. J Control Release. 2012; 161(3): 910–917. [PubMed: 22634093]
27. Hao Y, Creson T, Zhang L, Li P, Du F, Yuan P, et al. Mood Stabilizer Valproate Promotes ERK Pathway-Dependent Cortical Neuronal Growth and Neurogenesis. J Neurosci. 2004; 24(29):6590–6599. [PubMed: 15269271]
28. Wu F, Xing DM, Peng ZR, Rao T. Enhanced rat sciatic nerve regeneration through silicon tubes implanted with valproic acid. J Reconstr Microsurg. 2008; 24(4):267–276. [PubMed: 18496777]
29. Ferrer-Miralles N, Domingo-Espin J, Corchero JL, Vazquez E, Villaverde A. Microbial factories for recombinant pharmaceuticals. Microb Cell Fact. 2009; 8:17. [PubMed: 19317892]
30. Graslund S, Nordlund P, Weigelt J, Hallberg BM, Bray J, Gileadi O, et al. Protein production and purification. Nat Methods. 2008; 5(2):135–146. [PubMed: 18235434]

31. Goodman M. Market watch: Sales of biologics to show robust growth through to 2013. Nat Rev Drug Discov. 2009; 8(11):837. doi:http://www.nature.com/nrd/journal/v8/n11/suppinfo/nrd3040_S1.html. [PubMed: 19876035]
32. Martínez JL, Liu L, Petranovic D, Nielsen J. Pharmaceutical protein production by yeast: towards production of human blood proteins by microbial fermentation. Curr Opin Biotechol. 2012; 23(6): 965–971.
33. Redwan EL MR. Cumulative updating of approved biopharmaceuticals. Hum Antib. 2007; 16(3/4): 138–156.
34. Hariawala MD, D E. VEGF improves myocardial blood flow but produces EDRF-mediated hypotensionin porcine hearts. J Surg Res. 1996; 63(77–82)
35. Henry TD, Annex BH, McKendall GR, Azrin MA, Lopez JJ, Giordano FJ, et al. The VIVA Trial: Vascular Endothelial Growth Factor in Ischemia for Vascular Angiogenesis. Circ. 2003; 107(10): 1359–1365.
36. Lazarous DF, Shou M, Scheinowitz M, Hodge E, Thirumurti V, Kitsiou AN, et al. Comparative effects of basic fibroblast growth factor and vascular endothelial growth factor on coronary collateral development and the arterial response to injury. Circ. 1996; 94(5):1074–1082.
37. Eppler SM, Combs DL, Henry TD, Lopez JJ, Ellis SG, Yi JH, et al. A target-mediated model to describe the pharmacokinetics and hemodynamic effects of recombinant human vascular endothelial growth factor in humans. Clin Pharmacol & Ther. 2002; 72(1):20–32. [PubMed: 12152001]
38. Ramakrishna S, Kim Y-H, Kim H. Stability of Zinc Finger Nuclease Protein Is Enhanced by the Proteasome Inhibitor MG132. PLoS ONE. 2013; 8(1):e54282. [PubMed: 23365657]
39. Choo Y, Sanchez-Garcia I, Klug A. In vivo repression by a site-specific DNA-binding protein designed against an oncogenic sequence. Nature. 1994; 372(6507):642–645. [PubMed: 7990954]
40. Kang JS, Kim J-S. Zinc Finger Proteins as Designer Transcription Factors. J Biol Chem. 2000; 275(12):8742–8748. [PubMed: 10722717]
41. Li-Shan L, Yao-Guo Y, Wei L, Li-Long G, Heng G, Chang-Wei L, et al. Zinc finger protein-activating transcription factor up-regulates vascular endothelial growth factor-a expression in vitro. Chin Med Sci J. 2012; 27(3):171–175. [PubMed: 23062640]
42. Dai Q, Huang J, Klitzman B, Dong C, Goldschmidt-Clermont PJ, March KL, et al. Engineered zinc finger-activating vascular endothelial growth factor transcription factor plasmid DNA induces therapeutic angiogenesis in rabbits with hindlimb ischemia. Circ. 2004; 110(16):2467–2475.
43. Tsang, SH. Stem Cell Biology and Regenerative Medicine in Opthamology. New York, NY: Humana Press; 2012. Stem Cell Biology and Regenerative Medicine.
44. Sebastiano V, Maeder ML, Angstman JF, Haddad B, Khayter C, Yeo DT, et al. In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases. Stem cells (Dayton, Ohio). 2011; 29(11):1717–1726.
45. Soldner F, Laganiere J, Cheng AW, Hockemeyer D, Gao Q, Alagappan R, et al. Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. Cell. 2011; 146(2):318–331. [PubMed: 21757228]
46. Phase 1 Dose Escalation Study of Autologous T-cells Genetically Modified at the CCR5 Gene by Zinc Finger Nucleases in HIV-Infected Patients [database on the Internet]. ClinicalTrials.gov Identifier: NCT01044654. 2012. Accessed:
47. Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol. 2013; 14(1):49–55. doi:http://www.nature.com/nrm/journal/v14/n1/suppinfo/nrm3486_S1.html. [PubMed: 23169466]
48. Ding Q, Lee Y-K, Schaefer EAK, Peters DT, Veres A, Kim K, et al. A TALEN Genome-Editing System for Generating Human Stem Cell-Based Disease Models. Cell: Stem Cell. 2013; 12:238–251. [PubMed: 23246482]
49. Gabriel R, Lombardo A, Arens A, Miller JC, Genovese P, Kaeppel C, et al. An unbiased genome-wide analysis of zinc-finger nuclease specificity. Nat Biotech. 2011; 29(9):816–823. doi:http://www.nature.com/nbt/journal/v29/n9/abs/nbt.1948.html#supplementary-information.

50. Pattanayak V, Ramirez CL, Joung JK, Liu DR. Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection. Nat Meth. 2011; 8(9):765–770. doi:http://www.nature.com/nmeth/journal/v8/n9/abs/nmeth.1670.html#supplementary-information.
51. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. Nat Rev Drug Discov. 2010; 9(10):767–774. [PubMed: 20811384]
52. Dhoot DS, Kaiser PK. Ranibizumab for age-related macular degeneration. Expert Opin Biol Ther. 2012; 12(3):371–381. [PubMed: 22309606]
53. Keizer RJ, Huitema AD, Schellens JH, Beijnen JH. Clinical pharmacokinetics of therapeutic monoclonal antibodies. Clin Pharmacokinet. 2010; 49(8):493–507. [PubMed: 20608753]
54. Ng CM, Bruno R, Combs D, Davies B. Population Pharmacokinetics of Rituximab (Anti-CD20 Monoclonal Antibody) in Rheumatoid Arthritis Patients During a Phase II Clinical Trial. J Clin Pharmacol. 2005; 45(7):792–801. [PubMed: 15951469]
55. Getts DR, Getts MT, McCarthy DP, Chastain EML, Miller SD. Have we overestimated the benefit of human(ized) antibodies? Mabs. 2010; 2(6):682–694. [PubMed: 20935511]
56. Kormann MS, Hasenpusch G, Aneja MK, Nica G, Flemmer AW, Herber-Jonat S, et al. Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. Nat Biotechnol. 2011; 29(2):154–157. [PubMed: 21217696]
57. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nat. 2001; 411(6836):494–498.
58. Filleur S, Courtin A, Ait-Si-Ali S, Guglielmi J, Merle C, Harel-Bellan A, et al. SiRNA-mediated inhibition of vascular endothelial growth factor severely limits tumor resistance to Antiangiogenic thrombospondin-1 and slows tumor vascularization and growth. Cancer Res. 2003; 63(14):3919–3922. [PubMed: 12873985]
59. Kaiser PK, Symons RC, Shah SM, Quinlan EJ, Tabandeh H, Do DV, et al. RNAi-based treatment for neovascular age-related macular degeneration by Sirna-027. American journal of ophthalmology. 2010; 150(1):33–39 e2. [PubMed: 20609706]
60. Bumcrot D, Manoharan M, Koteliansky V, Sah DWY. RNAi therapeutics: A potential new class of pharmaceutical drugs. Nat Chem Biol. 2006; 2(12):711–719. [PubMed: 17108989]
61. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990; 249(4968):505–510. [PubMed: 2200121]
62. Ellington A, Keefe AD, Pai S. Aptamers as therapeutics. Nat Rev Drug Discov. 2010; 9(7):537–550. [PubMed: 20592747]
63. Ng EWM, Shima DT, Calias P, Cunningham ET, Guyer DR, Adamis AP. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. Nat Rev Drug Discov. 2006; 5(2):123–132. [PubMed: 16518379]
64. White RR, Sullenger BA, Rusconi CP. Developing aptamers into therapeutics. Journal of Clinical Investigation. 2000; 106(8):929–934. [PubMed: 11032851]
65. Cao J, Sun C, Zhao H, Xiao Z, Chen B, Gao J, et al. The use of laminin modified linear ordered collagen scaffolds loaded with laminin-binding ciliary neurotrophic factor for sciatic nerve regeneration in rats. Biomaterials. 2011; 32(16):3939–3948. [PubMed: 21397941]
66. Piantino J, Burdick JA, Goldberg D, Langer R, Benowitz LI. An injectable, biodegradable hydrogel for trophic factor delivery enhances axonal rewiring and improves performance after spinal cord injury. Exp Neurol. 2006; 201(2):359–367. doi:http://dx.doi.org/10.1016/j.expneurol.2006.04.020. [PubMed: 16764857]
67. Censi R, Di Martino P, Vermonden T, Hennink WE. Hydrogels for protein delivery in tissue engineering. J Control Release. 2012; 161(2):680–692. doi:http://dx.doi.org/10.1016/j.jconrel.2012.03.002. [PubMed: 22421425]
68. Sill TJ, von Recum HA. Electrospinning: Applications in drug delivery and tissue engineering. Biomaterials. 2008; 29(13):1989–2006. [PubMed: 18281090]
69. Curtin CMCG, Lyons FG, Bessho K, Dickson GR, Duffy GP, O'Brien FJ. Innovative Collagen Nano-Hydroxyapatite Scaffolds Offer a Highly Efficient Non-Viral Gene Delivery Platform for Stem Cell-Mediated Bone Formation. Advanced Materials. 2012; 24(6):749–754. [PubMed: 22213347]

70. Macaya D, Spector M. Injectable hydrogel materials for spinal cord regeneration: a review. Biomed Mater. 2012; 7(1):012001. [PubMed: 22241481]
71. Liao IC, Chew SY, Leong KW. Aligned core-shell nanofibers delivering bioactive proteins. Nanomedicine. 2006; 1(4):465–471. [PubMed: 17716148]
72. Chew SY, Mi R, Hoke A, Leong KW. Aligned Protein–Polymer Composite Fibers Enhance Nerve Regeneration: A Potential Tissue-Engineering Platform. Adv Funct Mater. 2007; 17(8):1288–1296. [PubMed: 18618021]
73. Marchesi C, Pluderi M, Colleoni F, Belicchi M, Meregalli M, Farini A, et al. Skin-derived stem cells transplanted into resorbable guides provide functional nerve regeneration after sciatic nerve resection. Glia. 2007; 55(4):425–438. [PubMed: 17203471]
74. Kloxin A, AM K, CN S, KS A. Photodegradable Hydrogels for Dynamic Tuning of Physical and Chemical Properties. Sci. 2009; 324:59–63.
75. Sershen SR, Westcott SL, Halas NJ, West JL. Temperature-sensitive polymer-nanoshell composites for photothermally modulated drug delivery. J Biomed Mater Res. 2000; 51(3):293–298. [PubMed: 10880069]
76. Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA. Hydrogels in Regenerative Medicine. Adv Mater. 2009; 21(32–33):3307–3329. [PubMed: 20882499]
77. Ehrbar M, Schoenmakers R, Christen EH, Fussenegger M, Weber W. Drug-sensing hydrogels for the inducible release of biopharmaceuticals. Nat Mater. 2008; 7(10):800–804. [PubMed: 18690239]
78. Tang M, Zhang R, Bowyer A, Eisenthal R, Hubble J. A reversible hydrogel membrane for controlling the delivery of macromolecules. Biotechnol Bioeng. 2003; 82(1):47–53. [PubMed: 12569623]
79. Zhang R, Tang M, Bowyer A, Eisenthal R, Hubble J. Synthesis and characterization of a D-glucose sensitive hydrogel based on CM-dextran and concanavalin A. Reactive & Functional Polymers. 2006; 66(7):757–767.
80. Wang F, Li Z, Khan M, Tamama K, Kuppusamy P, Wagner WR, et al. Injectable, rapid gelling and highly flexible hydrogel composites as growth factor and cell carriers. Acta Biomaterialia. 2010; 6(6):1978–1991. [PubMed: 20004745]
81. Banerjee I, Mishra D, Das T, Maiti TK. Wound pH-Responsive Sustained Release of Therapeutics from a Poly(NIPAAm-co-AAc) Hydrogel. Journal of Biomaterials Science-Polymer Edition. 2012; 23(1–4):111–132. [PubMed: 22133349]
82. Gao SQ, Maeda T, Okano K, Palczewski K. A Microparticle/Hydrogel Combination Drug-Delivery System for Sustained Release of Retinoids. Investig Ophthalmol Vis Sci. 2012; 53(10):6314–6323. [PubMed: 22918645]
83. Bidarra SJ, Barrias CC, Fonseca KB, Barbosa MA, Soares RA, Granja PL. Injectable in situ crosslinkable RGD-modified alginate matrix for endothelial cells delivery. Biomaterials. 2011; 32(31):7897–7904. [PubMed: 21784515]
84. Hastings CL, Kelly HM, Murphy MJ, Barry FP, O'Brien FJ, Duffy GP. Development of a thermoresponsive chitosan gel combined with human mesenchymal stem cells and desferrioxamine as a multimodal pro-angiogenic therapeutic for the treatment of critical limb ischaemia. J Control Release. 2012; 161(1):73–80. [PubMed: 22562065]
85. Sun J, Jiang G, Qiu T, Wang Y, Zhang K, Ding F. Injectable chitosan-based hydrogel for implantable drug delivery: Body response and induced variations of structure and composition. Journal of Biomedical Materials Research Part A. 2010; 95A(4):1019–1027. [PubMed: 20872751]
86. Choi JS. In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF). Biomaterials. 2007; 29:587–596. [PubMed: 17997153]
87. Liao IC, Leong KW. Efficacy of engineered FVIII-producing skeletal muscle enhanced by growth factor-releasing co-axial electrospun fibers. Biomaterials. 2011; 32(6):1669–1677. [PubMed: 21084118]
88. Cui W, Zhou Y, Chang J. Electrospun nanofibrous materials for tissue engineering and drug delivery. Sci Technol Adv Mater. 2010; 11(1):14109–14119.

89. Fu Y-C, Nie H, Ho M-L, Wang C-K, Wang C-H. Optimized bone regeneration based on sustained release from three-dimensional fibrous PLGA/HAp composite scaffolds loaded with BMP-2. Biotechnol and Bioeng. 2008; 99(4):996–1006.

90. Chew SY, Wen J, Yim EKF, Leong KW. Sustained release of proteins from electrospun biodegradable fibers. Biomacromolecules. 2005; 6(4):2017–2024. [PubMed: 16004440]

91. Xu XL, Yang LX, Xu XY, Wang X, Chen XS, Liang QZ, et al. Ultrafine medicated fibers electrospun from W/O emulsions. J Control Release. 2005; 108(1):33–42. [PubMed: 16165243]

92. Xu XL, Zhuang XL, Chen XS, Wang XR, Yang LX, Jing XB. Preparation of core-sheath composite nanofibers by emulsion electrospinning. Macromol Rapid Commun. 2006; 27(19): 1637–1642.

93. Zhang H, Zhao C, Zhao Y, Tang G, Yuan X. Electrospinning of ultrafine core/shell fibers for biomedical applications. Sci China Chem. 2010; 53(6):1246–1254.

94. Sun ZC, Zussman E, Yarin AL, Wendorff JH, Greiner A. Compound core-shell polymer nanofibers by co-electrospinning. Adv Mater. 2003; 15(22):1929–1932.

95. Liao IC, Chen S, Liu JB, Leong KW. Sustained viral gene delivery through core-shell fibers. J Control Release. 2009; 139(1):48–55. [PubMed: 19539680]

96. Jiang H, Hu Y, Zhao P, Li Y, Zhu K. Modulation of protein release from biodegradable core–shell structured fibers prepared by coaxial electrospinning. J Biomed Mater Res Part B: Appl Biomater. 2006; 79B(1):50–57. [PubMed: 16544305]

97. Bowman K, Leong KW. Chitosan nanoparticles for oral gene deliery. Int J Nanomedicine. 2006; 1(2):117–128. [PubMed: 17722528]

98. Pan J, Chan SY, Lee WG, Kang LF. Microfabricated particulate drug-delivery systems. Biotechnol J. 2011; 6(12):1477–1487. [PubMed: 22076813]

99. Kohane D. Microparticles and nanoparticles for drug delivery. Biotechnol Bioeng. 2007; 1(96): 203–209. [PubMed: 17191251]

100. Lee J, Bhang S, Park H, Kim B-S, Lee K. Active Blood Vessel Formation in the Ischemic Hindlimb Mouse Model Using a Microsphere/Hydrogel Combination System. Pharm Res. 2010; 27(5):767–774. [PubMed: 20221675]

101. Ise M, Ise H, Shiba Y, Kobayashi S, Goto M, Takahashi M, et al. Targeting N-acetylglucosamine-bearing polymer-coated liposomes to vascular smooth muscle cells. J Artif Organs. 2011; 14(4): 301–309. [PubMed: 21809097]

102. Auricchio A. Fighting Blindness with Adeno-Associated Virus Serotype 8. Human Gene Therapy. 2011; 22(10):1169–1170. [PubMed: 22044092]

103. Tang Y, Cummins J, Huard J, Wang B. AAV-directed muscular dystrophy gene therapy. Expert Opinion on Biological Therapy. 2010; 10(3):395–408. [PubMed: 20132060]

104. Hacein-Bey-Abina S, et al. LMO2-Associated clonal T-cell proliferation in two patients after gene therapy for SCID-X1. Sci. 2003; 302(5644):415–419.

105. Felgner JH, Kumar R, Sridhar CN, Wheeler CJ, Tsai YJ, Border R, et al. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J Biol Chem. 1994; 269(4):2550–2561. [PubMed: 8300583]

106. Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, et al. Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. J Control Release. 2001; 70(3):399–421. [PubMed: 11182210]

107. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, et al. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci U S A. 1995; 92(16):7297–7301. [PubMed: 7638184]

108. Su XF, Fricke J, Kavanagh DG, Irvine DJ. In Vitro and in Vivo mRNA Delivery Using Lipid-Enveloped pH-Responsive Polymer Nanoparticles. Molec Pharm. 2011; 8(3):774–787. [PubMed: 21417235]

109. Zhang Y, Satterlee A, Huang L. In Vivo Gene Delivery by Nonviral Vectors: Overcoming Hurdles. Mol Ther. 2012; 20(7):1298–1304. [PubMed: 22525514]

110. Romero NB, Braun S, Benveniste O, Leturcq F, Hogrel JY, Morris GE, et al. Phase I study of dystrophin plasmid-based gene therapy in Duchenne/Becker muscular dystrophy. Hum Gene Ther. 2004; 15(11):1065–1076. [PubMed: 15610607]

111. Guo X, Huang L. Recent Advances in Nonviral Vectors for Gene Delivery. Acc Chem Res. 2012; 45(7):971–979. [PubMed: 21870813]

112. Elsabahy M, Nazarali A, Foldvari M. Non-Viral Nucleic Acid Delivery: Key Challenges and Future Directions. Curr Drug Deliv. 2011; 8(3):235–244. [PubMed: 21291381]

113. Garcia, L.; Urbiola, K.; Duzgunes, N.; de Ilarduya, CT. Lipopolyplexes as nanomedicines for therapeutic gene delivery. In: Duzgunes, N., editor. Nanomedicine: Infectious Diseases, Immunotherapy, Diagnostics, Antifibrotics, Toxicology and Gene Medicine. Methods in Enzymology. San Diego: Elsevier Academic Press Inc; 2012. p. 327-338.

114. Grigsby CL, Leong KW. Balancing protection and release of DNA: tools to address a bottleneck of non-viral gene delivery. J R Soc Interface. 2010; 7:S67–S82. [PubMed: 19734186]

115. Grigsby CL, Ho YP, Leong KW. Understanding nonviral nucleic acid delivery with quantum dot-fret nanosensors. Nanomedicine (Lond). 2012; 7(4):565–577. [PubMed: 22471720]

116. Green JJ, Langer R, Anderson DG. A Combinatorial Polymer Library Approach Yields Insight into Nonviral Gene Delivery. Accounts Chem Res. 2008; 41(6):749–759.

117. Anderson DG, Lynn DM, Langer R. Semi-Automated Synthesis and Screening of a Large Library of Degradable Cationic Polymers for Gene Delivery. Angew Chem. 2003; 115(27):3261–3266.

118. Pelet J, Putnam D. A Combinatorial Library of Bi-functional Polymeric Vectors for siRNA Delivery In Vitro. Pharm Res. 2013; 30(2):362–376. [PubMed: 23054087]

119. Scudellari M. The Delivery Dilemma. Nat Rep Stem Cells. 2009:1754–8705.

120. Elbjeirami WM, West JL. Angiogenesis-like activity of endothelial cells co-cultured with VEGF-producing smooth muscle cells. Tissue Eng. 2006; 12(2):381–390. [PubMed: 16548696]

121. Cyranoski D. Stem cells cruise to clinic: Japanese study of induced pluripotent stem cells aims to demonstrate safety in humans. Nature News. 2013; 494

122. Murry CE, Pu WT. Reprogramming Fibroblasts into Cardiomyocytes. N Engl J Med. 2011; 364(2):177–178. [PubMed: 21226585]

123. T V, A O, ZP P, Y K, TC S MW. Direct conversion of fibroblasts to functional neurons by defined factors. Nat. 2010; 463(7284):1035–1041.

124. Abdullah A, Pollock A, Sun T. The Path from Skin to Brain: Generation of Functional Neurons from Fibroblasts. Mol Neurobiol. 2012; 45(3):586–595. [PubMed: 22614130]

125. Yim EKF, Darling EM, Kulangara K, Guilak F, Leong KW. Nanotopography-induced changes in focal adhesions, cytoskeletal organization, and mechanical properties of human mesenchymal stem cells. Biomaterials. 2009; (31):1299–1306. [PubMed: 19879643]

126. Murugan R, Ramakrishna S. Design strategies of tissue engineering scaffolds with controlled fiber orientation. Tissue Eng. 2007; 13(8):1845–1866. [PubMed: 17518727]

127. Kosen Y, Miyaji H, Kato A, Sugaya T, Kawanami M. Application of collagen hydrogel/sponge scaffold facilitates periodontal wound healing in class II furcation defects in beagle dogs. J Periodontal Res. 2012; 47(5):626–634. [PubMed: 22443229]

128. Archibald SJ, Krarup C, Shefner J, Li ST, Madison RD. A collagen-based nerve guide conduit for peripheral nerve repair: an electrophysiological study of nerve regeneration in rodents and nonhuman primates. J Comp Neurol. 1991; 306(4):685–696. [PubMed: 2071700]

129. Lin YC, Marra KG. Injectable systems and implantable conduits for peripheral nerve repair. Biomed Mater. 2012; 7(2):024102. [PubMed: 22456722]

130. Rodriguez FJ, Gomez N, Perego G, Navarro X. Highly permeable polylactide-caprolactone nerve guides enhance peripheral nerve regeneration through long gaps. Biomaterials. 1999; 20(16): 1489–1500. [PubMed: 10458562]

131. Jeffries EM, Wang YD. Biomimetic micropatterned multi-channel nerve guides by templated electrospinning. Biotechnol Bioeng. 2012; 109(6):1571–1582. [PubMed: 22179932]

132. Wang HB, Mullins ME, Cregg JM, McCarthy CW, Gilbert RJ. Varying the diameter of aligned electrospun fibers alters neurite outgrowth and Schwann cell migration. Acta Biomater. 2010; 6(8):2970–2978. [PubMed: 20167292]

133. Cellot G, Cilia E, Cipollone S, Rancic V, Sucapane A, Giordani S, et al. Carbon nanotubes might improve neuronal performance by favouring electrical shortcuts. Nat Nanotechnol. 2009; 4(2): 126–133. [PubMed: 19197316]

134. Patel ZS, Mikos AG. Angiogenesis with biomaterial-based drug- and cell-delivery systems. J Biomater Sci, Polym Ed. 2004; 15(6):701–726. [PubMed: 15255521]

135. De Coppi P, Delo D, Farrugia L, Udompanyanan K, Yoo JJ, Nomi M, et al. Angiogenic Gene-Modified Muscle Cells for Enhancement of Tissue Formation. Tissue Eng. 2005; 11(7–8):1034–1044. [PubMed: 16144439]

136. Incontinent Urinary Diversion Using an Autologous Neo-Urinary Conduit [database on the Internet]. ClinicalTrials.gov Identifier: NCT01087697. 2012 Accessed:

137. Reiffel AJ, Kafka C, Hernandez KA, Popa S, Perez JL, Zhou S, et al. High-Fidelity Tissue Engineering of Patient-Specific Auricles for Reconstruction of Pediatric Microtia and Other Auricular Deformities. PLoS ONE. 2013; 8(2):e56506. [PubMed: 23437148]

138. Risau W. Mechanisms of angiogenesis. Nat. 1997; 386(6626):671–674.

139. Naderi H, Matin MM, Bahrami AR. Review paper: Critical Issues in Tissue Engineering: Biomaterials, Cell Sources, Angiogenesis, and Drug Delivery Systems. J Biomater Appl. 2011; 26(4):383–417. [PubMed: 21926148]

140. Chen RR, Silva EA, Yuen WW, Mooney DJ. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. Pharmaceutical Research. 2007; 24(2):258–264. [PubMed: 17191092]

141. Matsui T, Nishino Y, Maeda S, Yamagishi S-i. PEDF-derived peptide inhibits corneal angiogenesis by suppressing VEGF expression. Microvascular Research. 2012; 84(1):105–108. doi:http://dx.doi.org/10.1016/j.mvr.2012.02.006. [PubMed: 22386653]

142. Bowers DT, Chhabra P, Langman L, Botchwey EA, Brayman KL. FTY720-loaded poly(DL-lactide-co-glycolide) Electrospun Scaffold Significantly Increases Microvessel Density over 7 Days in Streptozotocin-Induced Diabetic C57b16/J Mice: Preliminary Results. Transplant Proc. 2011; 43(9):3285–3287. [PubMed: 22099778]

143. Loyd CM, Diaconu D, Fu W, Adams GN, Brandt E, Knutsen DA, et al. Transgenic overexpression of keratinocyte-specific VEGF and Ang1 in combination promotes wound healing under nondiabetic but not diabetic conditions. Int J Clin Exp Pathol. 2012; 5(1):1–11. [PubMed: 22295141]

144. Cho S-W, Yang F, Son SM, Park H-J, Green JJ, Bogatyrev S, et al. Therapeutic angiogenesis using genetically engineered human endothelial cells. J Control Release. 2012; 160(3):515–524. [PubMed: 22450331]

145. Keeney M, van den Beucken JJJP, van der Kraan PM, Jansen JA, Pandit A. The ability of a collagen/calcium phosphate scaffold to act as its own vector for gene delivery and to promote bone formation via transfection with VEGF(165). Biomaterials. 2010; 31(10):2893–2902. [PubMed: 20044134]

146. Said SS, Pickering JG, Mequanint K. Advances in Growth Factor Delivery for Therapeutic Angiogenesis. Journal of Vascular Research. 2013; 50(1):35–51. [PubMed: 23154615]

147. Amsden BG. Delivery approaches for angiogenic growth factors in the treatment of ischemic conditions. Expert opinion on drug delivery. 2011; 8(7):873–890. [PubMed: 21644842]

148. Schiffelers RM, van Rooy I, Storm G. siRNA-mediated inhibition of angiogenesis. Expert Opinion on Biological Therapy. 2005; 5(3):359–368. [PubMed: 15833073]

149. Park H-J, Yang F, Cho S-W. Nonviral delivery of genetic medicine for therapeutic angiogenesis. Advanced Drug Delivery Reviews. 2012; 64(1):40–52. [PubMed: 21971337]

150. Mitsos S, Katsanos K, Koletsis E, Kagadis GC, Anastasiou N, Diamantopoulos A, et al. Therapeutic angiogenesis for myocardial ischemia revisited: basic biological concepts and focus on latest clinical trials. Angiogenesis. 2012; 15(1):1–22. [PubMed: 22120824]

151. Chu H, Wang Y. Therapeutic angiogenesis: controlled delivery of angiogenic factors. Therapeutic delivery. 2012; 3(6):693–714. [PubMed: 22838066]

152. Bhise NS, Shmueli RB, Sunshine JC, Tzeng SY, Green JJ. Drug delivery strategies for therapeutic angiogenesis and antiangiogenesis. Expert opinion on drug delivery. 2011; 8(4):485–504. [PubMed: 21338327]

153. Margolis, D.; Malay, D.; Hoffstad, O.; Leonard, C.; T, M.; Lopez de Nava, K., et al. Quality, AfHRa, editor. Prevalence of diabetes, diabetic foot ulcer, and lower extremity amputation

among Medicare beneficiaries, 2006 to 2008: Data Points #1. Data points publication series. http://www.ncbi.nlm.nih.gov/books/NBK63602/2011

154. Pham HT, Economides PA, Veves A. The role of endothelial function on the foot: Microcirculation and wound healing in patients with diabetes. Clin Podiatr Med Surg. 1998; 15(1):85–93. [PubMed: 9463769]
155. Reddy MA, Natarajan R. Epigenetic mechanisms in diabetic vascular complications. Cardiovasc Res. 2011; 90(3):421–429. [PubMed: 21266525]
156. Gene Therapy to Improve Wound Healing in Patients With Diabetes [database on the Internet]. Tissue Repair Company. 2007 Accessed:
157. G M, AJ T, VT M, D M, L P, GF P, et al. Treatment of nonhealing diabetic foot ulcers with a platelet-derived growth factor gene-activated matrix (GAM501): results of a phase 1/2 trial. Wound Repair Regen. 2009; 17(6):772–779. [PubMed: 19821960]
158. Doukas JCL, Gonzalez AM, Gu D, Hoganson DK, Ma C, Nguyen T, Printz MA, Nesbit M, Herlyn M, Crombleholme TM, Aukerman SL, Sosnowski BA, Pierce GF. Matrix immobilization enhances the tissue repair activity of growth factor gene therapy vectors. Hum Gene Ther. 2001; 12(7):783–798. [PubMed: 11339895]
159. Study of AdGVPEDF.11D in Neovascular Age-related Macular Degeneration (AMD) [database on the Internet]. ClinicalTrials.gov Identifier: NCT00109499. 2011. Accessed:
160. GenVec. AdPEDF for Macular Degeneration. 2013. In: http://www.genvec.com/go.cfm?do=Page.View&pid=30, editor.: Internet;

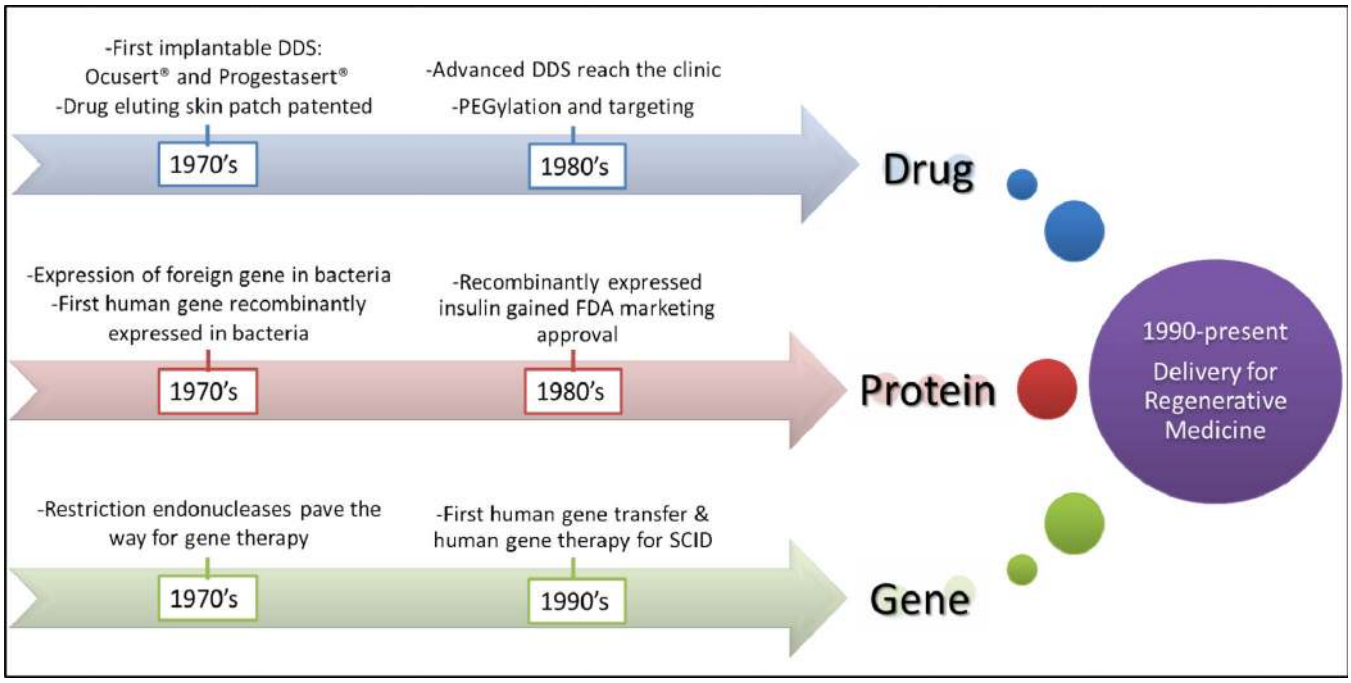

Fig. 1.

Parallel advancements in the use of drugs, proteins, and genes for regenerative therapeutics converged in the 1990's with the need for rationally designed delivery systems

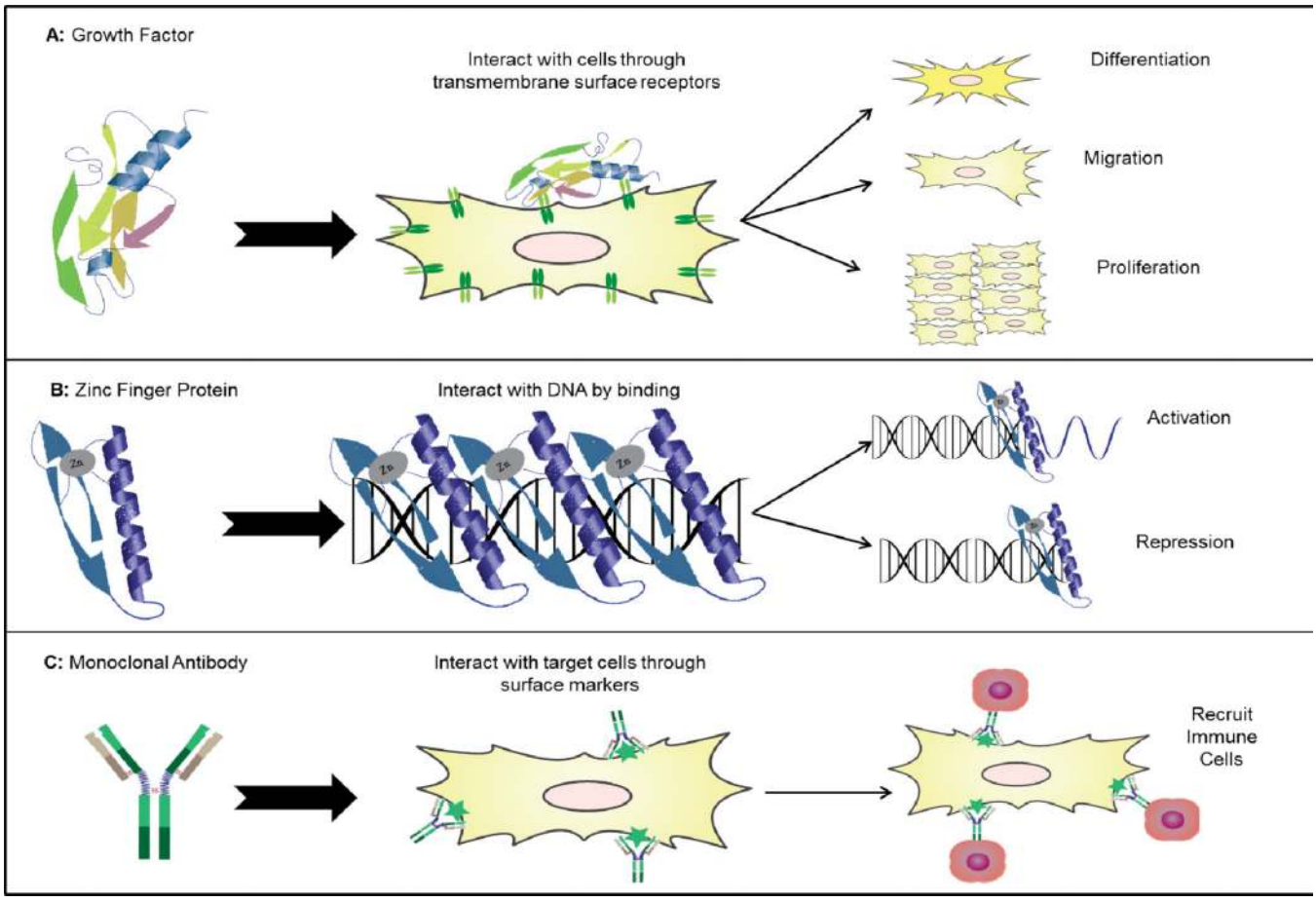

Fig. 2.

Visualization of protein-based cues. Growth factors interact with cells via transmembrane receptors to drive various processes such as differentiation, migration or proliferation (A). Each zinc finger protein recognizes 3–4 base pairs, such that a modular construct of multiple ZFPs can be generated to recognize a unique sequence in the genome (B). Monoclonal antibodies recognize specific antigens within the body, once bound to their target they can either physically inactivate it or recruit immune cells to destroy it (C)

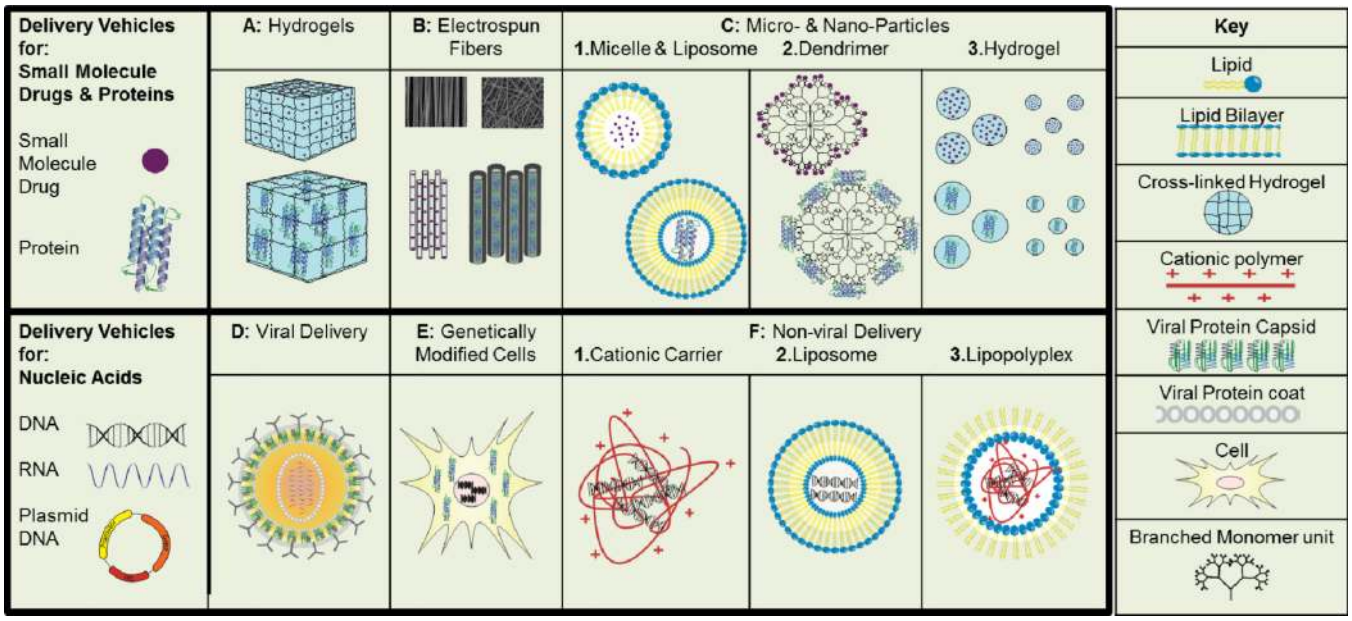

Fig. 3.

Delivery vehicles for small molecule drugs, proteins, and nucleic acids. Carriers for soluble factors can be macroscopic, such as hydrogels (A) and electrospun fibers (B), or microscopic, such as micelles and liposomes (C.1), dendrimers (C.2), or particulate hydrogel systems (C.3). Carriers for nucleic acids such as DNA, RNA and plasmid DNA have unique design requirements since they must be able to carry their negatively charged cargo across the negatively charged cellular membrane. Viral carriers (D) can be used to introduce DNA into cells, most commonly done *in vitro* to generate genetically modified cells (E). Non-viral delivery methods can include simple complexation with cationic polymers (F.1), or the use of particulate systems such as liposomes (F.2) and lipopolyplexes (F.3). These methods can also be used to generate genetically modified cells or deliver nucleic acid cargo *in vivo*

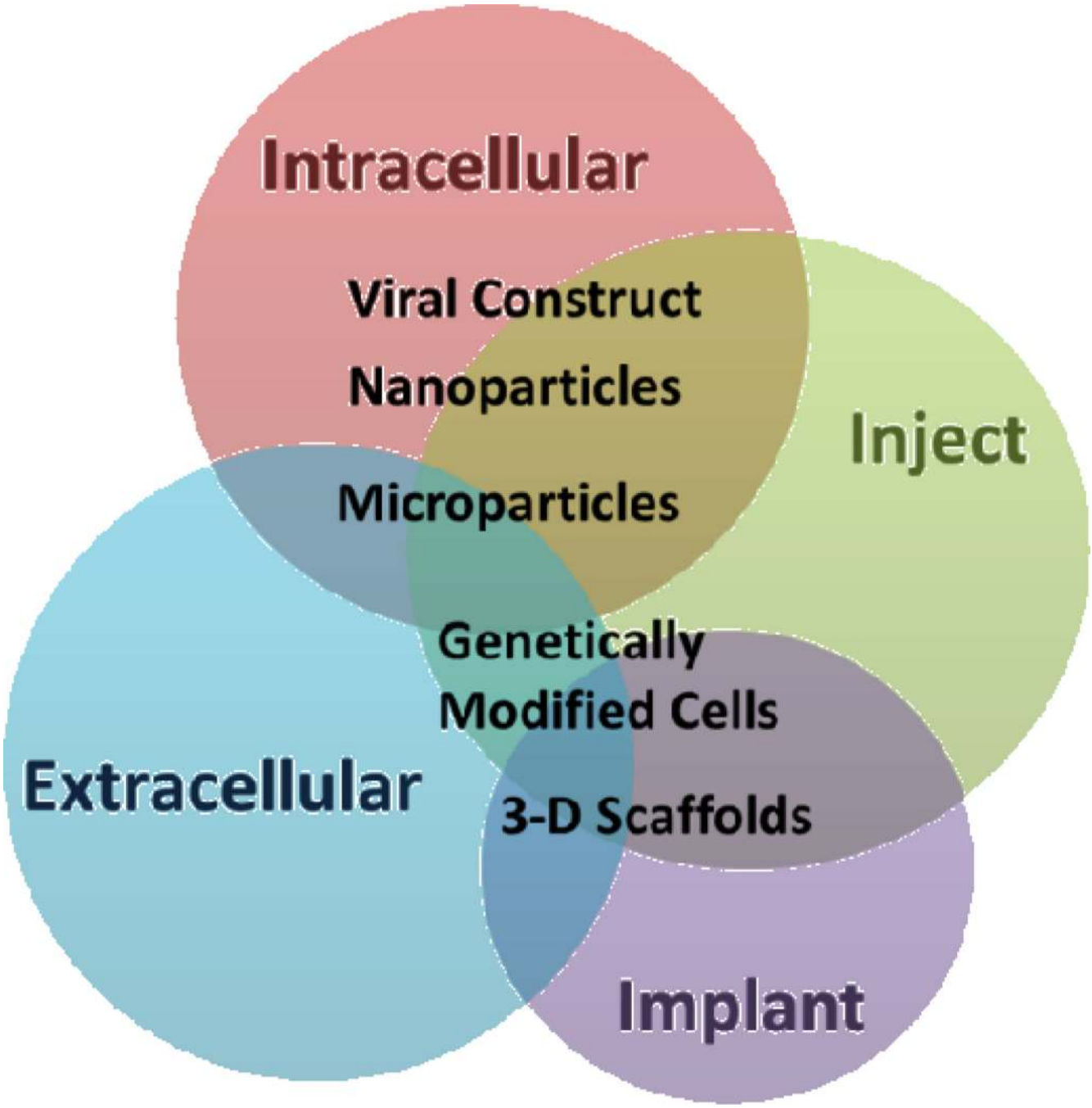

Fig. 4.

Carrier Selection Guide. Selection of the correct delivery vehicle must first consider whether the soluble factor is active intracellularly or if extracellular delivery is optimal. Once delivery method is chosen, the delivery method of the carrier should be selected based on disease pathogenesis. Injection of microscopic carriers may be optimal for systemic or localized pathogenesis, while implantation of 3D scaffold carriers are most effective when repairing a tissue defect

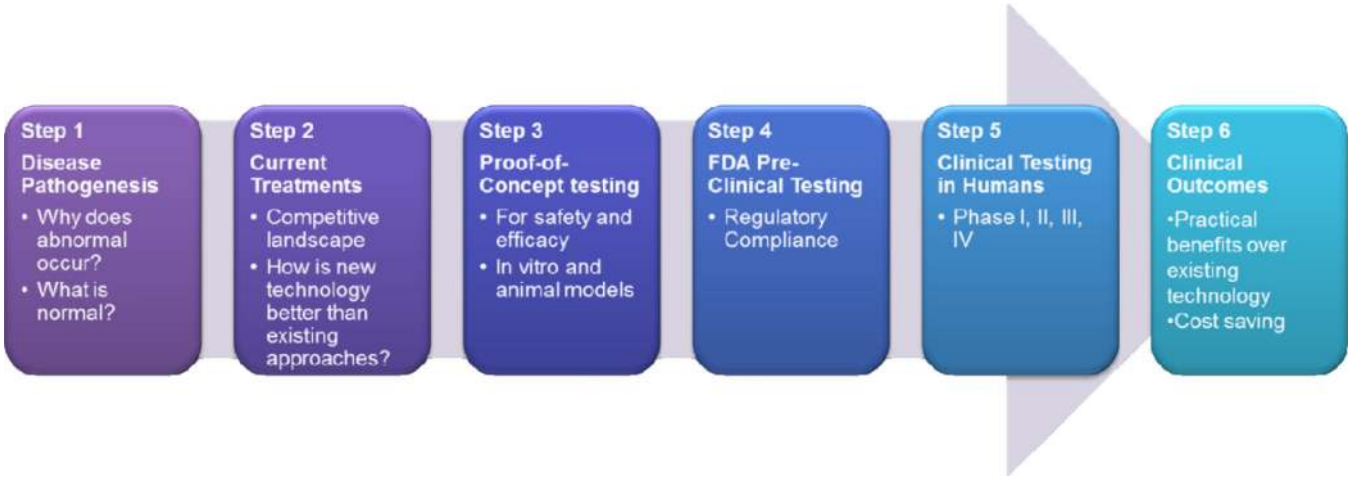

Fig. 5.
Technology Patent Protection and the steps to clinical translation

Table 1

Summary of soluble factors including their most common uses in regenerative medicine, as well as general pro's and con's of the system

Class of Drug	Soluble Factor	Most Common Regenerative Application	Pro's	Con's
SMDs	Small Molecule Drugs	Local delivery for nervous system regeneration	Infrastructure and popular acceptance exists for manufacture and use	Costly to identify develop novel formulations
Protein Drugs	Growth factor	Tissue Regeneration and angiogenesis	Naturally regulate cell function. Recombinant manufacturing	Can be toxic in excess, short half-life, subject to enzymatic cleavage
	Zinc Finger Proteins	Genome editing	Activate endogenous genes expressing all splice variants, or repress endogenous genes	High cost and difficulty of generation
	TALENs	Genome editing: repress endogenous genes	Simplistic design methods, low cost of production	Development of these as therapeutics is in its infancy
	Moncolonal Antibody	Inactivate pathways associated with disease states	Long half-life compared to other protein-based therapeutics, high specificity	Large size inhibits trafficing within tissues; immunogenecity
Nuclcic Acids	cDNA/pDNA	Introduce genes into cells	Well-developed technique for *in vitro* manipulation of cells to express a protein or gene of interest	Delivery method and risk of insertional mutageneis
	RNAi	Inhibit endogenous RNA to regulate gene expression	Regulate endogenous gene expression *in vivo*	Rapidly cleared *in vivo*, non-tissue-specific, negatively charged
	Aptamer	Inhibit action of target	Essentially non-immunogenic, and chemical generation results in little batch-to-batch variation	Rapidly degraded *in vivo*, & costly to generate