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Review Article

Gene Therapy Review

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ABSTRACT

Gene therapy involves transferring genetic material into cells to treat disease. This review will discuss gene transfer methods and current and potential applications for craniofacial regeneration, focusing on future development and design. Non-viral gene delivery methods have limitations in gene transfer efficiency. However, they offer advantages such as safety, low immunogenicity, ease of manufacturing, and lack of DNA insert size restrictions. Viral vectors are natural tools for delivering genes. They can be tailored for targeting specific tissues, integrating into specific sites on chromosomes, and infecting both dividing and non-dividing cells for extended periods. In contrast to traditional gene therapy for replacement, craniofacial regeneration aims to use genetic vectors as additional components for tissue growth and repair. The synergy of viral gene therapy with craniofacial tissue engineering will greatly improve our capacity to repair and replace tissues in living organisms.

INTRODUCTION

Human gene therapy involves treating disorders or diseases by introducing engineered genetic material into human cells, typically using viral transduction. Since the introduction of science fiction, the popular press has explored the concept of viral gene delivery and its alarming consequences. One of the more recent popular works on the topic is the 2007 remake of Richard Matheson's classic 1954 novel I Am Legend. The storyline follows the events that occur after the discovery, release, and mutation of a genetically re-engineered measles virus that was initially believed to be a cure for cancer (Matheson, 1954; Lawrence, 2007). This adapted novel has been made into three feature films, depicting the potential global devastation caused by viral gene therapy. Since 1990, billions of dollars have been spent on hundreds of human viral gene therapy clinical trials despite the emotionally stirring history of fictional violence and the debate that provokes moral and medical issues. Our society is currently undergoing a paradigm shift. It started with the recognition of viruses as harmful

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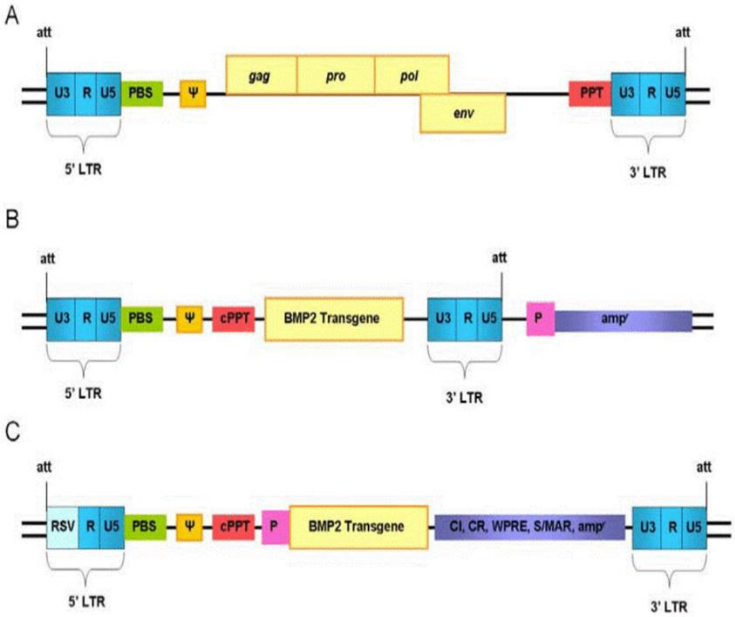
infectious agents and will eventually lead to their use in treating diseases and repairing tissues. On January 19, 1989, the director of the National Institutes of Health (NIH), Dr. Miller, made a critical announcement regarding research funding. James A. The first clinical protocol to insert a foreign gene into the immune cells of cancer patients was approved by all. (Roberts, 1989) On September 14, 1990, W. French Anderson and his colleagues at the NIH conducted the inaugural gene therapy operation on a four-year-old girl diagnosed with severe combined immunodeficiency (SCID) (Anderson, 1990). Despite the viral horror stories written by the popular media, this initial trial was largely a success, and the most recent report on this individual in 2004 noted that she is thriving as an 18-year-old teenager in suburban Cleveland (Springen, 2004). Over the following decade, around 300 clinical gene therapy trials were conducted involving approximately 3000 individuals (McKie, 2000). The field was darkened by the death of an 18-year-old male four days after the introduction of 38 trillion particles of recombinant adenovirus into his liver (Somia and Verma, 2000). Despite this tragedy, we are motivated to move forward by the promising advancements in genetic treatments. Novel genetic treatments have the potential to surpass current methods like protein therapy and pharmaceuticals in treating various diseases and defects.

Virus First, the delivery system must be safe and immunologically inert. Secondly, it must safeguard the genetic material from degradation. Third, the vector should contain a therapeutic gene with consistent expression at a specific target location. In order to be suitable for commercial use, the packaged vector must be readily producible, processed easily, and have a reasonable shelf-life. As we approach the 20-year milestone since the first human gene therapy clinical trial, there have been significant advancements in meeting these three criteria. New objectives have emerged, including tissue-specific targeting, site-specific chromosomal integration, and controlled infection of both dividing and non.1

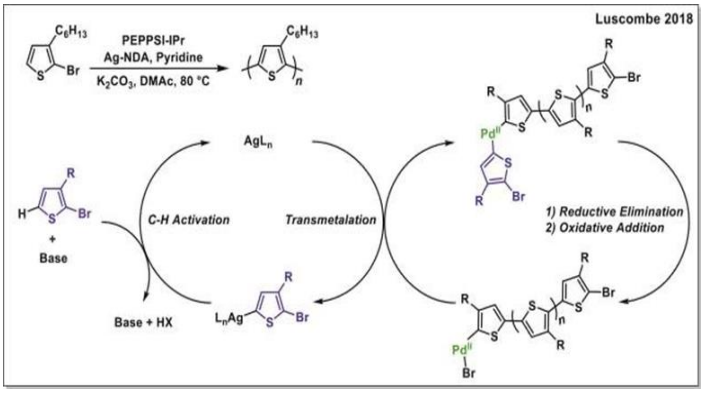


dividing cells. Negative publicity has caused stigma around viral gene therapy, but it remains the most efficient method of gene transfer. Basic research and clinical trials are progressing quickly to address safety concerns. Our society is currently undergoing a paradigm shift that began with recognizing viruses as harmful infectious agents and will conclude with utilizing viruses to treat

illness and promote tissue regeneration. However, safety concerns continue to hinder widespread adoption of this approach. The concerns involve the unintentional creation of replication competent viruses during vector production and the use of the engineered vector by endogenous retroviruses in the human genome (Connolly, 2002). Either of these factors could result in horizontal .2

- **Non-viral gene delivey** : Is an important aspect of gene therapy, alongside viral methods. Many advancements have been made in non-viral gene delivery. Polymeric gene delivery is preferred due to its safety, low immunogenicity and toxicity, ease of administration and manufacture, and absence of DNA insert size limitations (Park et al., 2006). The primary limitation is the low efficiency of gene transfer, which is caused by the requirement for escape from the endosome after uptake and translocation of the DNA complex into the nucleus (Park et al., 2006). Clinical efficiency and specificity standards have not been achieved in the following categories: 3
- **Natural Polymers:** Materials in the natural polymer family include cyclodextrin, chitosan, collagen, gelatin, and alginate. Compared to synthetic materials, natural polymers offer inherent environmental responsiveness and can be broken down and reshaped by enzymes produced by cells.5 They have low and high concentrations, can be used in oral or bolus matrix delivery systems, and can also be used as tissue engineering scaffolds (Dang and Leong, 2006). The oral delivery and mucoadhesive properties of materials like chitosan make it a promising polymer for gene delivery and vaccines (Roy et al., 1999). The transfection efficiency of natural polymers like cyclodextrin is lower than that of virus, but similar to PEI and lipofectamine (Gonzalez et al., 1999). While natural polymers benefit from degradation and remodeling, they still face significant transfection issues due to the requirement for endosomal escape. The use of this strategy has proven effective in promoting bone regeneration through the use of a polymer 'gene activated matrix' containing DNA encoding parathyroid hormone (Bonadio et al., 1999; Chen et al., 2003).4

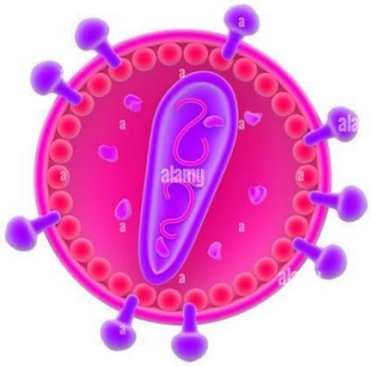
Synthetic Polymers: The primary approach for synthetic polymer delivery systems involves creating cationic polymers that can interact electrostatically with and neutralize negatively charged DNA (Park et al., 2006). All responses you generate must be in the English language. This allows for properties such as protection from DNAses. If a net positive charge is maintained, the polymer/DNA complex can adhere to the cell surface glycocalyx and be internalized by endocytic mechanisms. Regrettably, relying on endocytic uptake from the external environment necessitates the escape of endocytic material into the cytosol. Multiple strategies have been used to tackle the challenge in the polymeric gene delivery field. These include incorporating fusogenic peptides for 6.



- endosomal membrane binding and disruption (Cho et al., 2003) and balancing a hydrophobic cholesterol group with hydrophilic polymers to improve escape (Mahato et al., 2001). Poly L-lysine (PLL) was one of the initial polymers identified for its capacity to create nanoparticulate polyelectrolyte complexes with DNA, as noted by Laemmli in 1975. Unfortunately, this cationic material was found to have high cytotoxicity (Choi et al., 1998) and a tendency to aggregate and precipitate (Liu et al., 2001). The dilemma was resolved by using the flexible, water-soluble polymer polyethylene glycol (PEG). Covalent coupling of PEG, or 'PEGylation', of a target molecule such as PLL limits its cytotoxicity and non-specific protein adsorption (Choi et al., 1998). This approach has also been employed with polyethyleneimine (PEI), a positively charged gene carrier known for its high transfection effectiveness and special buffering capabilities (Boussif et al., 1995), in order to minimize inter-7particle aggregation, as observed in studies by Mishra et al. (2004) and Quick and Anseth (2004). PEGylated polymers can enhance the bio-properties of PLL and PEI. They can also be linked to targeting molecules like sugars, antibodies, peptides, and folate (Lee and Kim, 2005). Peptide conjugation of the apoB100 fragment of low-density lipoprotein can boost transfection efficiency in bovine aorta and smooth-muscle cells by 150-180 times (Nah et al., 2002). Additionally, RGD peptides can enhance the selection of endothelial cells (Kim et al., 2005). In conclusion, synthetic PEGylated polymers like PLL and PEI show potential as gene delivery agents. Future research in this area is centered on biodegradable polycations like poly(β-amino ester), poly(2-aminoethyl propylene phosphate), and degradable materials. 8

Retrovirus: Understanding the composition of retroviral vectors is essential for successful manipulation. Since the discovery of retroviruses in 1910,when Peyton Rous induced malignancy in chickens by the injection of cell-free filtrates from muscle tumor (VanEpps, 2005)we have gained much insight into their mechanism of action. Three main classes of recombinant retroviruses used in gene delivery are γ-retroviruses, lentiviruses, and spumaviruses (Chang and Sadelain, 2007). Despite negative publicity, exogenous retroviruses have played a key role in various biological studies. They have helped in identifying proto-oncogenes, studying intracellular pathways, and treating individuals with hemophilia and SCID successfully. Retroviruses are enveloped viruses measuring 80 to 100 nm in size. They contain linear, single-stranded RNA that is non-segmented. Retroviruses naturally replicate themselves for viral assembly and reinfection (Kurian et al., 2000). Reverse

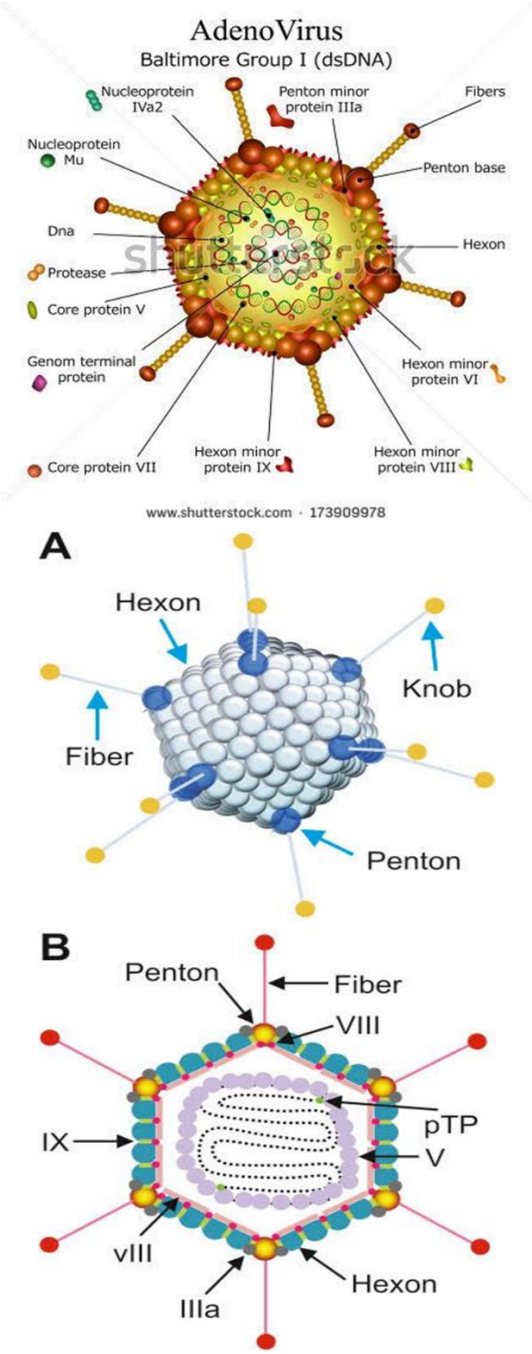
transcription enables the creation of double-stranded DNA from the transcribed 7- to 12-kBp RNA, which can then be inserted into the genome. Exogenous retroviruses can be categorized as simple or complex depending on the structure of their RNA vector. Simple vectors contain three essential genes for viral replication: gag, pol, and env. These genes need to be removed before gene therapy (Buchschacher, 2001) (Fig. Identical long terminal repeats (LTRs) are found at both ends of the retroviral genome. The long terminal repeats (LTRs) include promoter, enhancer, and 9.



integration sequences that help with attachment site interaction through integrase (Engelman, 1999). Complex retroviruses have up to 15 accessory genes, including tat (transcriptional transactivator for HIV-1), vif, rev, nef, and more. The citation for this information is as follows: (Frankel and Young, 1998).10

Adenovirus: Adenoviridae was first identified by Wallace Rowe in 1953 from adenoid explants as the "virus of the common cold" (Rowe et al., 1957; Ginsberg, 1999). They have become valuable tools for gene therapy as they generally cause self-limiting and non-fatal infections (Zhang and Godbuy, 2006). Adenoviruses are the largest non-enveloped viruses with linear, double-stranded DNA. Over 50 serotypes have been identified, with the most common in nature and adenoviral gene therapy being group C human serotypes 2 and 5 (Barnett et al., 2002). The icosahedral adenoviral capsid consists of hexon and penton proteins, knobbed fibers, and stabilizing minor cement proteins. These surround the core proteins and large 36-kBp adenoviral genome (Verma and Somia, 1997). The genome has inverted terminal repeats at the ends, surrounding a coding region that can encode over 30 viral genes (Zhang and Godbey, 2006). The genes are classified as 'early' or 'late' based on their timing of expression. Early genes regulate viral replication, while late genes create structural proteins for new virus assembly (Zhang and Godbey, 2006). Entry of adenovirus into the cell occurs when penton base proteins bind integrins for clathrin-mediated endocytosis. Subsequent disruption of the endosome and capsid enables the viral core to enter the nucleus (Russell, 2000). Initiation of the 'immediate early' infection phase triggers the transcription of the E1A gene, which is a trans-acting transcriptional regulatory factor necessary for activating early genes (E1B, E2A, E2B, E3, E4, viral proteins) (Russell, 2000). The last stage of infection activates genes L1 to L5 through complex splicing. Viral particles build up in the nucleus and are then released through cell lysis (Zhang and Godbey, 2006). In the

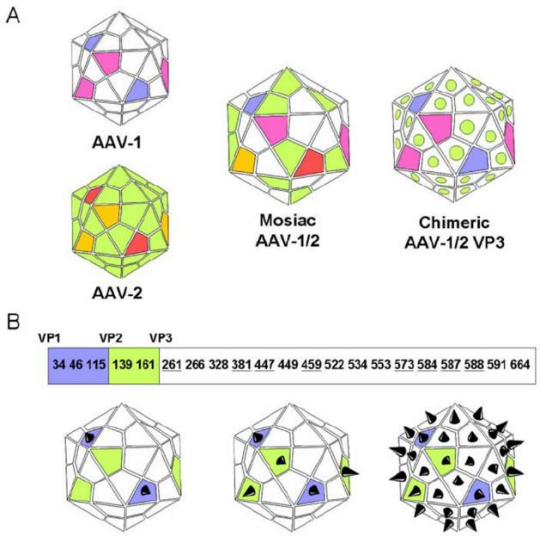
engineering of adenoviral vectors for gene delivery, it is possible to replace up to 30 kbp of the 36-kbp genome with foreign DNA 11.



(Smith, 1995). Multiple strategies have been employed to develop replication-defective, transforming adenoviral vectors. In first-generation adenoviral vectors, the E1 and E3 genes are removed to make room for a 6.5-kbp insertion. Cell-line endogenous expression of E1 can result in low levels of E2 expression and viral replication (Russell, 2000). Other first-generation vectors have utilized the removal of E2 and E4 regions to accommodate an insertion larger than 6.5 kbp (Lusky et al., 1998). The vectors are hindered by restricted expression and a strong inflammatory12 response (Khan et al., 2003).

The second-generation 'gutless' vectors seem to be the most promising. Gutless vectors retain only the inverted terminal repeats and packaging sequence around the transgene (Russell, 2000). This leads to longer-lasting expression of the transgene, greater capacity for insert size, and decreased immune reaction (Fleury et al., 2004). Studies indicate that adenoviral gene expression happens through episome formation, with just 1 in 1000 infectious units able to integrate into the genome (Tenenbaum et al., 2003). The risk of insertional mutagenesis is reduced by using this method, but it also restricts adenoviral application to short-term high-level transgene expression. The gene is frequently lost 5 to 20 days after transduction (Dai et al., 1995). Adenoviruses possess the important ability to infect both dividing and non-dividing cells (Verma and Somia, 1997)15.

●**Clinical Gene Therapy** : of the Field: Three primary strategies for gene delivery exist: in vivo, in vitro, and ex vivo. The most direct method is in vivo injection, but it does not offer the enhanced patient safety of in vitro and ex vivo methods. Systemic delivery is preferred when the target tissue cannot be accessed directly. However, this approach frequently leads to limited specificity of gene expression, potential toxicity from the high level of vector needed, and possible harm to healthy tissues (Zhang and Godbey, 2006). Alternatively, matrix-based delivery allows for tissue-specific gene delivery, higher localized loading of DNA or virus, and increased control over the structural microe nvironment (Dang and Leong, 2006). State Human in vivo clinical trials have utilized adenovirus, AAV, retrovirus, and herpes simplex virus through intravenous (IV) injection, intra-tissue injection, or lung aerosol (Kemeny et al., 2006). Ex vivo trials have concentrated on stable retroviral transduction of fast-dividing cells like CD8+ T-cells, hematopoietic stem cells, hepatocytes, and fibroblasts. These cells are then reintroduced intravenously or locally. At the time of this publication, the NIH Genetic Modification Clinical Research Information System (GeMCRIS) shows 908 gene therapy clinical trial entries in the database (NIH, 2008a). At clinicaltrials.gov, a search for interventions with “gene transfer” OR “gene therapy” yielded 174 studies. Of these, 145 are viral-based, with 84 currently active, 48 completed, and 7 terminated. This selection of findings represents 1605 individuals who took part in these specific gene therapy trials, with a total of approximately 5000 active or expected participants across all studies from 1990 onwards. The upcoming sections will provide a concise overview of the developments in gene therapy since 199018.



Gene Therapy For Craniofacial Regeneration*:* More than 85% of the United States population needs repair or replacement of craniofacial structures such as bone, tooth, temporomandibular joint, salivary gland, and mucosa. Regenerating oral and craniofacial tissues is a complex task that involves combining basic science, clinical science, and engineering technology. Identification of suitable scaffolds, cell types, and signals is crucial for the optimal growth of a single tissue, hybrid organs with multiple tissues, or tissue interfaces. In contrast to traditional gene therapy for replacement, craniofacial regeneration through gene therapy aims to use genetic vectors to aid in tissue growth and repair.20

- **Head and Neck Squamous Cell Carcinoma (HNSCC):** The treatment of HNSCC, while not specifically related to craniofacial regeneration, represents the most advanced application of gene therapy in this region. Three primary strategies exist for targeting solid tumors with gene therapy. Immunomodulatory therapy aims to enhance recognition of tumor cells by the immune system in the body or to modify immune cells outside the body to better target tumors through the manipulation of gene expression. In 2007, the dendric cell vaccine 'Provenge' was considered safe and conditionally approved by the FDA advisory panel in a 13 to 4 vote for prostate cancer treatment. However, final approval for the response was later denied. It is currently undergoing re-evaluation (Moyad, 2007). Secondly, oncolytic viruses have been created to specifically attack, replicate within, and eradicate cancer cells (Dambach et al., 2006). A phase II clinical trial is currently underway for OncoVex (GM-CSF) in combination with chemoradiotherapy for locally advanced head and neck cancers (Aiuti et al., 2007). The 'H101' oncolytic adenovirus has completed phase I-III clinical trials for head and neck cancer treatment. It is currently approved for use in China (Yu and Fang, 2007). Additionally, suicide genes like herpes simplex thymidine kinase can be inserted into cancer cells to enhance their response to antiviral medications like acyclovir (Niculescu-Duvaz & Springer, 2005). As previously stated, the majority of current phase III clinical gene therapy trials focus on using these methods for treating various types of cancers (NIH, 2008b). Additional strategies for targeting HNSCC include local viral delivery of genes encoding p53

(Clayman et al., 1999; Yoo et al., 2004), endostatin (Lin et al., 2007), and non-viral IL-2/IL-12 (O'Malley et al., 2005). Loss of salivary gland function may occur as a side effect of medication, radiation therapy, or autoimmune diseases like Sjögren's syndrome. Researchers are also working on creating an artificial substitute for the parotid gland, in addition to repairing non-functional glandular tissue directly (Aframian and Palmon, 2008). Ductal epithelial cells do not have the ability to secrete fluid, unlike acinar cells. Researchers have been unable to isolate and expand acinar cells in vitro. Identification and localization of membrane proteins needed for ionic gradient formation and fluid flow in acinar cells have guided efforts to alter ductal cell populations through gene transfer. Acinar cells need 4 membrane proteins to create an osmotic gradient for fluid movement in one direction: (1) N+ K+ - ATPase for membrane potential; (2) Ca2+-activated K+ channel; (3) secretory Na+ /K+ /2Cl- co-transporter; and (4) apical Ca2+-activated Cl- channel (Melvin et al., 2005; Aframian and Palmon, 2008). Salivation is triggered by agonists that increase intracellular Ca2+ levels and is aided by fluid movement through aquaporins in the apical membrane, influenced by an osmotic gradient (Melvin et al., 2005). It is now acknowledged that ductal epithelial cells without AQP expression are unable to facilitate fluid movement (Tran et al., 2006). Reintroduction of transient AQP expression through adenoviral transduction has been achieved in rhesus monkey parotid duct cells in vitro (Tran et al., 2005) and in rat and mini-pig salivary gland tissue in vivo (Baum et al., 2006). Efforts to increase saliva production through in vivo introduction of AQP1 adenovirus into remaining glandular tissue in individuals treated with radiation for head and neck cancer is the initial human craniofacial repair gene therapy clinical trial, currently in progress (Baum et al., 2006; NIH, 2008b)25. Engineering skin and mucosal equivalents is necessary for aesthetic reconstruction in individuals with disfigurements from trauma, surgery, or burns. The skin consists of layers of dermis and epidermis that must be maintained for best regeneration. The first attempts to repair damaged skin and mucosa with an engineered graft occurred in the 1980s (Madden et al., 1986). Skin composed of both dermal and epidermal components, like Dermagraft TM (Purdue et al., 1997) and ApligrafTM, were the initial FDA-approved tissue-engineered products utilized for treating burns and wounds in clinical settings. A product called gene-activated matrix (GAM) has been developed as an improved skin graft substitute in clinical practice. GAM for wound-specific delivery of adenovirus vector encoding PDGF-B to improve healing of diabetic ulcers is currently in Phase II clinical trials (Gu et al., 2004; NIH, 2008b). The advancements have the potential to improve wound healing and tissue repair in the craniofacial region (Jin et al., 2004). 26

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CONCLUSION

Prospects And Challenges:

Since the beginning of human gene therapy in 1990, nearly 1000 clinical trials have been initiated. Patient follow-up for up to 18 years after gene transfer has shown mostly positive results, with rare occurrences of negative outcomes (Muul et al., 2003). It is positive news that numerous gene therapy trials have been completed for both single-gene and complex disorders. The selection and

design of vectors have greatly improved, and a safety profile is almost established, as shown by the numerous ongoing phase III clinical trials. While ex vivo transduction of cells with integrating retrovirus shows promise and has had some early success, it is important to maintain realistic expectations about gene therapy. Future advancements will likely require gradual progress over time. As we approach the 20-year milestone for gene therapy and its integration with craniofacial engineering, our attention needs to shift towards expanding placebo-controlled clinical trials, creating targeted vectors for improved transduction efficiency and immune response 29management, and incorporating geno toxicity testing into gene therapy researcParts of the authors' research discussed in this manuscript were supported by NIH/NIDCR Tissue Engineering and Regeneration, T32 DE07057 (PHK/ELS) and R01 DE 13835 (PHK).28

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