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## **Scaffold-based Delivery of Nucleic Acid Therapeutics for Enhanced Bone and Cartilage Repair**

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### **Abstract**

Recent advances in tissue engineering have made progress towards the development of biomaterials capable of the delivery of growth factors, such as BMPs, in order to promote enhanced tissue repair. However, controlling the release of these growth factors on demand and within the desired localised area is a significant challenge and the associated high costs and side effects of uncontrolled delivery have proven increasingly problematic in clinical orthopaedics. Gene therapy may be a valuable tool to avoid the limitations of local delivery of growth factors. Following a series of setbacks in the 1990's, the field of gene therapy is now seeing improvements in safety and efficacy resulting in substantial

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clinical progress and a resurgence in confidence. Biomaterial scaffold-mediated gene therapy provides a template for cell infiltration and tissue formation while promoting transfection of cells to engineer therapeutic proteins in a sustained but ultimately transient fashion. Additionally, scaffold-mediated delivery of RNA-based therapeutics can silence specific genes associated with orthopaedic pathological states. This review will provide an overview of the current state-of-the-art in the field of gene-activated scaffolds and their use within orthopaedic tissue engineering applications.

## 1. Introduction

The current increasing trend in life expectancy will result in a proportional increase in musculoskeletal disorders, specifically orthopaedic pathologies, including fractures, bone metastases and osteoporosis, as well as a rise in rheumatic diseases such as osteoarthritis [1]. As a result, the coming decades will see a demand for more effective orthopaedic repair strategies [2]. Although there has been some success with current treatment methods, the limited regeneration potential of these approaches has led to the pursuit of advanced therapeutics, including those for functional bone and cartilage tissue regeneration. One such approach is the use of scaffold-based delivery systems for nucleic acid therapies. Introducing specific genetic sequences into a cell can correct for or replace a pre-existing gene, modulating their expression, to accomplish the desired effect [3]. Gene-activated platforms offer a method of delivering nucleic acid-based therapeutics in a sustained and controllable manner, thereby facilitating a safer and more efficient release of therapeutic factors. This review highlights ongoing research within the field, with a particular focus on gene-activated biomaterials for the promotion of stable cartilage formation and enhanced bone repair.

## 2. Orthopaedic Tissue Engineering

### a. Biomaterials

Biomaterial scaffolds have been used in orthopaedic tissue regeneration with limited success, particularly in cartilage repair. Scaffolds provide a 3D matrix that allows for and stimulates the attachment and proliferation of cells. Multiple reviews have reported extensively on the range of compositions, structures, fabrication methods and properties of available biomaterials [4, 5]. Ultimately, there are several tissue-specific considerations when selecting a biomaterial for use within orthopaedic tissue engineering. Ideally, scaffolds should be biocompatible, with a suitable degradation rate, exhibiting a porous architecture. Other factors such as ease of manufacture and handleability are also important with regards to clinical translation [4]. Typically, biomaterial scaffolds can be classified as either natural (e.g. collagen, proteoglycans, alginate or chitosan), synthetic (e.g. polystyrene, polyglycolic (PGA) acid or poly(lactic-co-glycolic acid) (PLGA)) or ceramic (e.g. hydroxyapatite,  $\beta$ -tricalcium phosphate), each demonstrating advantages and limitations. Natural materials provide cells with a suitable environment for growth and differentiation and while they often lack the desired mechanical properties of the tissue, the enhanced cell response may be enough to overcome this limitation. Conversely, synthetic materials allow for large-scale manufacture, with controllable mechanical properties and degradation rates, however, the by-products of degradation can be toxic, and the materials often require further treatment to allow for cell infiltration and integration into surrounding tissues. Ceramics on the other hand, provide high mechanical stiffness and biocompatibility, however are limited by their hard brittle surface [4]. The development of composite scaffolds offer the

advantages of combining the mechanical strength of ceramics with the biological activity of naturally derived materials. For example, the development of composite biomimetic scaffolds comprised of both collagen and hydroxyapatite, natural constituents of bone, resulted in increased mechanical strength compared to collagen alone, while maintaining the pore structure and bioactivity required to promote healing [6, 7]. While some reviews have reported on the successful use of scaffolds for bone and cartilage repair [1, 8-11] there are cases where treatment with a biomaterial scaffold alone will not suffice. As such, researchers, including our lab at the Royal College of Surgeons in Ireland (RCSI), have been investigating the use of biomaterial scaffolds to deliver bioactive therapeutics to the site of the defect, further enhancing the therapeutic efficacy of the platform [12].

#### **b. Bioactive Therapeutics**

Considering the significant role that growth factors play in the maintenance of tissue homeostasis, it is unsurprising that these biomolecules possess enormous therapeutic potential for use within tissue engineering applications. Multiple factors have demonstrated roles in stimulating chondrogenesis, osteogenesis and angiogenesis [13, 14]. Due to its potent osteogenic effects, recombinant human bone morphogenetic protein-2 (rhBMP-2), is widely studied for bone regeneration and is currently approved for use in patients. However, there remain concerns over the safety of rhBMP-2 and its association with unwanted side effects including carcinogenicity [15, 16]. Several other signalling molecules have been recognised for their beneficial role in bone repair [17]. These include cytokines such as transforming growth factor-beta (TGF- $\beta$ ) [18], insulin-like growth factor (IGF) [19], as well as fibroblast growth factor (FGF) [20].

Angiogenesis plays a vital role in bone regeneration, and angiogenic factors such as

vascular endothelial growth factor (VEGF) [21] and platelet-derived growth factor (PDGF) [22] have also been shown to encourage successful bone healing. Similarly, biomolecules targeting different processes in the development of cartilage have demonstrated roles in the management of cartilage disorders [23]. In addition to members of the TGF- $\beta$  superfamily [24], BMP-2 [25], and FGF-2 [26], members of the SOX family of transcription factors (SOX5, SOX6 and SOX9), collectively referred to as the “SOX-trio”, have been shown to play a vital role in the formation and maintenance of cartilage [27].

### **c. Therapeutic Delivery Systems**

Tissue regeneration requires prolonged exposure to bioactive molecules to ensure efficacy [10]; however, systemic intravenous administration and high dosage often result in undesirable effects in other tissues [28]. As a result, much ongoing research is focused on investigating alternative advanced approaches for their delivery.

Many next-generation biomaterial-based delivery systems aim to promote tissue regeneration through the controlled delivery of growth factors, commonly in the form of recombinant proteins [29-33]. Incorporation of growth factors into biomaterial platforms has demonstrated improved osteogenesis and vascularisation [10], as well as the ability to promote stable cartilage formation in joint repair [34]. While the criteria required for efficient growth factor delivery is beyond the scope of this review, multiple comprehensive reviews have previously been published on this topic [35, 36].

Of note, two such biomaterial-based delivery systems have been approved for use in bone regeneration; INFUSE (Medtronic) and OP-1 (Olympus Biotech – Operations

discontinued in 2014), containing rhBMP-2 and rhBMP-7 respectively, both of which are incorporated within collagen platforms. Although these products have demonstrated the ability to repair bone, some unwanted side effects including heterotopic ossification, and even an increased incidence of neurological deficits and cancer in patients, have been associated with high dosing strategies, poorly controlled growth factor release and non-specific delivery [37-39]. These unwanted side effects have led to concerns over the use of such products and have resulted in the demand for safer and more efficient delivery methods.

### **3. Gene Therapy in Orthopaedic Repair**

Recent positive clinical trial outcomes have begun to provide the much sought-after evidence of the potential of gene therapies to deliver lasting therapeutic benefit, catapulting these therapies back into the limelight [40]. At the turn of the century, early outcomes of the treatment of X-linked severe combined immunodeficiency reported promising results [41, 42]; however, this clinical trial was ultimately deemed a failure after the death of one patient and the development of acute leukaemia in multiple patients [43]. Shortly after, another death of an 18-year-old male participating in a pilot study for the treatment of ornithine transcarbamylase (OTC) deficiency [44] sent the field of gene therapy into a deep freeze. However, recent years have seen a revival of gene therapies with a growing number of studies demonstrating both safety and efficiency in treating several human diseases [3].

Advances in our understanding of the underlying biological process involved in these therapies have led to the European Medicines Agency approving the human gene therapy



product, Glybera [45], as well as the successful use of gene therapy, Strimvelis, in treating 18 children suffering from immunodeficiency resulting from adenosine deaminase deficiency [46]. Following this, the success stories have continued, so much so that 2017 was dubbed “the year of gene therapy breakthroughs” [47] with three treatments, Kymriah (Novartis), Yescarta (Gilead Sciences) and Luxturna (Spark Therapeutics), all coming to market after Food and Drug Administration (FDA) approval. Both Kymriah and Yescarta are immunotherapy approaches involving the introduction of synthetic chimeric antigen receptors (CAR) to T-cells for the treatment of acute lymphoblastic leukaemia and advanced lymphomas respectively, while Luxturna uses the direct injection of adeno-associated viral vectors expressing retinal pigment epithelium-specific protein for the treatment of rare retinal disorders.

While gene therapy is often associated with the treatment of rare or life-threatening genetic disorders, recent progress has shown that it also offers promise for more common applications such as the treatment of orthopaedic disorders, as evidenced by the recent approval of a gene therapy for osteoarthritis, Invossa<sup>TM</sup>, in South Korea. This treatment consists of a mixture of non-transformed and *ex vivo* retrovirally transduced chondrocytes for the overexpression of TGF- $\beta$ 1 and has demonstrated improvement in both the bone area and cartilage thickness in knee osteoarthritis with patients reporting a reduction in pain and increased patient quality of life compared to the placebo control [48].

Several small and large animal studies have also demonstrated the success of gene therapy for the treatment of bone and cartilage defects, with treatments focused mainly on the delivery of genes encoding for morphogenetic proteins [49]. Promising studies include the direct injection of adenovirus carrying BMP-2 demonstrating repair of

femoral defects in rodents [50]. Additionally, the *ex vivo* modification of cells for the overexpression of the angiogenic factor VEGF has demonstrated repair within rabbit tibiae [51], as well as the overexpression of the osteogenic factors BMP-2 [52] and BMP-4 [53] enhancing bone repair in both femoral and skull defects, respectively. With regards to cartilage repair, the direct delivery of recombinant adeno-associated viral vector (rAAV) with IGF-1[54], FGF-2 [55], or SOX 9 [56], has demonstrated enhanced repair in rabbits. Additionally, the genetic modification of chondrocytes or chondroprogenitors for the overexpression of TGF- $\beta$ 1 has resulted in the successful repair of osteochondral defects in a rodent [57], equine [58], and human defect model (human cartilage biopsies) [59].

Although these gene therapy approaches have demonstrated varying degrees of successful regeneration in bone and cartilage defects, consensus on the safest and most efficient delivery method remains unclear. Foreign genetic material is rapidly degraded by nucleases and is negatively charged making cell uptake difficult [60]. While the use of delivery vectors protects the genetic cargo and improves cellular uptake, rapid clearance of these vectors results in short therapeutic timeframes [61]. As an alternative, the use of 3D porous scaffold-based delivery systems in combination with vectors complexed with nucleic acid therapies demonstrate significant advantages over conventionally used delivery methods [12, 62, 63]. The delivery vector protects the genetic cargo from degradation by serum nucleases, while association of the vectors within the 3D microenvironment of the scaffold can prevent the clearance of complexes from the target site, allowing for a sustained therapeutic effect, with studies demonstrating bioactivity of released genetic cargo over a prolonged period (e.g. 3-6 weeks *in vitro*) [61].

#### **4. Gene-activated Scaffolds for Orthopaedic Tissue Engineering**

##### **a. Scaffold-mediated delivery of DNA**

##### **i. Bone**

The first scaffold-based gene delivery system for musculoskeletal defects was reported in 1996. Collagen scaffolds containing genes encoding for BMP-4 and pTH1-34, a plasmid coding for a fragment of parathyroid hormone (amino acids 1-34), were implanted into rat femoral defects resulting in new bone formation and the bridging of large segmental defects [64]. Following this, it was demonstrated that these platforms were capable of the retention of plasmid (p)DNA for up to six weeks in addition to inducing the formation of new bone in a canine tibial defect model in a manner dependent on time, plasmid dose, and defect gap size [65]. However, the direct incorporation of the genetic material into this scaffold required very high doses (up to 100 mg per scaffold) to exert a therapeutic effect [65, 66].

Delivery vectors can be used to overcome the requirement for high doses, ultimately improving the efficiency and therefore efficacy of the delivery system. Viral approaches demonstrate an efficient method of gene transfer; however, safety concerns such as insertional mutagenesis surround the use of these vectors limiting their clinical translation. Non-viral vectors are a relatively safer alternative, potentially having a clearer route to clinical translation in tissue regeneration [67]. However, non-viral vectors are typically hindered by low transfection efficiency and therefore efficacy. As such, a body of research is currently being carried out to enhance their efficiency through their incorporation into biomaterial scaffolds (Figure 1).

Combining non-viral vectors complexed with pDNA within a biomaterial scaffold facilitates enhanced gene transfer [68] and advancements in delivery methods over the last two decades have resulted in a reduction in the dose required from the milligram range (mg), reported in earlier studies, to the microgram range ( $\mu\text{g}$ ) (Figure 2). The incorporation of a calcium phosphate-pDNA (40  $\mu\text{g}$ ) precipitate mixed with collagen demonstrated enhanced efficiency of reporter plasmids *in vivo* [69]. Huang et al., demonstrated that condensing BMP-4-pDNA (200  $\mu\text{g}$ ) with a non-viral vector such as polyethyleneimine (PEI) before incorporation into a PLGA scaffold promoted enhanced bone formation in a rat cranial defect model compared to treatment with scaffolds incorporating uncondensed pDNA (200  $\mu\text{g}$ ) [70]. Following this, Curtin et al., developed an innovative osteoconductive and osteoinductive, biodegradable collagen nanohydroxyapatite (nHA) gene-delivery platform capable of stimulating mesenchymal stem cells (MSCs) towards the osteogenic lineage using as little as 1-5  $\mu\text{g}$  of BMP-2-pDNA [63].

Gene-activated scaffolds also allow for the incorporation and subsequent administration of more than one gene into target cells for a potentially heightened synergistic effect. Co-delivery of PEI-pDNA nanoplexes containing FGF-2 and BMP-2 (5  $\mu\text{g}$  pDNA) significantly increased osteogenesis in human adipose-derived MSCs *in vitro* compared to the delivery of either gene alone [71]. This combination of genes when embedded in a collagen scaffold was shown to be effective at regenerating bone in rodents with a diaphyseal long bone radial defect compared to treatment with PEI-pBMP-2 or PEI-pFGF-2 collagen scaffolds alone [72]. Interestingly, the model used in this study was a diabetic rat model, and the author suggests that these scaffolds could be advantageous in

promoting bone regeneration in diabetic patients. However, it is important to note that co-delivery does not always result in the desired synergistic effect. Following on from a study that demonstrated effective bone regeneration using a PDGF-B (1  $\mu$ g pDNA) collagen-based platform [73], D'Mello et al., carried out a pilot study, which investigated the co-delivery of the pro-angiogenic plasmid pVEGF with pPDGF-B. However, unlike the delivery of pPDGF-B alone, scaffolds incorporating pVEGF either alone or in combination with pPDGF-B (1  $\mu$ g pDNA each) failed to restore the bone defect [74].

Tissue engineering approaches should mimic the natural processes involved in tissue formation, and combinational gene therapy, delivering multiple genes can maximise regeneration. For example, the delivery of both pVEGF and pBMP-2 can recapitulate osteogenic-angiogenic coupling observed in bone development. Bone is highly vascularized, and the ability of a scaffold to enhance new blood vessel formation is critical to the successful repair of defects and disorders. Recent work from our lab compared the use of two different non-viral vectors, PEI and nHA, and investigated their ability to deliver both pVEGF and pBMP-2 alone and in combination. Results indicated that the delivery of both pVEGF and pBMP-2 by the nHA vector combined with a collagen-based scaffold exhibited the best healing profile [75]. This study again demonstrates that combinational gene delivery is a promising approach for efficient tissue repair; however, it also highlights that the ultimate therapeutic success is influenced by the choice of the vector [76]. More recently, a chitosan-based vector delivering both pBMP-2 and pVEGF (1  $\mu$ g each) on a collagen-hydroxyapatite scaffold resulted in the complete bridging of critical-sized rat calvarial defects within 28 days without the detection of off-target side effects. This study focused on the

neovascularisation induced by pVEGF, demonstrating that it plays a critical role in bone regeneration as the delivery of both pVEGF and pBMP-2 resulted in enhanced bone formation compared to the delivery of pBMP-2 alone [77-79].

## ii. Cartilage

By varying the scaffold composition, genetic material and vector within these platforms, gene-activated scaffolds have demonstrated the potential for use in the treatment of cartilage defects. TGF- $\beta$ 1 plays an essential role in the formation, growth, maintenance, and repair of articular cartilage [80, 81]. Tong et al. demonstrated the ability of scaffolds loaded with pTGF $\beta$ 1 (1  $\mu$ g, 2  $\mu$ g or 4  $\mu$ g per 1 mg scaffold) to act as a bioreactor (*in vitro*) for the production and secretion of collagen type-II and aggrecan by human bone marrow stem cells (hBMSCs) [82]. PLGA scaffolds filled with fibrin gel and the use of either N,N,N-trimethyl chitosan chloride (TMC) [83] or poly(ethylene oxide)-b-poly(L-lysine) (PEO-b-PLL) complexes [84] for the delivery of TGF- $\beta$ 1 (1 mg/ml), also demonstrated repair of both cartilage and osteochondral defects respectively in rabbits after 12 weeks.

A recent exciting development within cartilage repair is the use of gene-activated platforms that target specific aspects of the pro-inflammatory environment found in injured or diseased joints. The pro-inflammatory cytokine, interleukin 1 (IL-1), was shown to inhibit the chondrogenic development of cells seeded on a 3D woven poly ( $\epsilon$ -caprolactone) (PCL) scaffold [85]. Following this discovery, a gene-activated scaffold for the overexpression of an IL-1 receptor antagonist demonstrated the formation of cartilage with mechanical properties similar to that of native articular cartilage [86, 87]. These

studies highlight the promise for the use of gene-activated scaffolds capable of inducing the production of anti-inflammatory molecules or controlling the activation of pro-inflammatory cytokines within the diseased or injured joint. However, further research into the regulation of inflammation will be required.

### **b. Scaffold-mediated delivery of RNA**

Conventionally, gene therapy involves the induction of genes in the form of pDNA. However, state-of-the-art gene therapy has advanced to incorporate the use of RNA-based therapeutics, opening the door to a range of different targets and approaches for use within tissue regeneration including the ability to silence specific genes associated with pathological states in orthopaedics.

The use of RNA-based therapeutics eliminates the requirement for nuclear entry, often regarded to be the rate-limiting step in the delivery of pDNA-based therapeutics, as the plasmid must enter the nucleus to gain access to the machinery required for transcription [88]. RNA-based therapeutics offer several exciting approaches to gene therapy as RNA molecules such as messenger (m)RNA, small interfering RNA (si)RNA, short hairpin (sh)RNA, and micro (mi)RNA are involved throughout the transcriptional, regulatory and other functional activities of the cell. The use of these RNA molecules completely avoids the risk of insertional mutagenesis, although delivery of RNA is often hampered by its susceptibility to degradative enzymes, which present a significant limitation to its use. Recent studies have however reported the successful use of chemically modified mRNA demonstrating the enhanced stability of mRNA [73, 89]. The incorporation of chemically modified mRNA within biomaterial scaffolds demonstrated enhanced bone formation in a

rat calvarial bone defect model compared to pDNA-activated scaffolds [90]. Chemically modified mRNA encoding BMP-2 complexed with PEI induced enhanced BMP-2 over-expression by cells, ultimately resulting in enhanced Bone Volume/Tissue Volume (BV/TV) and greater defect bridging, compared to plasmid BMP-2 delivered by similar methods [90].

Other studies have supported the ability of chemically modified BMP-2 RNA to induce repair. Bone marrow and adipose-derived MSCs were transfected with chemically modified BMP-2 RNA, using lipofection and magnetofection procedures, resulting in new bone formation in a rat femoral defect model [89]. Further to this, the use of chemically modified mRNAs encoding for various BMPs has also demonstrated the promotion of bone regeneration *in vivo* when delivered using a fibrin gel. Chemically modified BMP-9 RNA demonstrated enhanced osteogenic differentiation by BMSCs in rat calvarial defect models, as evidenced by increased bone matrix production, with the connectivity of the newly formed bone greater following treatment with BMP-9 RNA compared to the delivery of BMP-2 RNA [91].

A particularly exciting approach to RNA-based therapeutics and scaffold-mediated delivery is the use of RNA molecules for the manipulation of the RNA interference pathway (RNAi), a naturally occurring mechanism, ultimately allowing for the silencing of specific genes [92]. The induction of RNA in the form of siRNA and microRNA allows for the targeting and degradation of specific mRNA sequences resulting in post-transcriptional gene silencing. The ability to silence specific genes represents a promising therapeutic approach for various orthopaedic disorders (Yin et al., 2014). A comprehensive review of scaffold-based microRNA delivery in regenerative medicine



was recently published by our lab [88]. Here, in addition to highlighting scaffold-based miRNA delivery, we also review some exciting scaffold-based delivery approaches using siRNA for the regeneration of cartilage.

Recently, our lab has demonstrated successful delivery of miRNA therapeutics from collagen-based scaffolds for sustained periods [94]. Delivery of miRNA therapeutics which either mimic (miR-mimics) or block (antagomiRs) the function of endogenous miRNAs, allows for the opportunity to modulate gene expression, depending on the specific application. Scaffolds incorporating an antagomiR blocking the function of miR-133a, an inhibitor of RunX2 expression, resulted in increased bone repair. Enhanced osteogenesis was confirmed by the upregulation of a series of osteogenic markers including enhanced expression of RunX2 itself, as well as an increase in osteocalcin expression and mineral deposition (*in vitro*) compared to gene-free scaffolds and scaffolds containing a scrambled antagomiR (Figure 3) [93].

Similarly, the use of RNAi allows for enhanced osteogenesis by silencing proteins that negatively regulate osteogenesis. Overexpression of Noggin, an antagonist of BMP activity, impairs bone formation and siRNA silencing of Noggin demonstrated enhanced osteogenic differentiation of hMSCs [95]. Interestingly, within the same study, the co-delivery of the siRNA with a miRNA (miRNA-20a), inhibiting the expression of peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ), a negative regulator of BMP-2 signalling, did not outperform the delivery of the silencing of Noggin by siRNA alone [61].

In the case of cartilage repair, a recent review highlights the wide range of known anti-chondrogenic factors and the potential of RNAi based therapies for enhanced chondrogenesis and cartilage repair [96]. Although there has been success in the development of scaffolds for cartilage repair, very few studies have combined the therapeutic potential of RNAi with these delivery systems and the use of RNAi-activated scaffolds remains an emerging area of research. One such study demonstrating the potential therapeutic application of advanced RNA-based delivery systems is a set of *in vitro* and *in vivo* studies aimed at silencing an anti-chondrogenic regulator, miRNA-221. Alginate pellets formed by hMSCs transfected with antagomiR-221, capable of silencing miRNA-221, demonstrated cartilage regeneration *in vivo* further highlighting the therapeutic potential of harnessing the RNAi pathway [97]. A major problem associated with *in vitro* expanded articular chondrocytes is that they tend to form fibrocartilage rather than the mechanically superior hyaline articular cartilage. One study described the delivery of siRNA targeting the COL1A1 gene in chondrocytes, demonstrating an improvement in the ratio of COL2A1, predominant in hyaline cartilage, compared to COL1A1, predominant in fibrocartilage, indicating a possible therapeutic for use in combination with autologous chondrocyte implantation procedures [98]. Similar work was also carried out on MSCs, with, siRNA-targeting COL1A1 having a positive effect, inducing more type II collagen expression over type I collagen. However, when implanted *in vivo*, the neo-cartilage tissues underwent endochondral ossification regardless of treatment [99]. Novel methods of controlling MSC differentiation is an area of research within our lab and many more labs worldwide.

## 5. Future perspectives

Since the first reports of a gene-activated matrix as an alternative method for musculoskeletal gene therapy [64], much excitement has followed this area of research. Scaffold-mediated gene therapy offers a promising opportunity to overcome the limitations associated with the local delivery of growth factors. Gene-activated platforms not only act as a template for cell infiltration and tissue formation but also enable autologous host cells to take up specific genes and engineer therapeutic proteins in a sustained but ultimately transient fashion. The introduction of genes in the form of plasmid DNA allows for gene expression and downstream therapeutic protein production, providing an enhanced therapeutic response. Alternatively, scaffold-mediated delivery of RNA molecules may be used for the silencing of specific genes associated with the negative regulation of tissue regeneration.

The regulatory landscape is also beginning to become clearer which should ensure a more transparent route to the market for these new novel therapeutics. For example, in Europe, these activated scaffolds, in particular those delivering pDNA, are likely to be classified as advanced therapy medicinal products (ATMPs) as defined by EC Regulation 1394/2007 [100]. Due to the complexity and innovative nature of such products relative to traditional medicinal products, new approaches to manufacturing and GMP are required in order to accelerate translation to the clinic. These challenges have been recognized by the regulator as reflected in the publication of the European Commission ‘Guidelines on GMP specific to ATMPs’ Nov. 2017 [101].

The field of genetic therapies is exploding and advances in the last two decades have led to a resurgence in the use of gene therapies with these approaches being applied to a number of regenerative medicine applications. In the last 5 years, 762 clinical trials related to gene therapy have been approved, ongoing or completed worldwide, with 2017 having the most ever in a single year (220 trials) [102]. With this ever-growing popularity, it is expected that more gene therapies will become available for orthopaedic applications in the coming years. Further to this, the recent FDA and EMA approval of Onpattro (Patisiran) in 2018 (marketed by Alnylam), the first ever non-viral RNA-based gene therapy, for the treatment of peripheral nerve disease caused by hereditary transthyretin-mediated amyloidosis (hATTR), further highlights the exciting therapeutic potential of RNA-based therapies. Additionally, the announcement by the FDA Commissioner Scott Gottlieb, M.D in July 2018, on the new framework for the development, review and approval of gene therapies, holds great promise in shaping the future of medicine. This “fast-tracking” of gene therapies by regulatory bodies, [103] along with the recent approval of several gene therapies and the first clinical case of scaffold-based gene delivery [104], will only further enhance the on-going drive among researchers for the creation and clinical approval of novel therapeutic strategies such as those described throughout this review.

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## References

1. Roseti, L., et al., *Scaffolds for Bone Tissue Engineering: State of the art and new perspectives*. Mater Sci Eng C Mater Biol Appl, 2017. **78**: p. 1246-1262.
2. Mehta, M., et al., *Biomaterial delivery of morphogens to mimic the natural healing cascade in bone*. Adv Drug Deliv Rev, 2012. **64**(12): p. 1257-76.
3. Ginn, S.L., et al., *Gene therapy clinical trials worldwide to 2017: An update*. J Gene Med, 2018. **20**(5): p. e3015.
4. O'Brien, F.J., *Biomaterials & scaffolds for tissue engineering*. Materials Today, 2011. **14**(3): p. 88-95.
5. Tataru, A.M. and A.G. Mikos, *Tissue Engineering in Orthopaedics*. The Journal of Bone and Joint Surgery. American volume, 2016. **98**(13): p. 1132-1139.
6. Gleeson, J., N. Plunkett, and F. O'Brien, *Addition of hydroxyapatite improves stiffness, interconnectivity and osteogenic potential of a highly porous collagen-based scaffold for bone tissue regeneration*. Eur Cell Mater, 2010. **20**(218): p. 30.
7. Murphy, C.M., et al., *A collagen-hydroxyapatite scaffold allows for binding and co-delivery of recombinant bone morphogenetic proteins and bisphosphonates*. Acta Biomaterialia, 2014. **10**(5): p. 2250-2258.
8. Hutmacher, D.W., *Scaffolds in tissue engineering bone and cartilage*, in *The Biomaterials: Silver Jubilee Compendium*, D.F. Williams, Editor. 2000, Elsevier Science: Oxford. p. 175-189.
9. Schieker, M., et al., *Biomaterials as Scaffold for Bone Tissue Engineering*. European Journal of Trauma, 2006. **32**(2): p. 114-124.
10. Bose, S., M. Roy, and A. Bandyopadhyay, *Recent advances in bone tissue engineering scaffolds*. Trends in biotechnology, 2012. **30**(10): p. 546-554.
11. Ghassemi, T., et al., *Current Concepts in Scaffolding for Bone Tissue Engineering*. Archives of Bone and Joint Surgery, 2018. **6**(2): p. 90-99.
12. Raftery, R., M., et al., *Delivering Nucleic-Acid Based Nanomedicines on Biomaterial Scaffolds for Orthopedic Tissue Repair: Challenges, Progress and Future Perspectives*. Advanced Materials, 2016. **28**(27): p. 5447-5469.
13. Tsiridis, E., N. Upadhyay, and P. Giannoudis, *Molecular aspects of fracture healing: which are the important molecules?* Injury, 2007. **38** Suppl 1: p. S11-25.

14. Devescovi, V., et al., *Growth factors in bone repair*. Chir Organi Mov, 2008. **92**(3): p. 161-8.
15. Weiss, K.R., "To B(MP-2) or Not To B(MP-2)" or "Much Ado About Nothing": Are Orthobiologics in Tumor Surgery Worth the Risks? Clin Cancer Res, 2015. **21**(13): p. 2889-91.
16. Skovrlj, B., et al., *Association Between BMP-2 and Carcinogenicity*. Spine (Phila Pa 1976), 2015. **40**(23): p. 1862-71.
17. Carano, R.A. and E.H. Filvaroff, *Angiogenesis and bone repair*. Drug Discov Today, 2003. **8**(21): p. 980-9.
18. Chen, G., C. Deng, and Y.-P. Li, *TGF- $\beta$  and BMP Signaling in Osteoblast Differentiation and Bone Formation*. International Journal of Biological Sciences, 2012. **8**(2): p. 272-288.
19. Schmidmaier, G., et al., *Improvement of fracture healing by systemic administration of growth hormone and local application of insulin-like growth factor-1 and transforming growth factor-beta1*. Bone, 2002. **31**(1): p. 165-72.
20. Fei, Y., G. Gronowicz, and M.M. Hurley, *Fibroblast growth factor-2, bone homeostasis and fracture repair*. Curr Pharm Des, 2013. **19**(19): p. 3354-63.
21. Street, J., et al., *Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover*. Proceedings of the National Academy of Sciences of the United States of America, 2002. **99**(15): p. 9656-9661.
22. Caplan, A.I. and D. Correa, *PDGF in bone formation and regeneration: new insights into a novel mechanism involving MSCs*. J Orthop Res, 2011. **29**(12): p. 1795-803.
23. Fortier, L.A., et al., *The Role of Growth Factors in Cartilage Repair*. Clinical Orthopaedics and Related Research, 2011. **469**(10): p. 2706-2715.
24. Wang, W., D. Rigueur, and K.M. Lyons, *TGF $\beta$  Signaling in Cartilage Development and Maintenance*. Birth defects research. Part C, Embryo today : reviews, 2014. **102**(1): p. 37-51.
25. Sekiya, I., et al., *Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma*. Cell and Tissue Research, 2005. **320**(2): p. 269-276.
26. Ellman, M.B., et al., *Fibroblast Growth Factor Control of Cartilage Homeostasis*. Journal of cellular biochemistry, 2013. **114**(4): p. 735-742.

27. Hardingham, T.E., R.A. Oldershaw, and S.R. Tew, *Cartilage, SOX9 and Notch signals in chondrogenesis*. *Journal of Anatomy*, 2006. **209**(4): p. 469-480.
28. Lieberman, J.R., A. Daluiski, and T.A. Einhorn, *The role of growth factors in the repair of bone. Biology and clinical applications*. *J Bone Joint Surg Am*, 2002. **84-a**(6): p. 1032-44.
29. Barrientos, S., et al., *PERSPECTIVE ARTICLE: Growth factors and cytokines in wound healing*. *Wound Repair and Regeneration*, 2008. **16**(5): p. 585-601.
30. Chen, F.M., M. Zhang, and Z.F. Wu, *Toward delivery of multiple growth factors in tissue engineering*. *Biomaterials*, 2010. **31**(24): p. 6279-308.
31. Quinlan, E., et al., *Long-term controlled delivery of rhBMP-2 from collagen-hydroxyapatite scaffolds for superior bone tissue regeneration*. *J Control Release*, 2015. **207**: p. 112-9.
32. Quinlan, E., et al., *Development of collagen-hydroxyapatite scaffolds incorporating PLGA and alginate microparticles for the controlled delivery of rhBMP-2 for bone tissue engineering*. *J Control Release*, 2015. **198**: p. 71-9.
33. Quinlan, E., et al., *Controlled release of vascular endothelial growth factor from spray-dried alginate microparticles in collagen-hydroxyapatite scaffolds for promoting vascularization and bone repair*. *J Tissue Eng Regen Med*, 2017. **11**(4): p. 1097-1109.
34. van den Berg, W.B., et al., *Growth factors and cartilage repair*. *Clin Orthop Relat Res*, 2001(391 Suppl): p. S244-50.
35. Lee, K., E.A. Silva, and D.J. Mooney, *Growth factor delivery-based tissue engineering: general approaches and a review of recent developments*. *Journal of the Royal Society Interface*, 2011. **8**(55): p. 153-170.
36. Vo, T.N., F.K. Kasper, and A.G. Mikos, *Strategies for controlled delivery of growth factors and cells for bone regeneration*. *Advanced drug delivery reviews*, 2012. **64**(12): p. 1292-1309.
37. Epstein, N.E., *Pros, cons, and costs of INFUSE in spinal surgery*. *Surg Neurol Int*, 2011. **2**: p. 10.
38. Carragee, E.J., E.L. Hurwitz, and B.K. Weiner, *A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned*. *Spine J*, 2011. **11**(6): p. 471-91.
39. Epstein, N.E., *Complications due to the use of BMP/INFUSE in spine surgery: The evidence continues to mount*. *Surg Neurol Int*, 2013. **4**(Suppl 5): p. S343-52.

40. Dunbar, C.E., et al., *Gene therapy comes of age*. Science, 2018. **359**(6372): p. eaan4672.
41. Kohn, D.B., *Gene Therapy for XSCID: The First Success of Gene Therapy*. Pediatric Research, 2000. **48**: p. 578.
42. Cavazzana-Calvo, M., et al., *Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease*. Science, 2000. **288**(5466): p. 669-72.
43. Hacein-Bey-Abina, S., et al., *Efficacy of gene therapy for X-linked severe combined immunodeficiency*. N Engl J Med, 2010. **363**(4): p. 355-64.
44. Raper, S.E., et al., *Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer*. Mol Genet Metab, 2003. **80**(1-2): p. 148-58.
45. Yla-Herttuala, S., *Endgame: glybera finally recommended for approval as the first gene therapy drug in the European union*. Mol Ther, 2012. **20**(10): p. 1831-2.
46. Aiuti, A., et al., *Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency*. New England Journal of Medicine, 2009. **360**(5): p. 447-458.
47. Mullin, E. *2017 was the year of gene-therapy breakthroughs*. 2018 [cited 2018 4th July]; Available from: <https://www.technologyreview.com/s/609643/2017-was-the-year-of-gene-therapy-breakthroughs/>.
48. Cho, J., et al., *A phase III clinical results of INVOSSA<sup>TM</sup> (TissueGene C): A clues for the potential disease modifying OA drug*. Cytotherapy, 2017. **19**(5): p. S148.
49. Evans, C.H. and J. Huard, *Gene therapy approaches to regenerating the musculoskeletal system*. Nature Reviews Rheumatology, 2015. **11**(4): p. 234.
50. Betz, O.B., et al., *Direct percutaneous gene delivery to enhance healing of segmental bone defects*. J Bone Joint Surg Am, 2006. **88**(2): p. 355-65.
51. Li, R., et al., *Effect of cell-based VEGF gene therapy on healing of a segmental bone defect*. J Orthop Res, 2009. **27**(1): p. 8-14.
52. Virk, M.S., et al., *"Same day" ex-vivo regional gene therapy: a novel strategy to enhance bone repair*. Mol Ther, 2011. **19**(5): p. 960-8.
53. Wright, V.J., et al., *BMP4-Expressing Muscle-Derived Stem Cells Differentiate into Osteogenic Lineage and Improve Bone Healing in Immunocompetent Mice*. Molecular Therapy, 2002. **6**(2): p. 169-178.



54. Cucchiarini, M. and H. Madry, *Overexpression of human IGF-I via direct rAAV-mediated gene transfer improves the early repair of articular cartilage defects in vivo*. *Gene Ther*, 2014. **21**(9): p. 811-9.
55. Cucchiarini, M., et al., *Improved tissue repair in articular cartilage defects in vivo by rAAV-mediated overexpression of human fibroblast growth factor 2*. *Molecular Therapy*, 2005. **12**(2): p. 229-238.
56. Cucchiarini, M., P. Orth, and H. Madry, *Direct rAAV SOX9 administration for durable articular cartilage repair with delayed terminal differentiation and hypertrophy in vivo*. *Journal of molecular medicine*, 2013. **91**(5): p. 625-636.
57. Pagnotto, M.R., et al., *Adeno-associated viral gene transfer of transforming growth factor-beta1 to human mesenchymal stem cells improves cartilage repair*. *Gene Ther*, 2007. **14**(10): p. 804-13.
58. Ortved, K.F., et al., *Implantation of rAAV5-IGF-I Transduced Autologous Chondrocytes Improves Cartilage Repair in Full-thickness Defects in the Equine Model*. *Molecular Therapy*, 2015. **23**(2): p. 363-373.
59. Rey-Rico, A., et al., *rAAV-mediated overexpression of TGF- $\beta$  via vector delivery in polymeric micelles stimulates the biological and reparative activities of human articular chondrocytes in vitro and in a human osteochondral defect model*. *International Journal of Nanomedicine*, 2017. **12**: p. 6985-6996.
60. Al-Dosari, M.S. and X. Gao, *Nonviral gene delivery: principle, limitations, and recent progress*. *The AAPS journal*, 2009. **11**(4): p. 671.
61. Nguyen, M.K., et al., *Sustained localized presentation of RNA interfering molecules from in situ forming hydrogels to guide stem cell osteogenic differentiation*. *Biomaterials*, 2014. **35**(24): p. 6278-6286.
62. Pan, Y., et al., *Development of a microRNA delivery system based on bacteriophage MS2 virus-like particles*. *Febs j*, 2012. **279**(7): p. 1198-208.
63. Curtin, C.M., et al., *Innovative collagen nano-hydroxyapatite scaffolds offer a highly efficient non-viral gene delivery platform for stem cell-mediated bone formation*. *Adv Mater*, 2012. **24**(6): p. 749-54.
64. Fang, J., et al., *Stimulation of new bone formation by direct transfer of osteogenic plasmid genes*. *Proc Natl Acad Sci U S A*, 1996. **93**(12): p. 5753-8.
65. Bonadio, J., et al., *Localized, direct plasmid gene delivery in vivo: prolonged therapy results in reproducible tissue regeneration*. *Nat Med*, 1999. **5**(7): p. 753-9.

66. Geiger, F., et al., *Vascular endothelial growth factor gene-activated matrix (VEGF165-GAM) enhances osteogenesis and angiogenesis in large segmental bone defects*. J Bone Miner Res, 2005. **20**(11): p. 2028-35.
67. Goldstein, S.A., *In vivo nonviral delivery factors to enhance bone repair*. Clin Orthop Relat Res, 2000(379 Suppl): p. S113-9.
68. Tierney, E.G., et al., *The development of non-viral gene-activated matrices for bone regeneration using polyethyleneimine (PEI) and collagen-based scaffolds*. Journal of Controlled Release, 2012. **158**(2): p. 304-311.
69. Kuroda, S., et al., *A new technique with calcium phosphate precipitate enhances efficiency of in vivo plasmid DNA gene transfer*. J Pharmacol Sci, 2005. **97**(2): p. 227-33.
70. Huang, Y.C., et al., *Bone regeneration in a rat cranial defect with delivery of PEI-condensed plasmid DNA encoding for bone morphogenetic protein-4 (BMP-4)*. Gene Ther, 2005. **12**(5): p. 418-26.
71. Atluri, K., et al., *Nanoplex-Mediated Codelivery of Fibroblast Growth Factor and Bone Morphogenetic Protein Genes Promotes Osteogenesis in Human Adipocyte-Derived Mesenchymal Stem Cells*. Mol Pharm, 2015. **12**(8): p. 3032-42.
72. Khorsand, B., et al., *Regeneration of bone using nanoplex delivery of FGF-2 and BMP-2 genes in diaphyseal long bone radial defects in a diabetic rabbit model*. J Control Release, 2017. **248**: p. 53-59.
73. Elangovan, S., et al., *The enhancement of bone regeneration by gene activated matrix encoding for platelet derived growth factor*. Biomaterials, 2014. **35**(2): p. 737-47.
74. D'Mello, S.R., et al., *A Pilot Study Evaluating Combinatorial and Simultaneous Delivery of Polyethylenimine-Plasmid DNA Complexes Encoding for VEGF and PDGF for Bone Regeneration in Calvarial Bone Defects*. Curr Pharm Biotechnol, 2015. **16**(7): p. 655-60.
75. Curtin, C.M., et al., *Combinatorial gene therapy accelerates bone regeneration: non-viral dual delivery of VEGF and BMP2 in a collagen-nanohydroxyapatite scaffold*. Adv Healthc Mater, 2015. **4**(2): p. 223-7.
76. Gonzalez-Fernandez, T., et al., *Mesenchymal stem cell fate following non-viral gene transfection strongly depends on the choice of delivery vector*. Acta Biomater, 2017. **55**: p. 226-238.

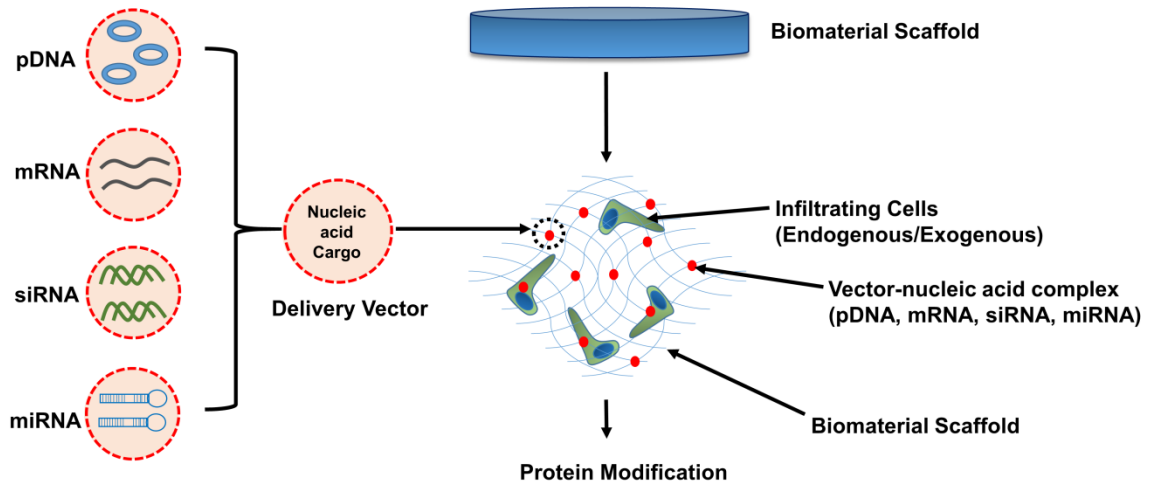
77. Raftery, R.M., et al., *Development of a gene-activated scaffold platform for tissue engineering applications using chitosan-pDNA nanoparticles on collagen-based scaffolds*. Journal of Controlled Release, 2015. **210**: p. 84-94.
78. Raftery, R.M., et al., *Translating the role of osteogenic-angiogenic coupling in bone formation: Highly efficient chitosan-pDNA activated scaffolds can accelerate bone regeneration in critical-sized bone defects*. Biomaterials, 2017. **149**: p. 116-127.
79. Raftery, R.M., et al., *Delivery of the improved BMP-2-Advanced plasmid DNA within a gene-activated scaffold accelerates mesenchymal stem cell osteogenesis and critical size defect repair*. Journal of Controlled Release, 2018. **283**: p. 20-31.
80. Finsson, K.W., et al., *TGF- $\beta$  signaling in cartilage homeostasis and osteoarthritis*. Front Biosci (Schol Ed), 2012. **4**: p. 251-68.
81. Tekari, A., et al., *Transforming growth factor beta signaling is essential for the autonomous formation of cartilage-like tissue by expanded chondrocytes*. PLoS One, 2015. **10**(3): p. e0120857.
82. Tong, J.-C. and S.-L. Yao, *Novel Scaffold Containing Transforming Growth Factor- $\beta$ 1 DNA for Cartilage Tissue Engineering*. Journal of Bioactive and Compatible Polymers, 2007. **22**(2): p. 232-244.
83. Wang, W., et al., *In vivo restoration of full-thickness cartilage defects by poly(lactide-co-glycolide) sponges filled with fibrin gel, bone marrow mesenchymal stem cells and DNA complexes*. Biomaterials, 2010. **31**(23): p. 5953-65.
84. Li, B., et al., *Fabrication of poly(lactide-co-glycolide) scaffold filled with fibrin gel, mesenchymal stem cells, and poly(ethylene oxide)-b-poly(L-lysine)/TGF- $\beta$ 1 plasmid DNA complexes for cartilage restoration in vivo*. J Biomed Mater Res A, 2013. **101**(11): p. 3097-108.
85. Ousema, P.H., et al., *The inhibition by interleukin 1 of MSC chondrogenesis and the development of biomechanical properties in biomimetic 3D woven PCL scaffolds*. Biomaterials, 2012. **33**(35): p. 8967-74.
86. Glass, K.A., et al., *Tissue-engineered cartilage with inducible and tunable immunomodulatory properties*. Biomaterials, 2014. **35**(22): p. 5921-31.
87. Moutos, F.T., et al., *Anatomically shaped tissue-engineered cartilage with tunable and inducible anticytokine delivery for biological joint resurfacing*. Proceedings of the National Academy of Sciences, 2016. **113**(31): p. E4513.

88. Curtin, C.M., I.M. Castaño, and F.J. O'Brien, *Scaffold-Based microRNA Therapies in Regenerative Medicine and Cancer*. Advanced Healthcare Materials, 2018. **7**(1): p. 1700695.
89. Balmayor, E.R., et al., *Chemically modified RNA induces osteogenesis of stem cells and human tissue explants as well as accelerates bone healing in rats*. Biomaterials, 2016. **87**: p. 131-146.
90. Elangovan, S., et al., *Chemically modified RNA activated matrices enhance bone regeneration*. J Control Release, 2015. **218**: p. 22-8.
91. Khorsand, B., et al., *A comparative study of the bone regenerative effect of chemically modified RNA encoding BMP-2 or BMP-9*. The AAPS journal, 2017. **19**(2): p. 438-446.
92. Fire, A., et al., *Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans**. Nature, 1998. **391**(6669): p. 806-11.
93. Castaño, I.M., et al., *Next generation bone tissue engineering: non-viral miR-133a inhibition using collagen-nanohydroxyapatite scaffolds rapidly enhances osteogenesis*. Scientific Reports, 2016. **6**: p. 27941.
94. Castaño, I.M., et al., *A novel collagen-nanohydroxyapatite microRNA-activated scaffold for tissue engineering applications capable of efficient delivery of both miR-mimics and antagomiRs to human mesenchymal stem cells*. Journal of Controlled Release, 2015. **200**: p. 42-51.
95. Devlin, R., et al., *Skeletal overexpression of noggin results in osteopenia and reduced bone formation*. Endocrinology, 2003. **144**(5): p. 1972-1978.
96. Lolli, A., et al., *Emerging potential of gene silencing approaches targeting anti-chondrogenic factors for cell-based cartilage repair*. Cellular and Molecular Life Sciences, 2017. **74**(19): p. 3451-3465.
97. Lolli, A., et al., *Silencing of Antichondrogenic MicroRNA-221 in Human Mesenchymal Stem Cells Promotes Cartilage Repair In Vivo*. Stem Cells, 2016. **34**(7): p. 1801-11.
98. Legendre, F., et al., *Enhanced hyaline cartilage matrix synthesis in collagen sponge scaffolds by using siRNA to stabilize chondrocytes phenotype cultured with bone morphogenetic protein-2 under hypoxia*. Tissue Engineering Part C: Methods, 2013. **19**(7): p. 550-567.
99. Legendre, F., et al., *Enhanced chondrogenesis of bone marrow-derived stem cells by using a combinatory cell therapy strategy with BMP-2/TGF- $\beta$ 1, hypoxia, and COL1A1/Htra1 siRNAs*. Scientific reports, 2017. **7**(1): p. 3406.

100. European Union, "*Regulation on Advanced Therapy Medicinal Products*," in *Regulation 1394/2007* (Brussels, 13 Nov. 2007).
101. European Commission, "*Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products*". 2017: (Brussels, 22 Nov. 2017).
102. Journal of Gene Medicine. "*Number of Gene Therapy Clinical Trials Approved Worldwide 1989-2018*". 2018; Available from: <http://www.abedia.com/wiley/index.html>.
103. Hanna, E., et al., *Gene therapies development: slow progress and promising prospect*. Journal of market access & health policy, 2017. **5**(1): p. 1265293.
104. Bozo, I.Y., et al., *World's First Clinical Case of Gene-Activated Bone Substitute Application*. Case Reports in Dentistry, 2016. **2016**: p. 6.

Figures

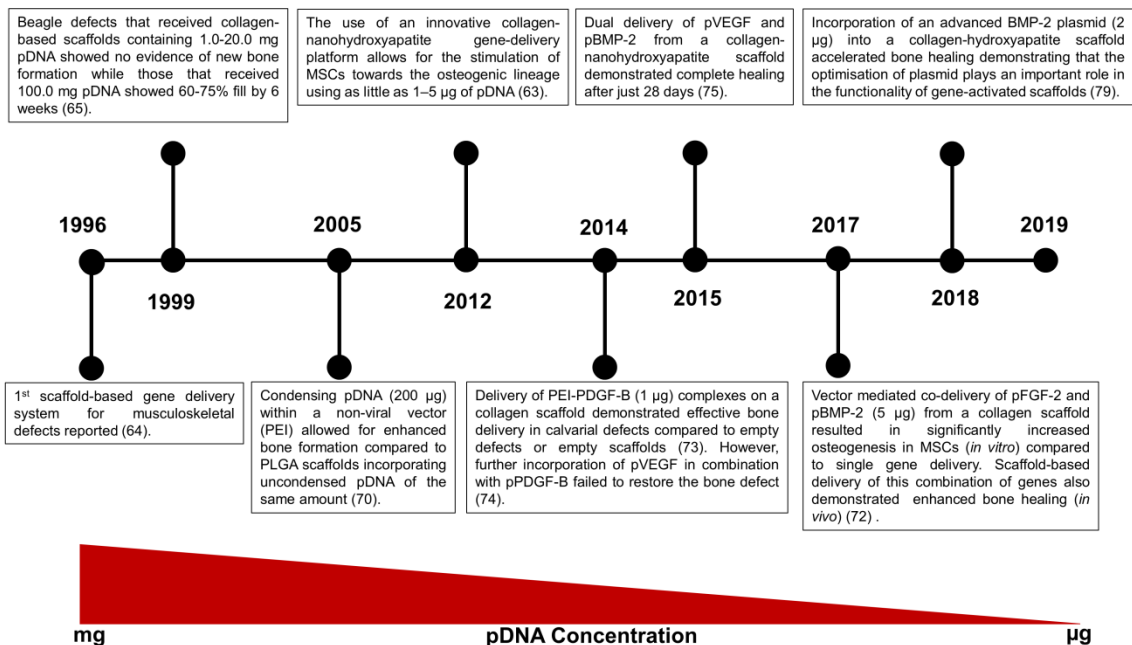
1. Gene-activated scaffold outline. Delivery vectors can be used to package and protect nucleic acid cargoes (such as pDNA, mRNA, siRNA, miRNA) facilitating cellular uptake. Vector-nucleic acid complexes are contained within the biomaterial scaffold. The scaffold itself provides structural support for the deposition of new functional tissue while infiltrating cells (endogenous/exogenous) engulf the complexes, internalising the genetic cargo, resulting in a modification of downstream protein expression. Image adapted from [12].



## 2. Refinements in the components of scaffold-based delivery of nucleic acid

therapeutics over the last two decades has resulted in the reduction of DNA dose required for efficient bone repair from milligrams (mg) to micrograms ( $\mu\text{g}$ ).

Research into improving the efficiency of non-viral vectors, combinational therapies and advanced plasmid design have led to more efficient delivery methods requiring lower and relatively safer doses of pDNA.



3. AntagomiR-133a activated gene scaffolds for bone tissue engineering. Scaffold mediated miR-133a inhibition by antagomiR-133a (a) resulted in increased calcium deposition in a hMSCS 3D in vitro culture over 28 days compared to gene-free scaffolds and scaffolds containing scrambled antagomiR. (b) Increased calcium deposits were observed with alizarin red staining at day 14 and day 28 in antagomiR-133a loaded scaffolds compared to controls. Figure re-used and adapted from [93].

