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Authors	Kelly, Domhnall C.;Raftery, Rosanne M.;Curtin, Caroline M.;O'Driscoll, Caitríona M.;O'Brien, Fergal J.
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Domhnall Kelly ORCID iD: 0000-0003-0374-856X

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

Domhnall C. Kelly[1,2,3,4], *Rosanne M. Raftery*[1,2,3], *Caroline M. Curtin*[1,2,3], *Caitriona M. O'Driscoll*[4,5], *Fergal J. O'Brien*[1,2,3,4]

[1]Tissue Engineering Research Group, Dept. of Anatomy, Royal College of Surgeons in Ireland (RCSI), Dublin, Ireland

[2]Trinity Centre of Bioengineering (TCBE), Trinity College Dublin (TCD), Dublin, Ireland

[3]Advanced Materials and Bioengineering Research Centre (AMBER), RCSI and TCD, Dublin, Ireland

[4]Centre for Research in Medical Devices (CÚRAM), Biomedical Sciences Building, National University of Ireland Galway (NUI, Galway), Galway, Ireland

[5]Pharmacodelivery Group, School of Pharmacy, University College Cork, Cork, Ireland

*Corresponding Author

Prof. Fergal O'Brien

Dept. Of Anatomy

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Royal College of Surgeons in Ireland

123, St. Stephens Green

Dublin D02 YN77

Ireland

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Running Title: Gene-activated Scaffolds for Orthopaedic Applications

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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.

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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 handle-ability 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, β-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

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advantages of combing 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-β) [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

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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-β 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

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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

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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^{TM}, in South Korea. This treatment consists of a mixture of non-transformed and *ex vivo* retrovirally transduced chondrocytes for the overexpression of TGF-β1 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

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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-β1 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].

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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).

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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 (μg) (Figure 2). The incorporation of a calcium phosphate-pDNA (40 μg) precipitate mixed with collagen demonstrated enhanced efficiency of reporter plasmids *in vivo* [69]. Huang et al., demonstrated that condensing BMP-4-pDNA (200 μg) 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 μg)[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 μg 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 μg 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

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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 μg 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 μg 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 μg 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

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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-β1 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 pTGFβ1 (1 μg, 2 μg or 4 μg 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-β1 (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 (ε-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

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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 reseach 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

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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

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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-ɣ), a negative regulator of BMP-2 signalling, did not outperform the delivery of the silencing of Noggin by siRNA alone [61].

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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.

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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].

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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 be 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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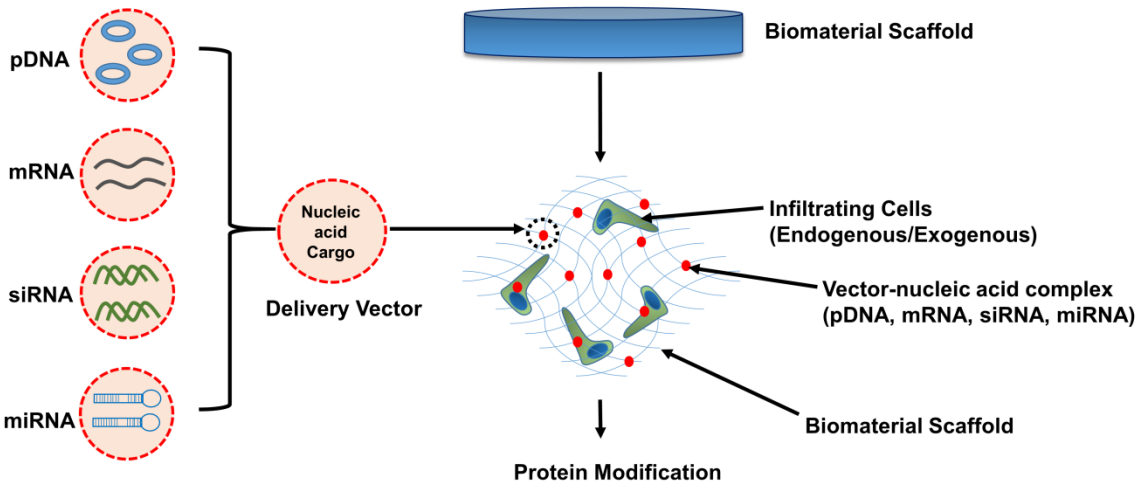
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Figures

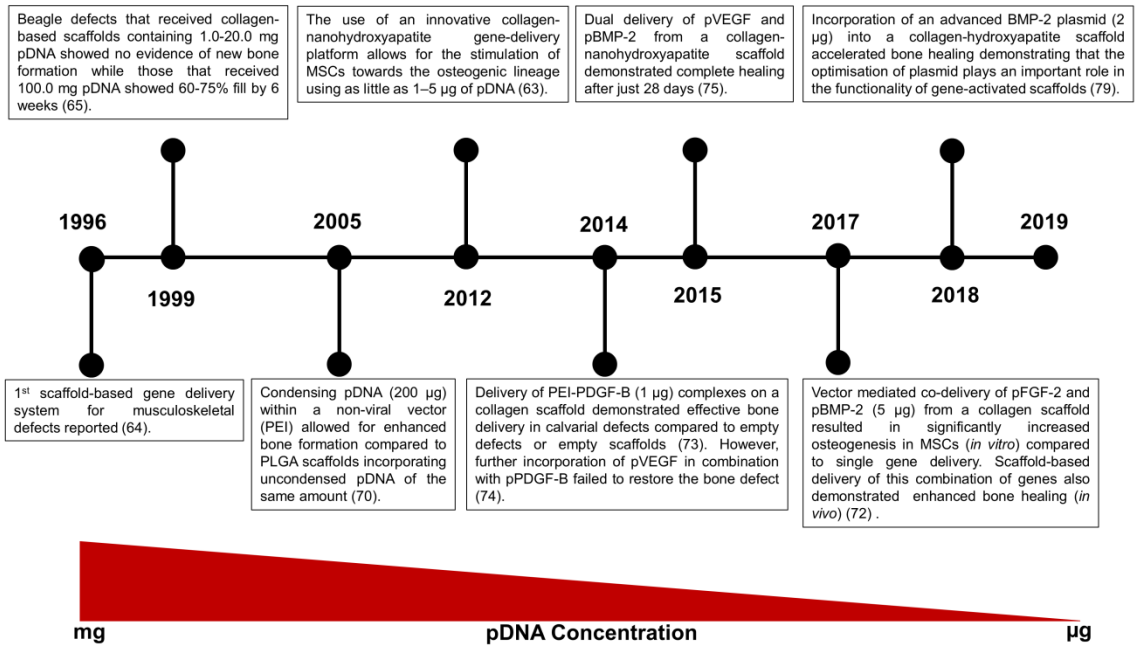
1. Gene-activated scaffold outline. Delivery vectors can be used to package and protect nucleic acid cargoes (such as pDNA, mRNA, siRNA, miRNA) facilitating cellular uptake. Vector-nucleic acid complexes are contained within the biomaterial scaffold. The scaffold itself provides structural support for 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].



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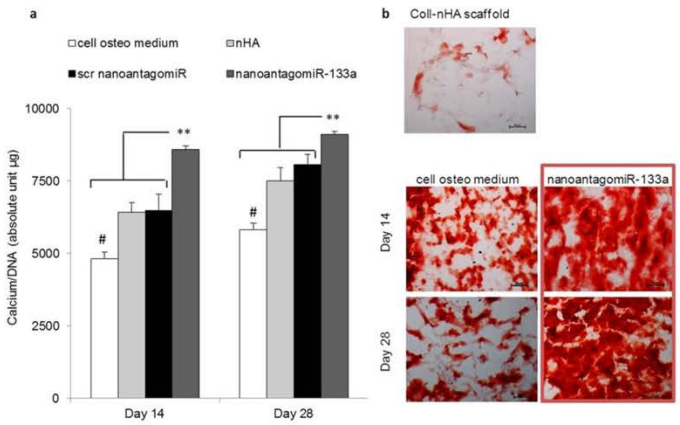
2. Refinements in the components of scaffold-based delivery of nucleic acid therapeutics over the last two decades has resulted in the reduction of DNA dose required for efficient bone repair from milligrams (mg) to micrograms (μg). Research into improving the efficiency of non-viral vectors, combinational therapies and advanced plasmid design have led to more efficient delivery methods requiring lower and relatively safer doses of pDNA.



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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].



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