Abstract: Scaffold-based bone tissue engineering has been introduced as an alternative treatment option for bone grafting due to limitations in the allograft. Not only physical conditions but also biological conditions such as gene expression significantly impact bone regeneration. Scaffolds in composition with bioactive molecules such as miRNA mimics provide a platform to enhance migration, proliferation, and differentiation of osteoprogenitor cells for bone regeneration. Among scaffolds, fibrous structures showed significant advantages in promoting osteogenic differentiation and bone regeneration via delivering bioactive molecules over the past decade. Here, we reviewed the bone and bone fracture healing considerations for the impact of miRNAs on bone regeneration. We also examined the methods used to improve miRNA mimics uptake by cells, the fabrication of fibrous scaffolds, and the effective delivery of miRNA mimics using fibrous scaffold and their processes for bone development. Finally, we offer our view on the principal challenges of miRNA mimics delivery by nanofibers for bone tissue engineering.

Keywords: bone regeneration; bone formation; nanofiber; miRNA delivery; scaffold

1. Introduction

Bone loss or damage can result from various causes, including degenerative diseases, fractures, and surgeries. Though bone can repair itself, the regeneration of damaged bone needs to be stimulated in many cases when complete bone regeneration cannot occur [1]. MiRNAs have been shown to alter expression levels in bone regeneration significantly. MiRNA, a class of non-coding RNA, plays a vital role in gene expression by targeting mRNAs to destabilize mRNAs and/or inhibit protein translation [2–4]. In recent years, miRNA mimic delivery has been investigated to treat various diseases and conditions, including cancer and neurodegenerative disorders [5].

A wide range of advanced scaffolds have been developed in the last decade for bone regeneration. Scaffold-based DDS (Table S1) for bone tissue engineering have evolved as an interdisciplinary approach to designing platforms from the perspective of both materials and functions. These platforms provide structures, helping reorganize the tissues of defective or lytic bone lesions [6]. Until recently, emphasis was only placed on microporous framework structures that can mimic physical features of ECM and support cell adhesion, migration, and morphogenesis [7]. Therefore, many scaffolds and methods are involved in the preparation of 3D micropores constructions. Amongst porous scaffolds, NFs are prominent in bone tissue engineering due to their ability to promote adhesion and osteogenic differentiation of bone borrow MSCs [8]. NFs are better candidates than hydrogels for drug delivery systems due to their high surface-to-volume ratio and porosity.
A notable feature of NFs is their ability to promote the transfer of drugs and waste products and making them an ideal scaffold for both DDS and tissue engineering [9].

The field of bone tissue engineering has recently been utilizing DDS for delivering functional molecules, including bioactive signal molecules [10]. Over the last decade, prominence has been placed on signaling molecules like non-coding RNAs, to improve the biocompatibility and biodegradation of scaffolds, and control cell adhesion and differentiation [8]. The miRNAs that regulate gene expression at the post-transcriptional level are evolutionally involved in several transcription factors and cytokines. It has been proven that, besides the architecture of the scaffolds that have been used for bone tissue engineering, miRNA mimics provide a link between the scaffold and activation of osteogenic markers. In the case of bone regeneration, a combination of NFs and miRNA mimic enhanced bone formation through cell signaling. For example, loading miRNA-22 and miRNA-126 mimics into PCL NFs could promote cell viability and osteogenic differentiation of human iPSCs with increased osteoblast marker gene expression, including RUNX2, BGLAP, ALPL, and SPARK [11].

To explain the application of fibrous scaffolds as miRNA mimic carriers to bone tissue engineering (see Figure 1), we describe fracture healing aspects of the impact of miRNAs on bone regeneration and methods to deliver miRNAs mimic into target cells. Moreover, we describe the impact of NF scaffolds to deliver miRNA mimics for bone tissue engineering. Finally, we discuss fundamental challenges and prospects in the application of a complex of NF and miRNA.

**Figure 1.** An illustration of the electrospinning system for NF formation. Electrospinning involves supplying an electric charge to a polymer solution in a syringe and subsequently stretching polymer droplets to form zones termed the Taylor cone and Jet, respectively, at the tip of the needle to form fibers. The produced fiber accumulates on a collector under the influence of electrostatic repulsion between the reference (polymer solution) and counter electrode (collector). Nanofibers can deliver liposomal synthetic oligonucleotides.
2. Bone Healing and MiRNAs Expression

The adult bone adults constantly remodel itself, responding to both various physiological and pathological demands, such as repairing bones weakened by macro- and microdamage. Bone regeneration is a complex process, involving both bone remodeling and cellular processes. In both endochondral and intramembranous ossification, a number of complex gene expressions are involved in reaching the remodeled bone. Over the past several decades, our understanding of either process has improved enormously [12]. In both cases, MSCs migrate to the damaged area and increase growth factors and cytokines levels followed by differentiation into chondrocytes (endochondral ossification) or osteoblasts (intramembranous ossification). After an injury, a cartilaginous callus forms at the injury site, which provides intermediate stabilization to the fracture segment. This cartilage callus subsequently becomes vascularized, remodeled, and finally will be replaced by bone. Conversely, in intramembranous ossification, MSCs differentiate directly into osteoblasts in a highly stabilized fracture healing without a prolonged cartilaginous intermediate [13].

Fracture healing is an apparent example of endochondral ossification that begins with fracture-induced vascular disruption. Exposure of vascular cells triggers a coagulation cascade (thrombin activation) and the subsequent formation of hematoma, where platelets and macrophages accumulate. A local inflammatory reaction is initiated to release inflammatory mediators from the fracture hematoma, causing vascular permeability and inflammatory cell migration, as shown in Figure 2. These processes activate osteoclasts to resorb bone debris and fibroblasts to transform the hematoma into granulation tissue. New vasculature in the fracture site provides MSCs that transformed to the cells with osteogenic potential and formed callus. A soft and hard callus could convert to the bone via endochondral ossification and intramembranous ossification, respectively.

Figure 2. An illustration of different stages of typical fracture healing. The metabolic phases (blue bars) overlapped with biological stages (brown bars) for 35 days (black bars). The three biological stages of bone fracture healing are inflammatory, endochondral bone formation, and coupled remodeling. The time scale denoted is based on the primary cell types present at each stage of healing of a mouse closed femur fracture. This figure was recreated from 2D to 3D with permission from reference [14]. Copyright 2021, Springer Nature.
Currently, more than 1000 articles in the PubMed database address questions about miRNAs in bone cells, including osteoblasts and osteoclasts. The microarray data from femoral fracture of twelve-week-old male Sprague-Dawley rats showed that 317 miRNAs were expressed more highly in normal healing fractures. However, eight miRNAs include rno-miR-140-3p, rno-miR-140-5p, rno-miR-181a-5p, rno-miR-181d-5p, rno-miR-208b-3p, rno-miR-451a, rno-miR-743b-5p, and rno-miR-879-3p, which were highly up-regulated by filtering with a fold change of >2.0, a low coefficient variation (<50%) during 28 days, as presented [15]. Recent studies demonstrated that miR-140-3p and miR-140-5p are highly expressed in the cartilage. Mmu-miR-140-3p and mmu-miR-140-5p are involved in osteoarthritis. Mmu-miR-140-3p with targeting the Cxcr4 and Rala promotes chondrogenesis and ameliorates osteoarthritis progression. Besides, the miR-140-5p with downregulating Il1b, Il6, Mmp13, Smad3, and Hmgb1 expression, inhibit inflammation. However, miR-140-5p with the upregulation of Dnpep, Tgfbr1, and Rala expression promotes chondrogenesis. Moreover, expression of miR-140-5p involves the upregulation of Hdac4 and Smad1, and results in the inhibition of chondrocyte hypertrophy and osteogenesis with downregulation Tlr4, Bmp2, and Tgfbr1 genes, respectively. MiR-181a-5p as well as miR-140-3p and miR-140-5p was identified as inflammation regulator at the early stage of fracture healing by targeting NCOA1, NRIP1, and IL1A gens. The treatment of human MSCs by miR-181d-5p mimic indicated that downregulation of miR-181d-5p promotes osteogenic differentiation by targeting RUNX2 through the MAPK pathway. Moreover, miR-181a-5p promotes osteoblastic differentiation via the procession of TGF-β secretion. Furthermore, both miR-181a-5p and miR-181d-5p, by upregulating BCL2, induce osteocyte apoptosis [16]. MiR-208b-3p was another miRNA that was expressed highly in bone fracture healing. Importantly, miR-208b-3p mimic suppressed osteoblastogenesis in MC3T3-E1 and inhibited bone formation by reducing Acvr1b translation, thereby decreasing Bmp2 and its downstream target Smad1/4/5 and Runx2 [17].

Moreover, the overexpression of mmu-miR-451a could inhibit the osteogenic differentiation of mice MSCs, accelerate bone loss via Bmp6 signaling, and elevate bone loss by regulating Smad1/5/8 expression [18]. Additionally, miR-451a downregulates the CELF2, thereby significantly increasing COX protein and inhibiting chondrocyte hypertrophy and inflammation. It is unfortunate that still, there is no significant evidence for the role of miR-743b-5p and miR-879-3p in the bone field. Possibly, miR-879-3p is only expressed in mice and rats. However, it is reported that negative expression of mmu-miR-743b-5p could enhance fibrogenesis and Tgfβ1 expression level [19]. TGFβ release from the bone matrix plays a vital role in the temporal and spatial regulation of bone remodeling during osteoclast bone resorption. Active TGFβ recruits MSCs to the bone resorption pit through the SMAD signaling pathway [20]. Moreover, activating the TGFβ pathway could promote angiogenesis in CRC cells [21]. The mi-RNAs involved in rat bone regeneration and considering their target genes applicable for human use are summarized in Figure 3.
Figure 3. Illustration of miRNAs highly involved in bone regeneration and their target genes.

Intermembranous bone ossification, such as alveolar bone healing, is well studied by Anderia Espindola Vieira et al. [22]. It was found that intramembranous bone healing process in mice follows by tooth extraction and initiates with clot formation. Based on the results of the mice model, it appears that blood clots are gradually resorbed and replaced by granulation tissue associated with Col1a1 and Col1a2 expression over the course of 14 days. Moreover, they showed that growth factors and cytokines such as BMP2/4/7, FGF2, TGFβ1, VEGFA, TNFα, and IL10 were highly expressed during the first week of healing, indicating a lack of osteochondral gene markers. Unfortunately, up to now, there is no evidence related to miRNA expression in intramembranous ossification. The development of scaffolds may depend on understanding molecular signaling pathways involved in bone regeneration besides the miRNAs that are highly expressed during bone fracture healing.

MiRNA stability is a concern when considering clinical use; for example, many miRNAs rapidly degrade at 37 °C when incubated with serum [23]. MiRNA mimics are oligonucleotides that have been chemically altered to increase their stability and affinity for target genes [24,25]. Moreover, antagonirs, synthetic antagonists of miRNA, are currently undergoing phase 1 and phase 2 clinical trials. Therefore, various miRNA mimics and antagonirs have been evaluated for bone regeneration purposes. Few articles have reviewed miRNA incorporation with hydrogel scaffolds for bone regeneration [26], but none have focused on NFs. Among the trials, several miRNAs have been loaded to hydrogels for bone regeneration, as updated and summarized in Table 1.
Table 1. miRNAs incorporated with hydrogel scaffolds for bone tissue engineering.

| miRNAs                  | Target Cell or Animal          | Target Genes                      | Refs  |
|-------------------------|--------------------------------|-----------------------------------|-------|
|                         | In vitro studies               |                                   |       |
| Let-7a                  | Human USSC                     | NLK                               | [27]  |
| Let-7f                  | Human MSCs                     | AXIN2                             | [28]  |
| miRNA-15b               | Human MSCs                     | BMPR2                             | [29]  |
| miRNA-20a               | Human MSCs                     | PPARγ, BAMBI, CRIM1               | [30]  |
| miRNA-27                | Human FOB1.19 cells            | APC                               | [31]  |
| miRNA-29a/b/c           | Mouse primary osteoblast,      | Bglap, Acvr2a, Ctnnbip, Dusp2,    | [32–34]|
|                         | MC3T3 cells                    | Hdac4, Tgfβ3                      |       |
| miRNA-130c              | Human MSCs                     | CAMTA1, CXCL12, ITGB1, FLT1       | [29]  |
| miRNA-96                | Human MSCs                     | FABP4                             | [35]  |
| miRNA-130b              | Human MSCs                     | CAMTA1, CD44, GDF6, PDGFRA, COL9A3| [29]  |
| miRNA-142-3p            | Human FOB1.19 cells            | APC                               | [36]  |
| miRNA-199a              | Human FOB1.19 cells            | SOX9                              | [35]  |
| miRNA-210               | Mouse ST2 cells                | Acvr1b                            | [37]  |
| miRNA-218               | Mouse MSCs                     | Dkk2, Sfrp2, Sost                 | [38]  |
| miRNA-355-5p            | MC3T3-E1, MLO-A5 cells         | Dkk1                              | [39]  |
| miRNA-1228              | Human primary osteoblast       | BMP2K                             | [40]  |
| miRNA-2861/miRNA-3960   | Mouse ST2 cells, Mouse         | Hdac4, Hoxa2                       | [41]  |
|                         | osteoblast                      |                                   |       |
| Anti-miRNA-133a         | Human MSCs                     | RUNX2                             | [42]  |
| miRNA-16                | Human MSCs                     | RUNX2                             | [43]  |
| miRNA-590-5p            | C3H10T1/2                      | Smad7                             | [44]  |
| miRNA-122               | Human MSCs                     | RUNX2, OSX                        | [45]  |
|                         | In vivo studies                |                                   |       |
| Anti-miRNA-214          | Ovariectomy, mouse             | Atf4                              | [46]  |
| Anti-miRNA-92a          | Femoral fracture, mouse        | Itga5, Mkk4                        | [47]  |
| Anti-miRNA-31           | Calvarium defect, rat and canine| Sath2                            | [48]  |
| Anti-miRNA-26a          | Calvarium defect, mouse        | Ptn                               | [49]  |
| Anti-miRNA-138          | Subcutaneous, mouse            | Runx2, Sp7, Bmp2                  | [50]  |
| miRNA-148b mimic        | Calvarium defect, mouse        | Runx2                             | [51]  |
| miRNA-5106              | Calvarium defect, mouse        | Runx2, Spp1, Alpl                 | [52]  |
| miRNA-34a               | Tibial defect, rat             | Notch1                            | [53]  |
| Anti-miRNA-222          | The refractory fracture, rat   | Col2a1, SOX9                       | [54]  |
| Anti-miRNA-432          | Ectopic bone formation, mouse  | Cdkn1b                            | [55]  |

3. MiRNA Mimic Uptake by Cells

The hydrophilic nature of synthetic oligonucleotides limits their ability to penetrate the hydrophobic cellular membranes [56]. To overcome these limitations, a variety of biocompatible nanocarriers have been used to deliver miRNA mimics into target cells, including calcium phosphate, gold nanoparticles [57–61], and liposomes [62]. The en-
capsulation of miRNA mimics in cationic liposomes, which have a high affinity for cell membranes with a net negative charge, is one approach to this problem [63]. Additionally, cellular uptake is facilitated by charge interactions between the cationic lipids and the anionic oligonucleotides, which result in a net positive charge in the complex [64]. Cationic liposomes might also provoke a pro-inflammatory response by inducing Th1 cytokine expression [65]. However, the immunogenicity and cytotoxicity of liposomes are challenging for clinical applications if high and frequent dosages are required [66]. Low toxicity, tissue-specific targeting, and proper cellular uptake are critical requirements for lipid-based nanoparticles in delivery of miRNA mimics [67]. For example, siPORT™ NeoFX™ [49], HiPerFect [68], and Lipofectamine® [69] are commercially available transfection reagents, which have been extensively used to deliver miRNA mimics.

As well as liposomes, biocompatible nanoparticles such as gold [70], silica [71], and calcium phosphate [58] have been used to carry miRNAs into cells. Alkyl-thiol-terminated oligonucleotides modified in gold nanoparticles showed a high affinity for miR-145 mimic, and subsequently increased miR-145 mimic uptake by PC3 cells and MCF7 cells [70]. The mesoporous silica nanoparticles demonstrate favorable drug delivery properties due to their large surface area and pore volume, tunable particle size and pore size, and biocompatibility [72]. Mesoporous silica nanoparticles with carrying a polyarginine-peptide: nucleic acid conjugate targeting oncogenic miR-221 mimic efficiently induced apoptosis in the T98G cells [73]. The long-chain miR-34a mimic conjugates (Lc-miR34a mimic) were introduced more efficiently into PC3 cells than lc-miR-34a mimic alone, with concomitant effects on cell proliferation and migration [74]. Several carriers, including transfection reagents, have been successfully used to deliver miRNAs into various cells in vitro. However, despite their success, miRNA mimics do not possess specific affinity for pathological sites.

To overcome the challenges encountered in delivering miRNA mimics to target cells, several approaches have been proposed. Effective drug delivery systems accumulate and retain active ingredients locally, thereby minimizing systemic adverse effects. [75]. Since miRNAs mimic are released into the circulation, delivering them into recipient cells at appropriate spatial and temporal scales [76], they needed appropriate scaffolds to carry miRNAs for targeting bone tissue. Therefore, further studies are required to develop platforms to accumulate the miRNAs at the specific sites of interest for tissue-specific targeting of miRNAs, as shown in Figure 4.
4. NFs Promote Osteogenesis

Electrospinning is an effective technique for manufacturing NFs from polymeric materials, when polymer concentration, solubility, and composition are controlled along with applied voltage, and can produce NFs with different features, including different topography, density, and fiber diameter (from a few nanometers to several micrometers) [77]. Figure 1 outlines the electrospinning technique, where a high voltage positive charge is applied to the electrostatic repulsion of the polymer solution. Droplets of positively charged polymer liquid could stretch when exposed to a negative charge, and eventually, the stretched droplets accumulated as fibers on a negatively charged metallic collector [78]. There has been considerable effort to mimic bone healing conditions with scaffolds. NFs have the excellent ability to capture drugs and complexes, such as liposome-encapsulated drugs, and release them based on parameters such as the chemical composition and structure of the fibers (e.g., the ratio of surface to volume) [75].

Interdisciplinary approaches have enabled us to design and develop scaffold-based cell engraftment platforms for bone tissue engineering [79]. Scaffolds with pore sizes of 100 to 300 µm and a porosity of more than 90% have been shown to enhance bone regeneration [80]. Many fabrication techniques have been introduced to facilitate the production of nanofibers from biocompatible polymers. A network of interconnected pores within highly porous microstructures have been found to promote tissue ingrowth in bone regeneration. Such 3D microporous frameworks structurally mimic the bone ECM and support cell adhe-
sion, migration, and proliferation [81]. However, in contrast, Beom Su Kim et al. showed that PCL/SF NFs with a pore size of around 60.6 µM improved the MSCs adhesion and growth in comparison to PCL NFs, with a pore size range around 121.7 µM [82]. Recently, attention has turned to polymeric NFs, allowing scaffold designs for tissue engineering and DDS [75].

In bone, osteoblasts produce ECM principally comprising type I collagen, and subsequently contribute to ECM mineralization [12]. Hence, a successful strategy for skeletal regeneration depends on whether enough active bone- or cartilage- and bone-forming cells can be recruited and appropriately differentiated. Electrospinning is a versatile method to produce NFs with variable features of such properties as orientation, morphology, and chemical composition [83]. The potential ability of NFs assemblies to support bone regeneration must be assessed using a combination of well-established detection methods for the proliferation-differentiation of osteoprogenitor cells and matrix mineralization phase of osteogenesis: e.g., MTT for proliferation, sequential expression of osteoblast/chondrocyte marker genes, and ALP activity for differentiation and extracellular calcium deposition for mineralization. In this direction, several studies have shown that random or aligned PLLA NFs fabricated by electrospinning induced osteogenesis in MG63 cells (Human osteosarcoma cell line) [84] and MSCs from Wistar rats [85]. Furthermore, both random and aligned PLLA NFs promoted osteogenic activities in human adipose tissue-derived MSC cultures, indicating that random NFs are more effective in cell proliferation and aligned NFs can stimulate osteogenic differentiation, as shown in Figure 5 [86]. In a recent example, the topography and chemistry of electrospun poly (butylene succinate)-based NFs affected adhesion and differentiation of Saos-2 cells suggesting that an ether linkage involved in the density of hydrogen bond acceptors on the surface of NFs may contribute to osteogenic activity (expression of osteogenic markers, differentiation, and mineralization of cells) [87]. Highly porous polymeric cellulose nanofibrils can also transfer glucose, proteins, oxygen, and waste products, which lead to promoting proliferation of MG63 cells [88].

**Figure 5.** Morphology of fabricated PLLA nanofibers, randomly oriented (A) and aligned oriented (B), the morphology of adipose tissue-derived MSC adhered to random (C) and aligned (D) nanofibers. Real-time PCR analysis of miRNAs expression during osteoblast differentiation (E). The expression of osteoblast-specific miRNAs (miR-15b, miR-30c, miR-20a, miR-125b, and miR-24) in osteo-differentiated cells cultured on random and aligned nanofibers during 21 days of osteogenic differentiation, *p < 0.05. This figure was reprinted with permission from reference [86], Copyright 2021, Elsevier.
5. Bone Tissue Regeneration by MiRNAs Mimic-NFs

Bone tissue engineering builds on the understanding of bone structure and aims to develop platforms that effectively regenerate bone defects. Bone derives its unique mechanical properties from an architectural design extending over nanoscale to macroscopic dimensions. A wide range of structural proteins are found in the ECM of bone, including serum-derived proteins, proteoglycans, glycosylated proteins, Gla-containing proteins, and small integrin-binding proteins. Mentioned proteins bind to strands of collagen fibrils (collagen type I, collagen type X, collagen type III, and collagen type V) [89], dominating at the nanometer level with a diameter scale between 35 to 60 nm. The hydroxyapatite crystals are embedded with collagen molecules and increase the rigidity of the bone. Besides, a wide range of proteins is engaged in the regulation of ECM formation [90]. Bone ECM also contains a number of biologically active molecules. For example, BMP2 [12], IGF1 [91], and TGF-β1 play crucial roles in osteoblast-osteoclast development and bone formation. RUNX2, a master transcriptional regulator of osteoblast differentiation [92], responds to numerous extracellular signals, including BMP and TGF-β1, and binds specific DNA sequences with several co-factors to regulate downstream target genes osteoblasts [93]. One such RUNX2 co-factor is SP7, and Sp subfamily member of the Sp/XKLF transcription factor family, leading to the upregulation of type I collagen [94]. SP7 also acts downstream of RUNX2 to regulate osteoblast development [36], exerting its regulatory effects by binding to guanine-rich sequences in specific target genes [95]. Several osteoblast-associated genes/gene products, including RUNX2, SP7, ALPL, COL1A1, IBSP, and BGLAP, are used as markers to demonstrate osteoblastogenesis in a developmental stage-dependent manner [96]. ALPL is a membrane-bound glycosylated enzyme that acts as a phosphatase to release inorganic phosphate from other molecules, increasing the concentration of phosphate required for calcification [97].

As of today, miRNA mimic-NF assemblies with osteogenic activity have been described only in a handful of publications (PubMed) summarizing cell culture models and laboratory animal models. James et al. provided the first report of the potential utility of a miRNA mimic-NF assembly [98]. In this example, electrospun and cross-linked gelatin nanofibers containing miRNA-29a inhibitors were supplied to MC3T3-E1 cells and mouse MSCs. Of the various target candidates tested, Tgfb1 and Igf1 were upregulated by the miRNA-29a inhibitor, leading to increased Sparc in the MC3T3-E1 cells and collagen deposition in MSCs. These NFs (35–50 µm thick) were stable for at least 7 days in the culture medium, and the release of miRNA-29a inhibitor into the medium was found as early as 2 h, gradually increasing and being sustained up to 72 h. To promote the differentiation switch toward osteoblast and accelerate the angiogenesis, osteogenic miR-22 and angiogenic miR-26 were introduced into electrospun PLC NFs [11]. In spite of their almost equal fiber diameter, the NFs derived from miR-22 and miR-26 had less mechanical strength and hydrophobicity. Cell adhesion, proliferation, and osteogenic differentiation of human iPS cells cultured under osteogenic conditions were enhanced by PLC NFs containing miR-22 and miR-26 compared to NFs without these miRNAs. In these cultures, more than 50% of the incorporated miRNAs were released during the first 72 h. Additionally, the 3D nanofiber aerogels containing miR-26a mimic were developed for the regeneration of calvaria bone defects in a rat model. The results of implantations indicate that aerogel- miR-26a mimic promotes bone formation up to 55%, while the aerogel loaded with negative control of miRNA could encourage bone formation up to 20%, as shown in Figure 6. Table 2 represents miRNA mimic-related molecules incorporated into NFs scaffolds for bone regeneration [99].
Two reports have extended our understanding of the effects of miRNA-NFs assemblies on skeletal regeneration in vivo [100,101]. Poly (L-lactic acid) (PLLA)-graft-poly (hydroxyethyl methacrylate) was employed to prepare nanofibrous porous solid microspheres. These solid microspheres with an average diameter between 30 and 60 µM and approximately 15 µM in pore size supported proliferation and chondrogenic differentiation of rabbit MSCs. When rabbit MSCs precultured on solid microspheres carrying antagomir-199a with a hyperbranched polymer vector were implanted into the subcutis of nude mice, and needle puncture-induced lumbar disc degeneration of rabbits, increased chondrogenesis and intervertebral disc regeneration, respectively, were seen. These results were attributed to the antagomir targeting \textit{Hif1a}, with the subsequent upregulation of \textit{Sox9} and other chondrocyte markers. The scaffold carrying hyperbranched polyplex-miR-10a mimic
(known as a T regulatory cell [Treg] marker) complexes, in combination with the Treg recruitment factors IL2 and TGFβ (2 mg of microspheres containing 0.8 nmol miR-10a mimic, 1 mg IL2, and 2 mg TGFβ protein), served as an injectable scaffold [101]. The nanofibrous scaffold was 60–90 nm in diameter, with multiple pores, and was designed to release IL2, TGFβ, and miR-10a mimic differentially for recruiting Tregs locally and promoting their differentiation effectively. miR-10a was expected to exert its effect on the PI3K-AKT-MTOR pathway by targeting IRS-1 and enhancing Treg differentiation. Since Tregs are a crucial regulator of the immune system in maintaining tolerance to self-antigens and suppressing the activities of other immune cells [102], nanofibrous microspheres carrying the active molecules as above were evaluated in a mouse model of periodontitis, a typical inflammatory disease that leads to gingival recession and alveolar bone defects [103]. Local injection of this assembly into the periodontal margin effectively inhibited the upregulation of gene expression of the primary cytokines of effector T cells in parallel with an increased ratio of Tregs/Th17 cells and concomitantly depressed osteoclastic bone resorption in the foci.

**Table 2.** miRNA mimic-related molecules incorporated into NFs.

| NF                  | miRNA          | Target Gene(s)         | Model/Application                                                                 | Ref    |
|---------------------|----------------|------------------------|-----------------------------------------------------------------------------------|--------|
| Crosslinked gelatin | TransIT-TKO®, Antagomir-29a complex | Tgfb1, Igf1             | Mouse MC3T3E1 cells, mouse MSCs in vitro                                            [98]   |
| PLC-gelatin NF      | Polypex-miR-22 and miR-26 mimic complex | RUNX2, SPARC, BGLAP     | Human iPS cells in vitro                                                            [11]   |
| PLL-PHN-NF-SMS      | HP-antagomir-199a complex                        | Hif1α                   | Rabbit MSCs in vitro, subcutaneous implantation in nude mice, implantation into an intervertebral disc degeneration rabbit model [100] |
| PLLA-NF-SMS         | HP-miR-10a mimic complex                        | Irs1                    | Mouse T cells, injection of complex plus TGFβ1 into a periodontitis mouse model     [101] |
| PLGA                | miR-2861 mimic                                       | RUNX2                   | Human iPS cells, in vitro                                                           [104] |
| PEG/PLGA            | mir-181a/b-1                                        | PTEN/PI3KK/AKT          | Adipose-derived mesenchymal stem cells, in vitro                                    [105] |
| HA-SS-PGEA          | mir-26a                                           | Runx2, Alpl, Bglap, and Bop | Rat MSCs, rat calvaria defect model                                                  [99]   |

Furthermore, human iPSCs were transduced with miR-2861 mimic, and then the osteogenic differentiation of cells was investigated when cultured on electrospun PLGA nanofibers. Surprisingly, the results indicated that osteogenic functions including RUNX2, ALPL, BGLAP, and SPARK in iPSCs improved when the transfected cells were cultured on the NFs and compared to the tissue culture plate. All these indicate the impact of NFs on osteogenic function [104]. Adipose-derived mesenchymal stem cells culturing on miR-181a/b-1 mimic incorporated with PLGA NFs compared with the same cells cultured on the tissue culture plate. The results revealed that adipose-derived mesenchymal stem cells cultured on NFs containing miR-181a/b-1 mimic expressed significantly more Runx2 and Spark than cells cultured on a tissue culture plate [105].

### 6. Principal Challenges

Several research studies have shown that nanofibrous scaffolds are useful in tissue engineering, particularly when combined with drugs and other bioactive molecules, including miRNAs, predicting their utility in many important clinical applications to come. [101]. Given our recent findings of selectively stored miRNAs in bone ECM, the development
of spatiotemporal-specific delivery platforms for miRNAs may benefit from an ability to harness the biomimetic properties of the skeletal tissue itself. Although further research will be required to address fundamental issues, such as ways to select the most advantageous miRNAs for skeletal tissue engineering. Considering a cocktail of multiple miRNAs, as discussed above [106], it may not only be the most advantageous, but also required for the desired outcome. To this end, an essential early step will be to develop a comprehensive miRNA picture of the bone microenvironment during regeneration. Furthermore, we believe that future approaches that incorporate miRNAs may use multifunctional nano scaffolds incorporating nanomaterials into miRNA-NF assemblies to control miRNA delivery more accurately and efficiently. Such a delivery system was the case when an NF platform coupling graphene oxide to gold nanoparticles carrying miR-101 had been used for the higher near-infrared thermal therapy of breast cancer. The prepared scaffold was induced cell death; not only by increasing the temperature inside cells, but also by the miR-101-dependent induction of apoptosis [107]. These biologically inspired nanomaterials may benefit patients with bone defects due to bone metastasis, for example.

Addressing all the issues that need to be considered, such as optimizing the release duration based on instability of miRNAs, improving the cellular uptake, and tissue-specific delivery to avoid systemic toxicity and off-target effect, delivery platforms, as well as NFs, will continue to be promising carriers for miRNAs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/mi12121472/s1, Table S1: The symbols and abbreviations.

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References
1. Maria, R.I.; Elisa, M.; Ilaria, B.; John, C.R.; Chiara, M.; Monica, M.; Simone, S.; Anna, T.; Mauro, T.; Fernanda, M. Adult Stem Cells for Bone Regeneration and Repair. Front. Cell Dev. Biol. 2019, 7, 268.
2. Huang, X.; Xiong, X.; Liu, J.; Zhao, Z.; Cen, X. MicroRNAs-containing extracellular vesicles in bone remodeling: An emerging frontier. Life Sci. 2020, 254, 117809. [CrossRef]
3. Sui, L.; Wang, M.; Han, Q.; Yu, L.; Zhang, L.; Zheng, L.; Lian, J.; Zhang, J.; Valverde, P.; Xu, Q.; et al. A novel Lipidoid-MicroRNA formulation promotes calvarial bone regeneration. Biomaterials 2018, 177, 88–97. [CrossRef] [PubMed]
4. Dexheimer, P.J.; Cochella, L. MicroRNAs: From mechanism to organism. Front. Cell Dev. Biol. 2020, 8, 409. [CrossRef] [PubMed]
5. Lee, S.W.L.; Paoletti, C.; Campisi, M.; Osaki, T.; Adriani, G.; Kamm, R.D.; Mattu, C.; Chiono, V. MicroRNA delivery through nanoparticles. J. Control. Release 2019, 313, 80–95. [CrossRef] [PubMed]
6. Lin, W.; Chen, M.; Qu, T.; Li, J.; Man, Y. Three-dimensional electrospun nanofibrous scaffolds for bone tissue engineering. J. Biomed. Mater. Res. Part B Appl. Biomater. 2020, 108, 1311–1321. [CrossRef]
7. Chi, H.; Chen, G.; He, Y.; Chen, G.; Tu, H.; Liu, X.; Yan, J.; Wang, X. 3D-HA Scaffold Functionalized by Extracellular Matrix of Stem Cells Promotes Bone Repair. Int. J. Nanomed. 2020, 15, 5825–5838. [CrossRef] [PubMed]
8. Rim, N.G.; Lee, J.H.; Jeong, S.I.; Lee, B.K.; Kim, C.H.; Shin, H. Modulation of Osteogenic Differentiation of Human Mesenchymal Stem Cells by Poly ([L-lactide]-co-(ε-caprolactone)]/Gelatin Nanofibers. Macromol. Biosci. 2009, 9, 795–804. [CrossRef]
9. Cleeton, C.; Keirouz, A.; Chen, X.; Radacsi, N. Electrospun Nanofibers for Drug Delivery and Biosensing. ACS Biomater. Sci. Eng. 2019, 5, 4183–4205. [CrossRef]
10. Dang, M.; Saunders, L.; Niu, X.; Fan, Y.; Ma, P.X. Biomimetic delivery of signals for bone tissue engineering. Bone Res. 2018, 6, 1–12. [CrossRef]

11. Tahmasebi, A.; Enderami, S.E.; Saburi, E.; Islami, M.; Yaslianifard, S.; Mahabadi, J.A.; Ardeshirylajimi, A.; Soleimanifar, F.; Moghadam, A.S. MicroRNA-incorporated electrospun nanofibers improve osteogenic differentiation of human-induced pluripotent stem cells. *J. Biomed. Mater. Res. Part A* 2020, 108, 377–386. [CrossRef]

12. Komori, T.; Yagi, H.; Nomura, S.; Yamaguchi, A.; Sasaki, K.; Deguchi, K.; Shimizu, Y.; Bronson, R.T.; Gao, Y.-H.; Inada, M. Targeted disruption of Cbfal results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 1997, 89, 755–764. [CrossRef]

13. Thompson, Z.; Miclau, T.; Hu, D.; Helms, J.A. A model for intramembranous ossification during fracture healing. *J. Orthop. Res.* 2002, 20, 1091–1098. [CrossRef]

14. Einhorn, T.A.; Gerstenfeld, L.C. Fracture healing: Mechanisms and interventions. *Nat. Rev. Rheumatol.* 2015, 11, 45. [CrossRef]

15. Waki, T.; Lee, S.Y.; Niikura, T.; Iwakura, T.; Dogaki, Y.; Okumachi, E.; Oe, K.; Kuroda, R.; Kurosaka, M. Profiling microRNA expression during fracture healing. *BMC Musculoskelet. Disord.* 2016, 17, 1–6. [CrossRef]

16. Liu, Y.; Wang, Y.; Cheng, X.; Zheng, Y.; Lyu, M.; Di, P.; Lin, Y. MiR-181d-5p regulates implant surface roughness-induced osteogenic differentiation of bone marrow stem cells. *Mater. Sci. Eng. C* 2021, 121, 111801. [CrossRef]

17. Arfat, Y.; Basra, M.A.R.; Shahzad, M.; Majeed, K.; Mahmood, N.; Munir, H. miR-208a-3p suppresses osteoblast differentiation and inhibits bone formation by targeting ACVR1. *Mol. Ther. Acids* 2018, 11, 323–336. [CrossRef]

18. Crane, J.L.; Xian, L.; Cao, X. Role of TGF-β Signaling in Coupling Bone Remodeling. In *Signaling*; Springer: New York, NY, USA, 2016; pp. 287–300.

19. Curtin, C.M.; Castaño, I.M.; O’Brien, F.J. Scaffold-Based microRNA Therapies in Regenerative Medicine and Cancer. *Adv. Healthc. Mater.* 2018, 7, 1700695. [CrossRef]

20. Leng, Q.; Chen, L.; Lv, Y. RNA-based scaffolds for bone regeneration: Application and mechanisms of mRNA, miRNA and siRNA. *Theranostics* 2020, 10, 3190. [CrossRef]

21. Vieira, A.E.; Repeke, C.E.; Ferreira Junior, S.D.B.; Colavite, P.M.; Biguetti, C.C.; Oliveira, R.C.; Assis, G.F.; Trombone, A.F.P.; Garlet, G.P. Intramembranous bone healing process subsequent to tooth extraction in mice: Micro-computed tomography, histomorphometric and molecular characterization. *PloS ONE* 2015, 10, e0128021. [CrossRef]

22. Coenen-Stass, A.M.L.; Pauwels, M.J.; Hanson, B.; Martin Perez, C.; Conceição, M.; Wood, M.J.A.; Mäger, I.; Roberts, T.C. Extracellular microRNAs exhibit sequence-dependent stability and cellular release kinetics. *RNA Biol.* 2019, 16, 696–706. [CrossRef]

23. Thompson, Z.; Miclau, T.; Hu, D.; Helms, J.A. A model for intramembranous ossification during fracture healing. *J. Orthop. Res.* 2002, 20, 1091–1098. [CrossRef]

24. Curtin, C.M.; Castaño, I.M.; O’Brien, F.J. Scaffold-Based microRNA Therapies in Regenerative Medicine and Cancer. *Adv. Healthc. Mater.* 2018, 7, 1700695. [CrossRef]

25. Wang, Z. The guideline of the design and validation of MiRNA mimics. *Methods Mol. Biol.* 2011, 676, 211–223.

26. Leng, Q.; Chen, L.; Lv, Y. RNA-based scaffolds for bone regeneration: Application and mechanisms of mRNA, miRNA and siRNA. *Theranostics* 2020, 10, 3190. [CrossRef]

27. Bakhshandeh, B.; Soleimani, M.; Hafizi, M.; Paylakhi, S.H.; Ghaemi, N. MicroRNA signature associated with osteogenic lineage commitment. *Mol. Biol. Rep.* 2012, 39, 759–7581. [CrossRef]

28. Egea, V.; Zahler, S.; Rieth, N.; Popp, T.; Kehe, K.; Jochum, M.; Ries, C. Tissue inhibitor of metalloproteinase-1 (TIMP-1) regulates mesenchymal stem cells through let-7f microRNA and Wnt/β-catenin signaling. *Proc. Natl. Acad. Sci. USA* 2012, 109, 309–316. [CrossRef]

29. Gao, J.; Yang, T.; Han, J.; Yan, K.; Qiu, X.; Zhou, Y.; Fan, Q.; Ma, B. MicroRNA expression during human multipotent mesenchymal stromal cells from bone marrow. *J. Cell. Biochem.* 2011, 112, 1844–1856. [CrossRef]

30. Zhang, J.; Fu, W.; He, M.; Xie, W.; Lv, Q.; Wan, G.; Li, G.; Wang, H.; Lu, G.; Hu, X. MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. *RNA Biol.* 2011, 8, 829–838. [CrossRef]

31. Wang, T.; Xu, Z. miR-27 promotes osteoblast differentiation by modulating Wnt signaling. *Biochem. Biophys. Res. Commun.* 2010, 402, 186–189. [CrossRef]

32. Li, Z.; Hassan, M.Q.; Jafferji, M.; Aqelain, R.I.; Garzon, R.; Croce, C.M.; van Wijnen, A.J.; Stein, J.L.; Stein, G.S.; Lian, J.B. Correction: Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. *J. Biol. Chem.* 2019, 294, 10018. [CrossRef]

33. Kapinas, K.; Kessler, C.; Ricks, T.; Gronowicz, G.; Delany, A.M. miR-29 modulates Wnt signaling in human osteoblasts through a positive feedback loop. *J. Biol. Chem.* 2015, 285, 25221–25231. [CrossRef]

34. Kapinas, K.; Kessler, C.B.; Delany, A.M. miR-29 suppression of osteoectin in osteoblasts: Regulation during differentiation and by canonical Wnt signaling. *J. Cell. Biochem.* 2009, 108, 216–224. [CrossRef]

35. Laine, S.K.; Alm, J.J.; Virtanen, S.P.; Aro, H.T.; Laitala-Leinonen, T.K. MicroRNAs miR-96, miR-124, and miR-199a regulate gene expression in human bone marrow-derived mesenchymal stem cells. *J. Cell. Biochem.* 2012, 113, 2687–2695. [CrossRef]

36. Hu, W.; Ye, Y.; Zhang, W.; Wang, J.; Chen, A.; Guo, F. miR-142-3p promotes osteoblast differentiation by modulating Wnt signaling. *Mol. Med. Rep.* 2013, 7, 689–693. [CrossRef]

37. Mizuno, Y.; Tokuzawa, Y.; Ninomiya, Y.; Yagi, K.; Yatsuka-Kanesaki, Y.; Suda, T.; Fukuda, T.; Katagiri, T.; Kondoh, Y.; Amemiya, T. miR-210 promotes osteoblast differentiation through inhibition of AcvR1b. *FEBS Lett.* 2009, 583, 2263–2268. [CrossRef]
38. Hassan, M.Q.; Maeda, Y.; Taipaleenmaki, H.; Zhang, W.; Jafferji, M.; Gordon, J.A.R.; Li, Z.; Croce, C.M.; Van Wijnen, A.J.; Stein, J.L. miR-218 directs a Wnt signaling circuit to promote differentiation of osteoblasts and osteomimicry of metastatic cancer cells. *J. Biol. Chem.* 2012, 287, 42084–42092. [CrossRef]

39. Zhang, J.; Tu, Q.; Bonewald, L.F.; He, X.; Stein, G.; Lian, J.; Chen, J. Effects of miR-335-5p in modulating osteogenic differentiation by specifically downregulating Wnt antagonist DKK1. *J. Bone Miner. Res.* 2011, 26, 1953–1963. [CrossRef]

40. Lisse, T.S.; Chun, R.F.; Rieger, S.; Adams, J.S.; Hewison, M. Vitamin D activation of functionally distinct regulatory miRNAs in primary human osteoblasts. *J. Bone Miner. Res.* 2013, 28, 1478–1488. [CrossRef]

41. Hu, R.; Liu, W.; Li, H.; Yang, L.; Chen, C.; Xia, Z.-Y.; Guo, L.-J.; Xie, H.; Zhou, H.-D.; Wu, X.-P. A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. *J. Biol. Chem.* 2011, 286, 12328–12339. [CrossRef]

42. Cañasto, I.M.; Curtin, C.M.; Duffy, G.P.; O’Brien, F.J. Next generation bone tissue engineering: Non-viral miR-133a inhibition using collagen-liposome mediated transfection and anti-proliferative effects of miR-101 in acute myeloid leukemia. *Sci. Rep.* 2016, 6, 1–10.

43. Mencía Cañasto, I.; Curtin, C.M.; Duffy, G.P.; O’Brien, F.J. Harnessing an inhibitory role of miR-16 in osteogenesis by human mesenchymal stem cells and advanced scaffold-based bone tissue engineering. *Tissue Eng. Part A* 2019, 25, 24–33. [CrossRef]

44. Balagangadharaan, K.; Chandran, S.V.; Arumugam, B.; Saravanan, S.; Venkatasubbu, G.D.; Selvamurugan, N. Chitosan/nano-hydroxyapatite/nano-zirconium dioxide scaffolds with miR-590-5p for bone regeneration. *Int. J. Macromol.* 2018, 111, 953–958. [CrossRef]

45. Shao, D.; Wang, C.; Sun, Y.; Cui, L. Effects of oral implants with miR-122-modified cell sheets on rat bone marrow mesenchymal stem cells. *Mol. Med. Rep.* 2017, 18, 1537–1540. [CrossRef]

46. Wang, X.; Guo, B.; Li, Q.; Peng, J.; Yang, Z.; Wang, A.; Li, D.; Hou, Z.; Lv, K.; Kan, G. miR-214 targets ATF4 to inhibit bone formation. *Nat. Med.* 2013, 19, 93–100. [CrossRef]

47. Murata, K.; Ito, H.; Yoshitomi, H.; Yamamoto, K.; Fukuda, A.; Yoshikawa, J.; Furu, M.; Ishikawa, M.; Shibuya, H.; Matsuda, S. Inhibition of miR-92a enhances fracture healing via promoting angiogenesis in a model of stabilized fracture in young mice. *J. Bone Miner. Res.* 2014, 29, 316–326. [CrossRef]

48. Deng, Y.; Bi, X.; Zhou, H.; You, Z.; Wang, Y.; Gu, P.; Fan, X. Repair of critical-sized bone defects with anti-miR-31-expressing bone marrow stromal stem cells and poly (glycerol sebacate) scaffolds. *Eur. Cell Mater.* 2014, 27, 13–25. [CrossRef]

49. Li, Y.; Fan, L.; Liu, S.; Liu, W.; Zhang, H.; Zhou, T.; Wu, D.; Yang, P.; Shen, L.; Chen, J. The promotion of bone regeneration through positive regulation of angiogenic–osteogenic coupling using microRNA-26a. *Biomaterials* 2013, 34, 5048–5058. [CrossRef]

50. Yan, J.; Zhang, C.; Zhao, Y.; Cao, C.; Wu, K.; Zhao, L.; Zhang, Y. Non-viral oligonucleotide antimir-138 delivery to mesenchymal stem cell sheets and the effect on osteogenesis. *Biomaterials* 2014, 35, 7734–7749. [CrossRef]

51. Qureshi, A.T.; Doyle, A.; Chen, C.; Coulon, D.; Dasa, V.; Del Piero, F.; Levi, B.; Monroe, W.T.; Gimble, J.M.; Hayes, D.J. Photoactivated miR-148b–nanoparticle conjugates improve closure of critical size mouse calvarial defects. *Acta Biomater.* 2015, 12, 166–173. [CrossRef]

52. Xue, Y.; Guo, Y.; Yu, M.; Wang, M.; Ma, P.X.; Lei, B. Monodispersed bioactive glass nanoclusters with exceptionally high miRNA loading for efficiently enhancing bone regeneration. *Adv. Healthc. Mater.* 2017, 6, 1700630. [CrossRef] [PubMed]

53. Liu, H.; Dong, Y.; Feng, X.; Li, L.; Jiao, Y.; Bai, S.; Feng, Z.; Yu, H.; Li, X.; Zhao, Y. miR-34a promotes bone regeneration in irradiated bone defects by enhancing osteoinduction of mesenchymal stromal cells in rats. *Stem Cell Res. Ther.* 2019, 10, 1–14. [CrossRef] [PubMed]

54. Yoshizuka, M.; Nakasa, T.; Kawanishi, Y.; Hachisuka, S.; Furuta, T.; Miyaki, S.; Adachi, N.; Ochi, M. Inhibition of microRNA-222 expression accelerates bone healing with enhancement of osteogenesis, chondrogenesis, and angiogenesis in a rat refractory fracture model. *J. Orthop. Sci.* 2016, 21, 852–858. [CrossRef] [PubMed]

55. Chang, C.-C.; Venø, M.T.; Chen, L.; Dittler, N.; Le, D.Q.S.; Dillschneider, P.; Kassem, M.; Kjems, J. Global microRNA profiling in human bone marrow stromal stem cells identified candidates for bone regeneration. *Mol. Ther.* 2018, 26, 593–605. [CrossRef]

56. Fortunato, O.; Iorio, M. V The therapeutic potential of MicroRNAs in cancer: Illusion or opportunity? *Pharmaceuticals* 2020, 13, 438. [CrossRef]

57. Cheng, Q.; Wei, T.; Farbiak, L.; Johnson, L.T.; Dilliard, S.A.; Siegwart, D.J. Selective organ targeting (SORT) nanoparticles for tissue-specific miRNA delivery and CRISPR–Cas gene editing. *Nat. Nanotechnol.* 2020, 15, 313–320. [CrossRef]

58. Di Mauro, V.; Iafisco, M.; Salvarani, N.; Vacchiano, M.; Croce, C.M.; Van Wijnen, A.J.; Stein, J.L. miR-218 directs a Wnt signaling circuit to promote differentiation of osteoblasts and osteomimicry of metastatic cancer cells. *J. Biol. Chem.* 2012, 287, 42084–42092. [CrossRef]

59. Yoshizuka, M.; Nakasa, T.; Kawanishi, Y.; Hachisuka, S.; Furuta, T.; Miyaki, S.; Adachi, N.; Ochi, M. Inhibition of microRNA-222 expression accelerates bone healing with enhancement of osteogenesis, chondrogenesis, and angiogenesis in a rat refractory fracture model. *J. Orthop. Sci.* 2016, 21, 852–858. [CrossRef] [PubMed]

60. Chang, C.-C.; Venø, M.T.; Chen, L.; Ditzler, N.; Le, D.Q.S.; Dillschneider, P.; Kassem, M.; Kjems, J. Global microRNA profiling in human bone marrow stromal stem cells identified candidates for bone regeneration. *Mol. Ther.* 2018, 26, 593–605. [CrossRef] [PubMed]

61. Ghosh, R.; Singh, L.C.; Shohet, J.M.; Gunaratne, P.H. A gold nanoparticle platform for the delivery of functional microRNAs into cancer cells. *Biomaterials* 2013, 34, 807–816. [CrossRef] [PubMed]

62. Morishita, Y.; Imai, T.; Yoshizawa, H.; Watanabe, M.; Ishibashi, K.; Muto, S.; Nagata, D. Delivery of microRNA-146a with polyethyleneimine nanoparticles inhibits renal fibrosis in vivo. *Int. J. Nanomed.* 2015, 10, 3475–3488. [CrossRef] [PubMed]

63. Ahmadzada, T.; Reid, G.; McKenzie, D.R. Fundamentals of siRNA and miRNA therapeutics and a review of targeted nanoparticle delivery systems in breast cancer. *Biophys. Rev.* 2018, 10, 69–86. [CrossRef] [PubMed]
63. Wu, Y.; Crawford, M.; Yu, B.; Mao, Y.; Nana-Sinkam, S.P.; Lee, L.J. MicroRNA delivery by cationic lipoplexes for lung cancer therapy. Mol. Pharm. 2011, 8, 1381–1389. [CrossRef]

64. Boussif, O.; Lezoualc'h, F.; Zanta, M.A.; Merghny, M.D.; Scherman, D.; Demeneix, B.; Behr, J.P. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: Polyethylenimine. Proc. Natl. Acad. Sci. USA 1995, 92, 7297. [CrossRef]

65. Szebeni, J.; Moghimi, S.M. Liposome triggering of innate immune responses: A perspective on benefits and adverse reactions: Biological recognition and interactions of liposomes. J. Liposome Res. 2009, 19, 85–90. [CrossRef]

66. Zatevskii, T.S.; Kotelevtsev, Y.V.; Koteliansky, V. Lipid nanoparticles for targeted siRNA delivery—going from bench to bedside. Int. J. Nanomed. 2016, 11, 3077.

67. Scheideler, M.; Vidakovic, I.; Prassl, R. Lipid nanocarriers for microRNA delivery. Chem. Phys. Lipids 2020, 226, 104837. [CrossRef]

68. Chang, M.; Lin, H.; Fu, H.; Wang, B.; Han, G.; Fan, M. MicroRNA-195-5p regulates osteogenic differentiation of periodontal ligament cells under mechanical loading. J. Cell. Physiol. 2017, 232, 3762–3774. [CrossRef]

69. Yang, H.; Qin, X.; Wang, H.; Zhao, X.; Liu, Y.; Wo, H.T.; Liu, C.; Nishiga, M.; Chen, H.; Ge, J.; et al. An in Vivo miRNA Delivery System for Restoring Infarcted Myocardium. ACS Nano 2019, 13, 9880–9894. [CrossRef]

70. Ekin, A.; Karatas, O.F.; Culha, M.; Ozen, M. Designing a gold nanoparticle-based nanocarrier for microRNA transfection into the prostate and breast cancer cells. J. Gene Med. 2014, 16, 331–335. [CrossRef]

71. Lin, Y.-S.; Abadeer, N.; Hurley, K.R.; Haynes, C.L. Ultrastable, dispersible, small, and highly organomodified mesoporous silica nanothearapeutics. J. Am. Chem. Soc. 2011, 133, 20444–20457. [CrossRef]

72. Zhang, Q.; Wang, X.; Li, P.; Nguyen, K.T.; Wang, X.; Luo, Z.; Zhang, H.; Tan, N.S.; Zhao, Y. Biocompatible, uniform, and dispersible mesoporous silica nanoparticles for cancer-targeted drug delivery in vivo. Adv. Funct. Mater. 2014, 24, 2450–2461. [CrossRef]

73. Bertucci, A.; Prasetyanto, E.A.; Zanta, M.A.; Manicardi, A.; Brognara, E.; Gambari, R.; Corradi, R.; De Cola, L. Combined Delivery of Temozolomide and Anti-miR-221 PNA Using Mesoporous Silica Nanoparticles Induces Apoptosis in Resistant Glioma Cells. Small 2015, 11, 5687–5695. [CrossRef]

74. Jung, H.; Kim, S.A.; Yang, Y.G.; Yoo, H.; Lim, S.-J.; Mok, H. Long chain microRNA conjugates in calcium phosphate nanoparticles for efficient formulation and delivery. Arch. Pharm. Res. 2015, 38, 705–715. [CrossRef]

75. Kharaghani, D.; Gitigard, P.; Ohtani, H.; Kim, K.O.; Ullah, S.; Saito, Y.; Khan, M.Q.; Kim, I.S. Design and characterization of dual systems. J. Biomed. Mater. Res. Off. J. Soc. Biomater. Jpn. Soc. Biomater. Aust. Soc. Biomater. Korean Soc. Biomater. 2015, 10, 12640. [CrossRef]

76. Pereira-da-Silva, T.; Cruz, M.C.; Carrusca, C.; Ferreira, R.C.; Napoleão, P.; Carmo, M.M. Circulating microRNA profiles in different arterial territories of stable atherosclerotic disease: A systematic review. Am. J. Cardiovasc. Dis. 2018, 8, 1.

77. Lee, H.; Nishino, M.; Sohn, D.; Lee, J.S.; Kim, I.S. Control of the morphology of cellulose acetate nanofibers via electrospinning. Cellulose 2018, 25, 2829–2837. [CrossRef]

78. Taylor, G.I. Disintegration of water drops in an electric field. Proc. R. Soc. Lond. Ser. A Math. Phys. Sci. 1964, 280, 383–397.

79. Howard, D.; Buttery, L.D.; Shakesheff, K.M.; Roberts, S.J. Tissue engineering: Strategies, stem cells and scaffolds. J. Anat. 2008, 213, 66–72. [CrossRef]

80. Hu, Y.; Grainger, D.W.; Winn, S.R.; Hollinger, J.O. Fabrication of poly (ε-hydroxy acid) foam scaffolds using multiple solvent systems. J. Biomed. Mater. Res. Off. J. Soc. Biomater. Jpn. Soc. Biomater. Aust. Soc. Biomater. Korean Soc. Biomater. 2002, 59, 563–572. [CrossRef] [PubMed]

81. Dong, H.P.; Vaquette, C.; Shahab, T.; Perez, R.A.; Yang, Y.; Dargaville, T.R.; Shafii, A.; Tran, P.A. Porous 3D Printed Scaffolds for Guided Bone Regeneration in a Rat Calvarial Defect Model. Appl. Mater. Today 2020, 20, 100706. [CrossRef]

82. Kim, B.S.; Park, K.E.; Kim, M.H.; You, H.K.; Lee, J.; Park, W.H. Effect of nanofiber content on bone regeneration of silk fibroin/poly (ε-caprolactone) nano/microfibrous composite scaffolds. Int. J. Nanomed. 2015, 10, 485.

83. Rogina, A. Electrospinning process: Versatile preparation method for biodegradable and natural polymers and biocomposite systems applied in tissue engineering and drug delivery. Appl. Surf. Sci. 2014, 296, 221–230. [CrossRef]

84. Wang, B.; Cai, Q.; Zhang, S.; Yang, X.; Deng, X. The effect of poly (l-lactic acid) nanofiber orientation on osteogenic responses of human osteoblast-like MG63 cells. J. Mech. Behav. Biomater. Mater. 2011, 4, 600–609. [CrossRef]

85. Maj, J.; He, X.; Jabbari, E. Osteogenic differentiation of marrow stromal cells on random and aligned electrospun Poly(l-lactic acid) nanofibers. Ann. Biomed. Eng. 2011, 39, 14–25. [CrossRef]

86. Izadpanahi, M.; Seyedi-Jafari, A.; Arefian, A.; Hamta, A.; Hosseinzadeh, S.; Kehtari, M.; Soleimani, M. Nanotopographical cues of electrospin PLLA efficiently modulate non-coding RNA network to osteogenic differentiation of mesenchymal stem cells during BMP signaling pathway. Mater. Sci. Eng. C 2018, 83, 96–100. [CrossRef]

87. Cristofaro, F.; Gigli, M.; Bloise, N.; Chen, H.; Bruni, G.; Munari, A.; Moroni, L.; Lotti, N.; Visai, L. Influence of the nanofiber chemistry and orientation of biodegradable poly (butylene succinate)-based scaffolds on osteoblast differentiation for bone tissue regeneration. Nanoscale 2018, 10, 8689–8703. [CrossRef]

88. Courtenay, I.C.; Filgueiras, J.G.; Deazavedo, E.R.; Jin, Y.; Edler, K.J.; Sharma, R.I.; Scott, J.L. Mechanically robust cationic cellulose nanofibril 3D scaffolds with tuneable biomimetic porosity for cell culture. J. Mater. Chem. B 2019, 7, 53–64. [CrossRef]

89. Boskey, A.L.; Robey, P.G. The Composition of Bone. In Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2018; pp. 84–92.

90. Gong, T.; Xie, J.; Liao, J.; Zhang, T.; Lin, S.; Lin, Y. Nanomaterials and bone regeneration. Bone Res. 2015, 3, 15029. [CrossRef]
91. Breeland, G.; Menezes, R.G. Embryology, Bone Ossification. In StatPearls [Internet]; StatPearls Publishing: Treasure Island, FL, USA, 2020.

92. Reznikov, N.; Bilton, M.; Lari, L.; Stevens, M.M.; Kröger, R. Fractal-like hierarchical organization of bone begins at the nanoscale. Science 2018, 360, eaao2189. [CrossRef]

93. Sartori, M.; Giavaresi, G.; Parrilli, A.; Ferrari, A.; Aldini, N.N.; Morra, M.; Cassinelli, C.; Bollati, D.; Fini, M. Collagen type I coating stimulates bone regeneration and osteointegration of titanium implants in the osteopenic rat. Int. Orthop. 2015, 39, 2041–2052. [CrossRef]

94. Milona, M.; Gough, J.E.; Edgar, A.J. Expression of alternatively spliced isoforms of human Sp7 in osteoblast-like cells. BMC Genomics 2003, 4, 43. [CrossRef] [PubMed]

95. Liu, T.M.; Lee, E.H. Transcriptional regulatory cascades in Runx2-dependent bone development. Tissue Eng. Part B. Rev. 2013, 19, 3. [CrossRef] [PubMed]

96. Komori, T. Regulation of bone development and extracellular matrix protein genes by RUNX2. Cell Tissue Res. 2010, 339, 189–195. [CrossRef]

97. Orimo, H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. J. Nippon Med. Sch. 2010, 77, 4–12. [CrossRef]

98. James, E.N.; Delany, A.M.; Nair, L.S. Post-transcriptional regulation in osteoblasts using localized delivery of miR-29a inhibitor from nanofibers to enhance extracellular matrix deposition. Acta Biomater. 2014, 10, 3571–3580. [CrossRef]

99. Li, R.; Wang, H.; John, J.V.; Song, H.; Teusink, M.J.; Xie, J. 3D Hybrid Nanofiber Aerogels Combining with Nanoparticles Made of a Bio-cleavable and Targeting Polycation and MiR-26a for Bone Repair. Adv. Funct. Mater. 2020, 30, 2005531. [CrossRef]

100. Feng, G.; Zhang, Z.; Dang, M.; Rambhia, K.J.; Ma, P.X. Nanofibrous spongy microspheres to deliver rabbit mesenchymal stem cells and anti-miR-199a to regenerate nucleus pulposus and prevent calcification. Biomaterials 2020, 256, 120123. [CrossRef]

101. Liu, Z.; Chen, X.; Zhang, Z.; Zhang, X.; Saunders, L.; Zhou, Y.; Ma, P.X. Nanofibrous Spongy Microspheres to Distinctly Release miRNA and Growth Factors to Enrich Regulatory T Cells and Rescue Periodontal Bone Loss. ACS Nano 2018, 12, 9785–9799. [CrossRef]

102. Sakaguchi, S.; Sakaguchi, N.; Shimizu, J.; Yamazaki, S.; Sakihama, T.; Itoh, M.; Kuniyasu, Y.; Nomura, T.; Toda, M.; Takahashi, T. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: Their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immuno. Rev. 2001, 182, 18–32. [CrossRef]

103. Kinane, D.F.; Stathopoulou, P.G.; Papapanou, P.N. Periodontal diseases. Nat. Rev. Dis. Prim. 2017, 3, 1–14. [CrossRef]

104. Abazari, M.F.; Zare Karizi, S.; Kohandani, M.; Nasiri, N.; Nejati, F.; Saburi, E.; Nikpoor, A.R.; Enderami, S.E.; Soleimanifar, F.; Mansouri, V. MicroRNA-2861 and nanofibrous scaffold synergistically promote human induced pluripotent stem cells osteogenic differentiation. Polym. Adv. Technol. 2020, 31, 2259–2269. [CrossRef]

105. Qi, P.; Niu, Y.; Wang, B. MicroRNA-181a/b-1-encapsulated PEG/PLGA nanofibrous scaffold promotes osteogenesis of human mesenchymal stem cells. J. Cell. Mol. Med. 2021, 25, 5744–5752. [CrossRef]

106. Xiao, J.; Low, W.C.; Milbreta, U.; Lu, Q.R.; Chew, S.Y. Nanofiber-mediated microRNA delivery to enhance differentiation and maturation of oligodendroglial precursor cells. J. Control. Release 2015, 208, 85–92. [CrossRef]

107. Assali, A.; Akhavan, O.; Adeli, M.; Razzazan, S.; Dinarvand, R.; Zanganeh, S.; Soleimani, M.; Dinarvand, M.; Atyabi, F. Multifunctional core-shell nanoplatforms (gold@graphene oxide) with mediated NIR thermal therapy to promote miRNA delivery. Nanomed. Nanotechnol. Biol. Med. 2018, 14, 1891–1903. [CrossRef]