Table 1. The application of miRNAs in bone tissue engineering *in vivo*

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| miRNA | Other bioactive factor | Transfection agent | Biomaterial scaffold | Cell type | Animal model | timepoint | Results | Reference |
| miR let-7d |  | Layered double hydroxide (LDH) nanoparticles |  Fibrin Gel | BMSCs | subcutaneous pockets on the backs of the athymic nude mice (Subcutaneous Ectopic Osteogenesis Model) | 2 weeks | Significant improvement of bone volume fraction (bone volume/total volume, BV/TV) | (Yang et al., 2021) |
| miR-10a | IL-2, TGF-β | PLLA/PEG co-functionalized MSN, PLGA MS | PLLA nanofibrous spongy microspheres (NF-SMS) | -- | mouse periodontal disease model | 10 days | substantially rescue the alveolar bone loss | (Liu et al., 2018b) |
| antimiR-138 | SDF-1α | chitosan/tripolyphosphate/hyaluronic acid/antimiRNA-138 nanoparticles (CTH/antimiR-138 NPs) | thermosensitive chitosan/β-glycerol phosphate (CS/GP) hydrogel | -- | 8 mm calvarial defect in rats | 8 weeks | higher regenerated bone (32.74 ± 4.89%), higher BMD of newly formed bone, greater Tb.N (0.90 ± 0.05) | (Wu et al., 2018) |
| miR-19b-3p |  | lentivirus | PLLA/POSS | BMSCs | 8 mm calvarial defect in rats | 3 months | almost complete repair of bone defects and higher bone mineral density | (Xiong et al., 2020) |
| miR-20a |  | poly(ethylene glycol) (PEG) hydrogels  |  | hMSCs  | 5 mm calvarial defect in rats | 12 weeks | significantly higher average bone volume fraction (24.51%), significantly higher trabecular number and lower trabecular separation | (Nguyen et al., 2018) |
| miR-21  |  | nanocapsules | O-carboxymethyl chitosan (CMCS) | -- | 2 mm diameter bilateral bone defects of the proximal tibia in rats with bilateral ovaries removal | 4 weeks | At 4 weeks: significantly higher BV/TV and Tb.Th, higher calcium nodule formation; At 8 weeks: less new cancellous bone, lower BV/TV, lower Tb.Th, the new cancellous bone had been absorbed and the marrow cavity had been dredged. | (Sun et al., 2020) |
| miR-21  |  | nanocapsules and O-carboxymethyl chitosan (CMCS) powder mixed gel | titanium cylinders | -- | New Zealand White rabbits | 3 months | increased content of mineral (Ca and P), plenty of nodules, a dense structure combined with collagenous fiber and apatite, similar to mature bone | (Geng et al., 2020) |
| miR-21  |  | lentivirus | β-TCP | rBMSCs | 5 mm calvarial defect in rats | 60 days | higher BMD and Tb.Th | (Yang et al., 2019) |
|  | lentivirus | β-TCP | BMSCs | 20 mm × 10 mm osteoperiosteal segmental defect in canine mandibular | 6 months | higher BV/TV, BMD, bone mineral content (BMC), improved percentage of new bone area (52.21 ± 3.87%), lower percentage of the β-TCP residual area (6.82 ± 1.43%) |
| miR-21  |  | N-(3-aminopropyl) methacrylamide, acrylamide and ethylene glycol dimethacrylate nanocapsules | titanium (Ti)-based SrHA/miR-21 composite coating | Osteoblast-like MG63 cells | 4 mm defeact at distal femur and tibia in New Zealand white rabbits | 3 months | significantly higher bone-implant contact, higher biomechanical strength (287 ± 25 N), highest υ1PO43−/amide I values (13.1±1.4) | (Geng et al., 2018) |
| miR-21 |  | nanocapsules | O-carboxymethyl chitosan (CMCS) network |  | 3 mm tibial plateau bone defect in rats | 8 weeks | significantly higher BV/TV, 2.4-fold bone formation | (Meng et al., 2016b) |
| miR-26a |  | injectable poly(ethylene glycol) (PEG) hydrogel | -- | hMSCs | 7 mm calvarial defect in rats | 8 weeks | statistically increase in BV/TV and bone surface density (bone surface/ bone volume, BS/BV) | (Gan et al., 2021) |
| miR-26a |  | a comb-shaped polycation (HA-SS-PGEA) consisting of Hyaluronic acid (HA), disulfide groups and ethanolamine (EA)-functionalized poly(glycidyl methacrylate) (PGMA)  | Three-dimensional (3D) hybrid nanofiber aerogels | BMSCs | 8 mm diameter cranial defect in rats | 4 weeks | Much larger defect healing area. (new bone volumes: 21.8 mm3, corresponding closure percentages: 62.2%, coverage: 56.4% | (Li et al., 2020) |
| antimiR-26a-5p  |  | lentivirus | biphasic calcium phosphate (BCP) | adipose-derived mesenchymal stem cells (ADSCs) | 4 mm-long ×2 mm-deep femoral defect in rats | 2\4\8 weeks | significantly higher BV/TV (8 weeks), higher BMD (2,4 weeks), higher Tb.N. (2,4 weeks), higher Tb.Th (2,4 weeks), lower residual bcp/TV | (Yuan et al., 2019) |
| miR-26a |  | lentivirus | β-TCP | mBMSCs | 5 mm calvarial defect in mice | 2 months | A marked increase in the volume of newly formed bones, which almost filled the whole defect area | (Liu et al., 2018a) |
| miR-26a  |  | siPORT NeoFX transfection agent | HyStem-HP™ hydrogel | mBMMSCs | ectopic bone formation model of immunocompromised mice | 8 weeks | significantly more bone formation and high density of blood vessels | (Li et al., 2013) |
|  |  | siPORT NeoFX transfection agent | HyStem-HP™ hydrogel | mBMMSCs | 5 mm calvarial defect in mice | 12 weeks | increasing vascular volume showed by immunofluorescence staining for CD31 |
| miR-29b-3p  | pTRE2-Tet-on plasmid | microbubble-ultrasound system | -- | -- | femoral fracture in mice | 6 weeks | significant reduction in callus area, higher BV/TV (including BVh/TV, BVl/TV, BV1 /TV) and BMD, enhanced stiffness and relative stiffness. | (Lee et al., 2016) |
| miR-29b |  |  O-carboxymethyl chitosan (CMCS) coating nanocapsules | titanium Alloy | -- | 3 mm tibial defect in rats | 8 weeks | significantly higher rate of calcification (2.80-fold), 24% increase in BIC, more new bone (~2.01-fold at 2 weeks) | (Meng et al., 2016a) |
| as-miR-31 |  | lentivirus | β-tricalcium phosphate (β-TCP) | rat ASCs | 5 mm calvarial defect in rats | 8 weeks | higher BMD (0.553 ± 0.081 g/ cm3), BV/TV (35.42±6.12%, ), new bone formation at 2 weeks: 4.58±0.51%, 4 weeks: 7.62±1.18%, 6 weeks: 8.11±0.89%, 8 weeks: 36.81±3.54%) | (Deng et al., 2013) |
| antimiR-31 |  | lentivirus | poly(glycerol sebacate) (PGS) | rat BMSCs | 8 mm calvarial defect in rats | 8 weeks | higher BV/TV (41.82±6.54 %), BMD (0.492±0.062 g/cm3), and percentage of new bone area (60.92±7.34 %) | (Deng et al., 2014) |
| miR-33a-5p |  | Lipofectamine 3000 | collagen-based hydrogels | hASCs | ectopic bone formation model of nude mice  | 8 weeks | more newly constructed bone, more collagen fiber bundles arranged compactly | (Shen et al., 2020) |
| miR-34a |  | Lipofectamine 2000 |  | rBMSCs | ectopic bone formation model of nude mice | 8 weeks | significantly higher percentage of bone area to total area (BA/TA) | (Liu et al., 2019a) |
|  | Lipofectamine 2000 | collagen-based hydrogel | -- | 3 mm tibial defect in rats | 8 weeks | signigicantly higher BV/TV |
|  miR-92b |  | lentivirus |  | MSC | ectopic bone formation model of nude mice  | 8 weeks | miR-92b was superior to GFP in ectopic bone formation by HE staining. | (Hou et al., 2021) |
| open femur fracture model of rats | 3 weeks | higher newly formed bone, higher volume of low-density bone/total tissue volume, higher percentage of bone in callus |
| miR-93-5p inhibitor |  | Lipofectamine 2000 | -- | rabbit BMSCs | Trauma-induced osteonecrosis of the femoral head (TIONFH) rabbit model | 8 weeks | significantly fewer empty lacunae and more osteoblasts | (Zhang et al., 2021b) |
| miR-106a Inhibitor |  | Liposome 2000 | autologous oxygen release nano bionic scaffold | rBMSCs | rat tibia fracture model | 6 weeks | markedly higher BMD, significantly promoted collagen II production | (Sun et al., 2018) |
| miR-129-5p |  | lentivirus | matrigel | BMSCs | 3 mm diameter defect on each side of the calvaria in mice | 8 weeks | much higher BV/TV (0.702±0.027), significantly higher BMD (1296±53g/cm3), more bone-like structures and collagen deposits | (Zhao et al., 2021) |
| miR-133a |  | aspartate, serine, serine (AspSerSer)6-liposome | -- | osteoblast | hindlimb unloading (HU)-challenged mice | 3 weeks | less bone loss and osteoclast numbers, enhanced BMD, BV/TV, Tb.Th and Tb.N, lower Tb.Sp, trabecular bone pattern factor (TbPF) and BS/BV | (Zhou et al., 2021) |
| antagomiR-133a |  | collagen-hydroxyapatite (coll-HA) | coll-nHA | -- | 7 mm calvarial defect in rats | 4 weeks | statistically more calcified tissue (8.71 ± 7.48%), statistically more and thicker new trabeculae, statistically more de novo bone (≥70% increase) | (Castaño et al., 2020) |
| antagomiR-133a/b |  | CTH nanoparticle (chitosan solution (CS), Sodium tripolyphosphate (TPP), hyaluronic acid (HA)) | -- |  | 5 mm calvarial defect in mice | 12 weeks | significantly increased new bone area  | (Jiang et al., 2020) |
|  miR-135  |  | lentivirus | PSeD | rat ADSCs | 8 mm calvarial defect in rats | 8 weeks | significantly higher BV/TV (50.53±4.45%), BMD (0.0165±0.0012 g/cc) and Tb.N (0.3352±0.0529), larger newly formed bone (820.4±77.3 mm2), higher percentage of newly formed bone in the total area of bone tissue (40.13± 1.94%), larger area of fluorochrome stained bone | (Xie et al., 2016) |
| miR-142-5p |  | periosteal injection at the fracture site | -- | -- | femoral fracture in mice | 4 weeks | significantly higher BMD | (Tu et al., 2017) |
| miR-146a inhibitor |  | lentivirus | poly(sebacoyl diglyceride) (PSeD) porous scaffold | rat ADSCs | 8 mm calvarial defect in rats | 8 weeks | significantly higher BV/TV, Tb.N and BMD (49.8 ± 5.49%, 0.4094 ± 0.0687, 0.01581 ± 0.00299 g/cc), larger nwe bone areas in weeks 2~4: 92.38 ± 16.69 mm2, weeks 4~6: 115.32 ± 11.87 mm2, and weeks 6~8: 90.93 ± 9.95 mm2 | (Xie et al., 2017) |
| miR-148a-3p |  | lentiviruses | -- | BMSCs | ovariectomy (OVX)-induced osteoporosis model in mice | 6 weeks | highr BMD, BV*/*TV ratio, Tb.N and Tb.Th, lower trabecular spacing (Tb.Sp) | (Liu and Sun, 2021) |
| miR-148b  |  | (hydroxypropyl) cellulose (HPC)-modified silver nanoparticles (SNPs) | collagen-infilled 3D printed hybrid | rBMSCs | 5 mm in diameter and 1 mm in thickness calvarial defect in rats | 8 weeks | significantly higher BV/TV (%), higher normalized BMD (34.7 ± 8.9%), larger bone coverage area (78.1 ± 20.8%), higher connectivity density (2.86 ± 1.23) | (Moncal et al., 2019) |
| miR-148b  |  BMP-2 | baculovirus | poly (L-*lactide*-co-*glycolide*) (PLGA) | hASCs | 4 mm calvarial defect in nude mice | 12 weeks | the new bone nearly filled the entire defect after 12W, higher bone area (94.7 ± 0.8%), volume (89.4 ± 11.1%) and density (95.7 ± 3.9%) | (Liao et al., 2014) |
| miR-187 |  | lentivirus |  | hMSCs | osteoporosis (OP) mouse model | 28 days | significantly reverse the decreased bone healing rate | (Zhang et al., 2021a) |
| agomiR-199a-5p |  | chitosan nanoparticles | hydroxyapatite/collagen (HA/collagen) | hMSC | ectopic bone formation model of NOD/SCID mice | 6 weeks | higher density, darker MSCs and more collagen deposition in Masson trichrome staining for collagen | (Chen et al., 2015) |
|  | chitosan nanoparticles | fibrin gel | -- | 3 mm tibial defect in rats | 8 weeks | higher density, more regenerated bone in the center of the repaired area |
| miR-200c |  | pDNA | 3D-printed β-tricalcium phosphate (β-TCP) with collagen coatings | BMSCs | 9 mm diameter parietal defect in rats | 4 weeks | statistically increase in bone formation | (Remy et al., 2021) |
| miR-205 |  | lentivirus |  | endothelial colony-forming cells (ECFCs) | mandibular distraction osteogenesis (MDO) canine model | 4 weeks | the distraction gap was fully bridged | (Jiang et al., 2021) |
| miR-210 | simvastatin (Siv) | dual-sized pore structure calcium-silicon nanospheres (DPNPs) | β-TCP | -- | 5 mm calvarial defect in mice | 8 weeks | the highest bone density and bone volume, strong positive expression of CD31 (the platelet endothelial cell adhesion molecule-1) | (Liu et al., 2021) |
| miR-222 | aspirin  | mesoporous silica nanoparticles (MSN) | injectable colloidal hydrogel | -- | 5 mm mandibular defect in rats | 10 weeks | higher BV/TV% (21.97% ± 3.99%), significant increased neurogenic proteins expression | (Lei et al., 2019) |
| antimiR-222 |  | Lipofectamine 2000 | HA/tricalcium phosphate (HA/TCP) | hBMSCs | ectopic bone formation model of non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice | 8 weeks | statistically higher quantified bone volume (% bone/total area) | (Chang et al., 2018) |
| miR-335-5p |  | lipidoid nanoparticles | empty silk scaffold (SS)  | mBMSCs | 4 mm calvarial defect in mice | 5 weeks | significantly higher BV/TV | (Sui et al., 2018) |
| miR-335-5p |  | tetrahedral DNA nanostructures (TDNs) | Heparin lithium hydrogel (Li-hep-gel) | BMSCs | 3 mm femoral defect in rabbits | 12 weeks | 70% new bone area, new blood vessels 12n, empty lacunae 22% ± 6% | (Li et al., 2022) |
| antimiR-467g |  | Lipofectamine RNAi MAX | -- | mouse calvarial osteoblast (MCO) cells | 0.8mm femoral defect in mice | 21 days | significantly higher BV/TV, Tb.Th and Tb.N | (Kureel et al., 2017) |
| miR-672 |  | lentivirus | CPC | ADSCs | 5 mm critical size skull defect in rats | 8 weeks | highest blood vessel volume and number (3.56 ± 6.46 mm3, 15.43 ± 7.67 mm−2), enhanced BMD(0.78 g ± 4.28 cm-3), BV/TV (17.83±8.42%), significantly higher new bone area and new bone area/total area  | (Chen et al., 2021) |
| miR-2861 |  | sticky-end tetrahedral framework nucleic acids (stFNAs) |  | BMSCs | 1 mm spherical femoral defect in mice | 2 weeks | The surface defect had almost completely healed after 2 weeks. | (Li et al., 2021) |
| miR-5106 |  | novel monodispersed bioactive glass nanoclusters (BGNCs) with PEI | hydrogel | BMSCs | 5 mm calvarial defect in rats | 4 weeks | significantly high new bone volume and trabecular thickness | (Xue et al., 2017) |

Table 2. The mechanism of miRNAs tested *in vivo* regulating osteogenic differentiation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRNAs | pathway | related signaling pathways | cell types | Reference |
| miR-1-3p | SOX9 pathway | miR-1-3p targets and decreases Sox9 transcription factor activity. Sox9 negatively regulates Runx2 and type X collagen expression to modulate endochondral ossification-related disorders.(Ding et al., 2021) | BMSCs | (Ding et al., 2021) |
| miR-10a | TGF-β pathway | Interleukin 2 (IL-2) and transforming growth factor beta (TGF-β) are cytokines known to enhance Treg recruitment, proliferation, and differentiation. miR-10a can facilitate naïve T cells to differentiate to Tregs. The higher number and possibly more mature Tregs substantially suppressed the destructive osteoclastogenesis and enhanced the osteoblastic activity, synergistically rescuing periodontal bone loss. | mice T cells | (Liu et al., 2018b) |
| miR-let-7d | BMP pathway | miRNA-let-7d targets the 3′-UTR of HMGA2, resulting in the suppression of the expression of GSK3β protein,positively regulating osteogenic differentiation and negatively regulates adipogenic differentiation of hADSCs | human adipose-derived mesenchymal stem cells (hADSCs) | (Wei et al., 2014) |
| BMSCs | (Yang et al., 2021) |
| miR-19b-3p | BMP pathway | miR-19b-3p could bind to the 3′UTR of Smurf1, suppressing the expression of Smurf1 which is a negative regulator of osteogenesis. Smurf1 could mediate Runx2 degradation to inhibit osteoblast differentiation and bone formation. Smurf1 can also mediate the degradation of Smad1/5 which is the down-stream factor of BMP signal channel, resulting in the suppression of the osteoblast differentiation. | BMSCs | (Xiong et al., 2020) |
| miR-20a | BMP pathway | miRNA-20a has a positive effect on hMSC osteogenic differentiation by inhibiting the expression of PPAR-γ, a down regulator of BMP signaling in osteogenesis. | hMSCs | (Nguyen et al., 2018) |
| miR-21  | PI3K-AKT signaling pathway | miR-21 directly targets and inhibits PTEN by binding its 3′-UTR, thus leading to the activation of AKT and HIF-1α. The PI3K-AKT signaling pathway activity has an increasing tendency responding to miR-21 up-regulation. This enhancement promotes the phosphorylation of GSK-3β, leading to the stabilization and high concentration accumulation of β-catenin in cytoplasm to activate the transcription of RUNX-2, and finally increases the osteogenesis of hUMSCs. | hUMSCs | (Meng et al., 2015) (Yang et al., 2019) |
| miR-26a | BMP pathway | miR-26a interacts with the 3′-UTR of the Smad1 mRNA, diminishing the availability of the active SMAD1 transcription factor to participate in the differentiation process of hADSCs and elevating the mRNA and protein expression levels of Runx2. SMAD1 is the downstream effector of BMP signaling, and it is phosphorylated by BMP type I receptors. | hADSCs | (Gan et al., 2021) (Liu et al., 2018a) |
| Wnt/β-catenin pathway | miRNA-26a targets the 3′-UTR of GSK3 β to activate Wnt signaling for promoting osteogenic differentiation of BMSCs by inhibiting the expression of GSK3β and increasing the level of active β-catenin. | BMSCs | (Su et al., 2015) |
| miR-26a-5p inhibits the translation of Wnt5a by directly binding to the 3'-UTR of Wnt5a. WNT5A is a noncanonical Wnt ligand and activates two noncanonical Wnt pathways, one of which is the Wnt/Ca2+ signaling pathway.(Yuan et al., 2019) | ADSCs | (Yuan et al., 2019) |
| miR-29b  | M-CSF and RANK-L signaling pathways | miR-29b targets C-FOS and MMP2 within osteoclasts (OCLs). In OCL precursors, M-CSF promotes RANK expression through C-FOS and sustains survival and cytoskeletal reorganization. RANK controls NFKB activation, which in turn leads to upregulated expression of NFATc-1, the master transcription factor for OCL generation and function. MMP2 belongs to the gelatinase protein family and participates to bone matrix degradation. | osteoclast (OCL) | (Rossi et al., 2013) |
| miR-31 | BMP pathway | miR-31 typically binds to the mRNA and targets and inhibits the translation of the master transcription factor special AT-rich sequence-binding protein 2 (Satb2). SATB2 interacts with and enhances the transcriptional activity of Runx2 and activating transcription factor 4 (ATF4)(Yan et al., 2011). As-miR-31 promotes bone regeneration and bone defect repair. | BMSCs | (Deng et al., 2014) |
| miR-33a-5p | circFOXP1/miR-33a-5p/FOXP1 pathway | miR-33a-5p inhibits osteogenesis by targeting forkhead box P1 (FOXP1) 3'-UTR and down-regulating FOXP1 expression(Shen et al., 2020). FOXP1 regulates cell-fate choice of MSCs through interactions with the CEBPβ/δ complex and recombination signal binding protein for immunoglobulin κ J region (RBPjκ), key modulators of adipogenesis and osteogenesis, respectively(Li et al., 2017a). | hASCs | (Li et al., 2017a; Shen et al., 2020) |
| miR-34a | Notch signaling pathway | miR-34a directly targets Notch1, improving the osteogenic differentiation of irradiated BMSCs by suppressing NOTCH1, since downregulation of NOTCH1 enhanced the mRNA and protein expression of RUNX2 and OCN. | BMSCs | (Liu et al., 2019a) |
| mir-92b | ERK and JNK signaling pathways | Ezh2 is a potential target of mir-92b and down-regulated by it. Ezh2 is the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2) and catalyzes tri-methylation of histone H3 at lysine 27 (H3K27me3) to silence target genes. And extracellular signal-regulated kinases (ERK) and c-Jun N-terminal protein kinase (JNK) signaling pathways were activated by mir-92b, which could finally lead to the enhanced osteogenesis of MSCs.  | MSCs | (Hou et al., 2021) |
| miR-93-5p | BMP-2/Smad5 pathway | miR-93-5p suppresses osteogenic differentiation of BMSCs by binding the 3′-UTR of Smad5 and reducing BMP-2 and RUNX2. | BMSCs | (Zhang et al., 2021b) |
| miR-106a | BMP-2/Smad5 pathway | miR-106b-5p regulates Smad5 expression negatively, and they functioned as a inhibitory factor in the physiological process of bone formation and osteoblast differentiation. Smad5 is a downstream transcription factor phosphorylated and activated by of BMP-2 receptors which is a key signaling component in osteoblast differentiation, a member of TGF-β superfamily. The phosphorylated Smad5 forms a complex with Smad4 (Co-Smad), then translocates into the nucleus to activate transcription factor Cbfa1/Runx2 | BMSCs | (Fang et al., 2016) |
| miR-129-5p | Wnt/β-catenin pathway | miR-129-5p targets the 3′-UTR of Dickkopf3 (Dkk3) and repress it to enhance osteoblast differentiation. Dkk3 could bind to β-catenin, mediating Wnt signaling pathway. | BMSCs | (Zhao et al., 2021) |
| miR-133 | BMP pathway | MiR-133 directly regulates the 3′UTR of distal-less homeobox 3 (Dlx3), a member of the Dlx family of homeobox proteins. It is a transcriptional activator of runt-related transcription factor 2 (Runx2) during osteogenic differentiation. Mir-133a inhibits Dlx3 expression via direct targeting of the Dlx3 3'-UTR. | MSCs | (Qadir et al., 2018) |
| miR-133 inhibits the bone formation by targeting the the 3′-UTR of RUNX2 and decreasing the expression level of RUNX2(Peng et al., 2018; Jiang et al., 2020). | osteoblasts | (Zhou et al., 2021) |
| miR-135  | Hoxa2/Runx2 pathway | miR-135 negatively regulates Hoxa2 expression by targeting the 3′-UTR of Hoxa2. And Hoxa2 negatively regulates Runx2 expression in ADSCs. The overexpression of miR-135 enhances the expression of bone markers and extracellular matrix calcium deposition | ADSCs | (Xie et al., 2016) |
| antimiR-138 | ERK1/2 pathway | The antimiR-138 delivery down-regulates the endogenous miR-138 levels in BMSC sheets, activates the the extracellular signal regulated kinases 1/2 (ERK1/2) pathway and enhances the expression of RUNX2 finally leading to enhanced osteogenesis. | BMSCs | (Yan et al., 2014) |
| miR-142-5p | Ubiquitination pathway | miR-142-5p promotes osteoblast activity and matrix mineralization by targeting the gene encoding WW-domain-containing E3 ubiquitin protein ligase 1. And miR-142-5p stimulates osteocalcin and Runx2 expression by targeting Wwp1. Agomir-142-5p in the fracture areas stimulates osteoblast activity. | preosteoblast cells | (Tu et al., 2017) |
| miR-146a | BMP pathway | miR-146a exertes its repressive effect on Drosophila mothers against decapentaplegic protein 4 (SMAD4) through interacting with 3′-untranslated region (3′-UTR) of SMAD4 mRNA which is an important co-activator in the BMP signaling pathway. | ADSCs | (Xie et al., 2017) |
| miR-148a-3p | Nrf2 pathway | miR-148a-3p negatively regulates p300 expression in osteoblasts by binding to the 3-UTR of p300 mRNA, which could inactivate the Nrf2 pathway, consequently down regulating RUNX2\ALP activity, and blunting osteoblast differentiation and subsequent bone reconstruction, ultimately leading to osteoporosis. | osteoblastics | (Wang et al., 2013) (Liu and Sun, 2021) |
| miR-148b  | BMP pathway | miR-148b directly targets *NOG*, whose gene product (noggin) is an antagonist to BMPs and negatively regulates BMP-induced osteogenic differentiation and bone formation. | rBMSCs | (Li et al., 2017b) |
| miR-187 | BMP pathway | miR-187 downregulates human BarH-like homeobox 2 (BARX2) through targeted regulation, inducing osteogenic differentiation of hMSCs. (33550149) Barx2 regulates the expression of several genes encoding cell-adhesion molecules and extracellular matrix proteins, including NCAM and collagen II (Col2a1) in the limb bud. Two members of the BMP family that are crucial for chondrogenesis, GDF5 and BMP4, regulate the pattern of Barx2 expression in developing limbs. Barx2 acts downstream of BMP signaling and in concert with Sox proteins to regulate chondrogenesis(Meech et al., 2005). | hMSCs | (Zhang et al., 2021a) |
| miR-199a-5p | HIF1α-Twist1 pathway | At early stage of differentiation, hypoxia induces HIF1a-Twist1 pathway to enhance osteogenesis by up-regulating miR-199a-5p, while at late stage of differentiation, miR-199a-5p enhances osteogenesis maturation by inhibiting HIF1α-Twist1 pathway. And Runx2 might be negatively regulated by HIF1α, which is the direct target of miR-199a-5p. | hMSCs | (Chen et al., 2015) |
| miR-200c | Wnt/β-catenin pathway | miR-200c overexpression is shown to downregulate SRY (sex detg. region Y)-box 2 (Sox2) and Kruppel-like factor 4 by directly targeting 3'-untranslated regions and upregulate the activity of Wnt signaling inhibited by Sox2. | hBMSCs | (Akkouch et al., 2019) |
| BMP pathway | miR-200c effectively inhibits Noggin, an antagonist of BMP signals, by directly targeting the 3’UTR of Noggin.  | human embryonic palatal mesenchyme (HEPM) | (Hong et al., 2016) |
| miR-205 | Notch signaling pathway | miR-205 targets the 3′-untranslated region (UTR) of cfa-NOTCH2, which is a unique transcription regulator in bone angiogenesis. Inhibit miR-205 increases NOTCH2 expression, resulting in the elevated secretion of VEGF proteins and thereby stimulating angiogenesis and osteogenesis within the skeletal system. | endothelial colony-forming cells (ECFCs) | (Jiang et al., 2021) |
| miR-214 | BMP pathway | miR-214 targets the the 3′-UTR of the transcription factor ATF4 to inhibit bone formation. There is a runt‑related transcription factor 2 (Runx2) binding site in Atf4 promoter(Wang et al., 2013). | preosteoblast cells (MC3T3-E1 cells) | (Wang et al., 2021) |
| Pten/PI3k/Akt pathway | miR-214 targets the the 3′-UTR of phosphatase and tensin homolog (Pten). It has been demonstrated that Pten regulates RANKL-induced osteoclast differentiation from RAW 264.7 osteoclast precursors through PI3K/Akt pathway. | Osteoclasts | (Zhao et al., 2015) |
| miR-222 | Wnt/β-catenin pathway | miR-222 promotes neural differentiation of hBMSCs *in vitro* by targeting Nemo-like kinase (NLK) and decreasing NLK protein level. NLK is an inhibitor of Wnt/β-catenin signaling, which plays as a vital role in neuronal differentiation. | hBMSCs | (Lei et al., 2019) |
| TGFβ pathway | Anti-miR-222 enhances *in vivo* ectopic bone formation through targeting to the 3′UTR of cyclin-dependent kinase inhibitor 1B (CDKN1B), a cell-cycle inhibitor(Chang et al., 2018). CDKN1B regulate osteoblast differentiation through cell-cycle arrest, and cell-cycle arrest is a prerequisite for differentiation. | hMSCs | (Chang et al., 2018) |
| STAT5A signaling pathway | MiR-222 was found to negatively modulate angiogenesis by targeting the c-Kit receptor (Mazziotta et al., 2021) together with the signal transducer and activator of transcription 5A (STAT5A) (Zhang et al., 2021b). The c-Kit receptor is the receptor for the angiogenic activity of stem cell factor (SFC), and is expressed on the surface of ECs (Mazziotta et al., 2021). STAT5A activates bFGF and IL-3, which in turn trigger vascular EC morphogenesis in the STAT5A signaling pathway (Zhang et al., 2021b). The present study demonstrated that although the target genes of miR-222 related to angiogenesis were not validated, down-regulation of the c-kit receptor and STAT5A by the miR-222 inhibitor might contribute to the enhanced neovascularization at the fracture site. | hMSCs | (Yoshizuka et al., 2016) |
| miR-335-5p | Wnt/β-catenin pathway | MiR335-5p may inhibit Wnt antagonist Dickkopf-1 (DKK1) expression and upregulate the Wnt pathway, promoting osteogenesis and angiogenesis as well as enhancing bone regeneration in steroid-associated osteonecrosis (SAON). | BMSCs | (Li et al., 2022) (Sui et al., 2018) |
| miR-467g | Ihh/Runx-2 signaling pathway | miR-467g targets the 3′-UTR of Runx-2, and down regulates Runx-2, inhibiting osteoblast differentiation. | osteoblast | (Kureel et al., 2017) |
| miR-590-5p | BMP pathway | The 3'-untranslated region of Smad7 was directly targeted by miR-590-5p. Smad7 inhibits osteoblast differentiation via Smurf2-mediated Runx2 degradation.miR-590-5p promotes osteoblast differentiation by indirectly protecting and stabilizing the Runx2 protein by targeting Smad7 gene expression(Vishal et al., 2017). | mMSCs | (Brenner et al., 2021) |
| Wnt/β-catenin pathway | miR-590-3p binds to 3'UTR of APC mRNA. miR-590-3p can promote osteogenic differentiation via suppressing APC expression and stabilizing β-catenin. | hMSCs | (Wu et al., 2016b) |
| miR-672 | TGFβ pathway | miR-672 negatively regulated the expression of TIMP2 by interacting with 3′-UTR of TIMP2 mRNA, regulating ADSCs angiogenesis *in vitro*. TIMP2, a member of the TIMP family, regulates the proteolytic activity of matrix metalloproteinases (MMPs), a group of proteolytic enzymes, and maintain the balance between extracellular matrix (ECM) breakdown and synthesis. | ADSCs | (Chen et al., 2021) |
| miR-2861 | BMP pathway | MiR-2861 bound to the amino acid coding sequences (CDSs) of histone deacetylase 5 (HDAC5) mRNA with complementarity to the miR-2861 seed region, inhibiting the expression of HDAC5 protein at the translational level, thereby upregulating the expression of the runt-related transcription factor 2 (Runx2) protein and ultimately promoting the osteogenic differentiation of BMSCs. | BMSCs | (Li et al., 2021) |
| miR-5106 | Wnt/β-catenin pathway | miR-5106 targets and increases Sox9 transcription factor activity(Xue et al., 2017). Sox9 negatively regulates Runx2 and type X collagen expression to modulate endochondral ossification-related disorders(Ding et al., 2021). | BMSCs | (Xue et al., 2017) |

Table 3. The information of miRNA transfection vectors applied to bone tissue engineering

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Types of vector | Transfection agent | Loading methods and loading efficiency | Unloading | Stability | Cells | Cellular uptake | Cytotoxicity | miRNAs | Reference |
| Nucleic acids | sticky-end tetrahedral framework nucleic acids (stFNAs) | complementary base pairing | RNase H cuts | degradation in >0.8 U mL−1 RNase A, still existed at 35% FBS | BMSCs | the BMSCs had adsorbed a large amount of stFNA after 12 h | decreased cell viability when concentration >300 nm, altered cell viability only when carrying 2000 nm miR | miR-2861 | (Li et al., 2021) |
| tetrahedral DNA nanostructures (TDNs) | complementary base pairing | -- | -- | BMSCs | ≈37.6% of BMSCs absorbed MiR@TDNs | -- | miR-335-5p | (Li et al., 2022) |
| Calcium phosphates | collagen-hydroxyapatite (coll-HA) |  |  | extending its half-life | rMSCs | 55.4 ± 9.76% and 10.8 ± 6.37% at 3 and 7 days respectively |  | antagomiR-133a | (Castaño et al., 2020) (Mencía Castaño et al., 2016) |
| nanohydroxyapatite (nHA) particles | electrostatic interaction (Bose and Tarafder, 2012) | CaPs dissoluted in the acidic environment of the endocytic vesicle (Bose and Tarafder, 2012) |  | hMSCs | precipitates at the cell surface and undergo endocytosis, 33.5 ± 1.5% and 39.6 ± 4.7% respectively for the 10 and 20 nM doses Dy547 nanoantagomiR by day 3 (Bose and Tarafder, 2012; Mencía Castaño et al., 2015) | no cytotoxic effects | antagomiR-16 | (Mencía Castaño et al., 2019) |
| Nano systems | Layered double hydroxide (LDH) nanoparticles | electrostatic interactions with mild orbital agitation | proton sponge effect | protective effect of LDH against serum degradation | BMSCs | clathrin-mediated endocytosis, the buffering capacity facilitates endosomal escape, internalization was increased in 24 h | did not remarkably affect cell proliferation at any concentration tested | miRNA let-7d | (Yang et al., 2021) |
| mesoporous silica nanoparticles (MSN) | miR222 filled the pores of MSNs, optimal loading capacity: around 6.6 wt% (MSN : miR222 = 15 : 1) | disulfide bonds will break up by glutathione (GSH), 15% released on the first day, and 80% at the end of 35 days | disulfide bonds and amino groups to stabilize miR222, release <3% after 3 days in phosphate-buffered saline (PBS) | hBMSCs | endocytos | non-toxic | miR-222 | (Lei et al., 2019) |
| CTH nanoparticle (chitosan solution (CS), Sodium tripolyphosphate (TPP), hyaluronic acid (HA)) | The loading efficiency was over 90% when the N/P ratio was 15:1. | 40-50% at 21days |  | murine BMSCs | reached to the greatest transfection efficiency with 2 mM CTH-antagomiR-133a/b. | did not impair BMSCs proliferation and exhibited no cytotoxicity in BMSCs | antagomiR-133a/b | (Jiang et al., 2020) |
| (hydroxypropyl) cellulose (HPC)-modified silver nanoparticles (SNPs) | a nitrobenzyl photocleavable linker between the 3′ terminal and miR-148b sequence, NP ratio: 5000:1 | 405 nm irradiation |  | rBMSCs | 56.1 ± 2.2% of cells contained SNP-miR148b-TAMRA 12 h post-transfection, just before illumination. After photo-activation, the percentage decreased. | no any major inhibition on the proliferation of rBMSCs | miR-148b  | (Moncal et al., 2019) |
| dual-sized pore structure calcium-silicon nanospheres (DPNPs) | coordination bond formatio between calcium ions in the DPNPs and phosphate in miR-210, miR-210 could be adsorbed on the surface of the mesoporous structure of DPNPs | reaches maximum releasing amount after 4 days | the electrophoretic bands can be detected even after 10 hours | mBMSCs | 79.20% | no any mass cell death | miR-210 | (Liu et al., 2021) |
| PLLA/polyethylene glycol (PEG) co-functionalized mesoporous MSN， poly(lactic acid- co-glycolic acid) microspheres (PLGA MS) | electrostatic interactions between an amino-functionalized multi-armed cationic polymer and miR | 90% by day 50 at 260 nm |  | mice T cells | the cationic polymer can bind and transfer the miRNA into T cells | no inhibitory effect on T cells | miR-10a | (Liu et al., 2018b) |
| chitosan/tripolyphosphate/hyaluronic acid/antimiRNA-138 nanoparticles (CTH/antimiR-138 NPs) | ionic gelation and encapsulation of miR | 26.8% in the first 2 d, and 52.4% by 21 d |  | rMSCs |  |  | antimiR-138 | (Wu et al., 2018) |
| chitosan nanoparticles | electrostatic interactions, 82% | About 30%, 55% and 65% within 7, 14 and 21 days, respectively | nanoparticle/agomir complexes showed constant expression of miRNA in long-term culture | hMSCs | Chitosan binding to negatively charged cellular membranes can enhance cellular uptake. | no significant cytotoxicity | agomiR-199a-5p | (Chen et al., 2015) |
| electrospun polycaprolactone (PCL) nanofibers |  | >50% in the first 72 hr, release sustained up to 14 days |  | induced pluripotent stem cells (iPSCs) |  | iPSCs showed an increasing proliferation trend | miR-22 and miR-126  | (Tahmasebi et al., 2020) |
| bioactive glass nanoparticles (BGNs) silica nanoparticles (SNs) polyethylenimine | SNs: physical adsorption, BGNs: a strong surface interaction, BGNs showed a higher miRNA binding amount (∼200 μg/mg) at 40–320 μg/mL nanoparticle concentrations compared to that of SNs (∼20 μg/mg).  | miRNA was released from BGNs after degradation | > 82% of the intact miRNA left after 3 h of incubation in 25% FBS, in BGN/miRNA and PEI/LIPO groups, while almost completely degraded in SN/miRNA group | BMSCs | BGN group: ∼45%, PEI: 25%, LIPO: 35% | live cell attachment: BGNs group: good at 30–240 μg/mL, PEI 25K and LIPO groups: significantly low; cell viability: significantly higher with 30 and 60 μg/mL BGNs after 72 h | miR-5106 | (Yu et al., 2017) |
| monodispersed BGNs with polyethyleneimine (PEI) | a strong binding affinity bwteen the Ca2+ in BGs framework and the phosphates groups in miRNA |  | >80% of the intact miRNA after 24 h nuclease incubation, but only 50% and 35% in BGN and Lipo group | BMSCs | BGNCs group: 45.3%, BGNs group: 40.1%, Lipo group: 35.1% (after 48 h) | good cells attachment morphology, no dead cells after 1 d | miR-5106 | (Xue et al., 2017) |
| chitosan/hyaluronic acid nanoparticles (CS/HA NPs) | electrostatic interactions, N/P ratio was 20:1 | release sustained up to 14 days |  | hBMMSCs | a moderate and long-lasting transfection process up to 14 days | no any obvious cytotoxicity after 24 hours | miR-21 | (Wang et al., 2016) |
| chitosan (CS)/tripolyphosphate (TPP)/Hyaluronic Acid (HA) nanoparticles (CTH NPs) | electrostatic interactions, N/P ratio > 20:1 | losing the tight binding between the gene and the carrier, and prolong circulation time of the delivery system by reducing their non-specific interactions with serum proteins. | With N/P ratio increasing, the antmiR-138 in complexes was subject to less RNase degradation. | rMSCs | 40% to nearly 70% with the antmiR-138 concentration from 50 to 150 nM | no toxicity | antimiR-138 | (Wu et al., 2016a) |
| polyethylenimine (PEI) bound to magnetic nanoparticles (MNPs) | a salt-induced aggregation called "magnetofection" | Released the DNA in the perinuclear region due to strong biotin-streptavidin connections of MNPs.(Delyagina et al., 2011) | Appropriate condensation of miR protects miR from early enzymatic degradation. | hMSCs | miR/PEI/MNP group (N/P ratio of 2.5): 79%, miR/PEI/CombiMag: 56%, miR/Magnetofectamine: 75% | no significant cell mortality  | miR | (Schade et al., 2014) |
| photocleavable (PC) silver nanoparticle | The light activated technology links a truncated single stranded miRNA to SNP surface via a PC linker. | PC linker release miRNA from the particle by a discrete photo-trigger.  | The HPC was displaced with negatively charged PC-miR-148b, increasing colloidal stability in aqueous solution | hASCs |  | minimal cytotoxicity (90.08 ± 2.12% viable hASCs | miR-148b | (Qureshi et al., 2013) |
| gold nanoparticles | miR can be attached to the gold nanoparticle. |  |  | hMSCs | At 1 hour GNPs were mainly at the cell periphery, whilst at 48 hours the NPs were within the cell, mainly packaged into endosomes. | no adverse effects | antagomiR-31 | (McCully et al., 2018) |
| PEI-capped gold nanoparticles (AuNPs) | electrostatic interactions |  | stable in serum for 6 h | hMSCs and MC3T3-E1 cells | AuNPs/Cy3-miR-29b: 54±0.71% and 88±1.42% for hMSCs and MC3T3-E1 cells, lipo/miR-29b: 65.12±1.85% and 80.57±1.77% | no significant cytotoxicity, lower toxicity than lipo | miR-29b | (Pan et al., 2016) |
| GNP SNP | A stable covalently bound linker, amide bond, conjugated molecular cargo to surfaces. | photothermal release at temperatures ≥60 °C or at ≈400 nm irradiation | the 2'-O-methyl modified RNA mimics prolonged the lifetime of RNA in serum | hASCs |  | toxic cellular response to the 405 nm LED light source | miR-148b and miR-21 | (Abu-Laban et al., 2019) |
| lyophilized mesoporous silica nanoparticles with core-cone structure and coated with polyethylenimine (MSN-CC-PEI) | electrostatic attraction, the loading efficiency achieved 60% with 40 μg/mL nanoparticles | proton sponge (proton buffering) |  | rBMSCs |  | considerably more cytotoxicity at 40 μg mL−1 PEI coated particles, no significant cytotoxicity in uncoated particles, the optimal exposure conditions for the PEI coated NPs would be less than 24 hours | rno-miRNA-26a-5p | (Hosseinpour et al., 2021) |
| MSNs-PEI-KALA peptide | miR-26a is bonded to the MSN surface. | KALA's membrane disrupting activity | no release of miRNA in RNase A | rBMSCs | ~23% fluorescence intensity at 12 h with 20 μg/mL complexes | no significant cytotoxicity | miR-26a | (Yan et al., 2020) |
| lipidoid nanoparticles | electrostatic interactions |  |  | mBMSCs | a similar or higher transfection efficiency than Lipofectamine 2000. | no significant cytotoxicity | miR-335-5p | (Sui et al., 2018) |
| R9-LK15 nanocomplexes | electrostatic interactions |  | stable in serum for up to 24 h | rBMSCs | R9-LK15/miR-29b nanocomplexes: 78.33% ± 5.90, Lipo/miR-29b nanocomplexes: 36.43% ± 1.75 | no significant cytotoxicity, much less cytotoxic than Lipo | miR-29b  | (Liu et al., 2019b) |
| [nanocapsules and O-carboxymethyl chitosan (CMCS) powder mixed gel](https://www.sciencedirect.com/science/article/pii/S0928493119310173?via=ihub) |  | a fast release within the first 20 h (~50%)， ~10% remained in the coating after 100 h |  | MSCs |  | good biocompatibility | miR-21  | (Geng et al., 2020) |
| N-(3-aminopropyl) methacrylamide, acrylamide and ethylene glycol dimethacrylate nanocapsules | electrostatic interactions, hydrogen bonding, free-radical polymerization wraps the miRNA molecules with thin shells of network polymer (Liu et al., 2015) | The crosslinker molecules are degradable in acidic environment (pH 5.4) (Liu et al., 2015) | no extraction of As-miR-21 by heparin from the nanocapsules, better stability against RNase and serum than lipo/AS-miR-21 (Liu et al., 2015) | Osteoblast-like MG63 cells | two and fivefold higher than that of lipo/AS-miR-21 (Liu et al., 2015) | no any obvious rejection phenomenon 1 month after surgery (Geng et al., 2018), low cytotoxicity (Liu et al., 2015) | miR-21  | (Geng et al., 2018) |
| CMCS nanocapsules | electrostatic interactions, hydrogen bonding, free-radical polymerization wraps the miRNA molecules with thin shells of network polymer |  | no extraction from nanocapsules by heparin, compared to the lipo/miR-21 complex | hUMSCs | 61.6% after 48 h (nearly 3.6-fold that of the CMCS/lipo/miR-21 group), 1.6-fold greater at 3 days | 97.6 ± 9.3% cell viability at a miR-21 concentration of 50 nM | miR-21  | (Meng et al., 2016b) |
| CMCS coating nanocapsules |  |  |  |  | 77.14% cells presented green fluorescence after 4 h of incubation, while 67.56% in lipo2000 group | 78.28% hUMSCs viability at the nanocapsule concentration of 500 nM, and 93.05% at 50 nM | miR-29b | (Meng et al., 2016a) |
| nanocapsules | Electrostatic interaction and hydrogen bonding interaction formed a polymer shell around the miR-21. |  |  | rMSCs | high efficiency | increased cytotoxicity when concentration >100 nm, metabolic activity was 98.06% at 50 nm | miR-21  | (Sun et al., 2020) |
| Liposomes | aspartate, serine, serine (AspSerSer)6-liposome |  |  |  | osteoblasts |  |  | mir-133a | (Zhou et al., 2021) |
| Lipofectamine 3000 |  |  |  | hASCs |  |  | miR-33a-5p | (Shen et al., 2020) |
| Lipofectamine 2000 |  |  |  | rBMSCs, hBMSCs, rabbit BMSCs |  |  | miR-34a, miR-106a Inhibitor, antimiR-222, antimiR-138, miR-93-5p inhibitor | (Liu et al., 2019a) (Sun et al., 2018) (Chang et al., 2018) (Yan et al., 2014) (Zhang et al., 2021b) |
| Lipofectamine RNAi MAX (Carthew et al., 2020) |  | Complexes gradually diffused, with full release taking ~7 days.(Carthew et al., 2020) |  | MCO cells | transfection efficiency: 97%(Carthew et al., 2020) |  | antimiR-467g | (Kureel et al., 2017) |
| siPORT NeoFX transfection agent |  | proton sponge |  | hBMMSCs, mBMMSCs, ADSCs |  |  | miR-26a, anti-hsa-miR-221 | (Li et al., 2013) (Hoseinzadeh et al., 2016) |
| X-tremeGENE transfection reagent |  |  |  | mBMSCs, mMSCs  |  |  | miR-590-5p, miR-590-5, miR-15b | (Balagangadharan et al., 2018) (Vishal et al., 2017) (Vimalraj et al., 2016) |
| Other types | injectable poly(ethylene glycol) (PEG) hydrogel | covalently connected by an ultraviolet (UV) light-cleavable linker | The release rate was ~70% upon 365nm UV irradiation for 5 min. | no miR-26a release in the absence of UV irradiation | hMSCs | The internalization efficiency was about 60% in 1 day. | The biocompatibility of the gel are suitable for the surface-cultured cells. | miR-26a | (Gan et al., 2021) |
| poly(ethylene glycol) (PEG) hydrogels  |  |  |  | hMSCs  |  |  | miR-20a | (Nguyen et al., 2018) |
| a comb-shaped polycation (HA-SS-PGEA) consisting of Hyaluronic acid (HA), disulfide groups and ethanolamine (EA)-functionalized poly(glycidyl methacrylate) (PGMA) | electrostatic interactions | DL-dithiothreitol (DTT) induced disulfide bond cleavage; Burst release in first 3 days, and sustained release for one month. | The HA-SS-PGEA completely retarded miRNA migration at the N/P ratio of 1 and enabled NPs stable to resist anionic macromolecules to prevent premature failure of miRNA. | BMSCs | The ability of HA-SS-PGEA to transport miRNA through cellular membrane is stronger than PEI and PGEA. | The cell viability reached above 90%. | miR-26a | (Li et al., 2020) |
| Bio-inspired bioactive glasses | The miR-7–FAM labelling efficiency > 90% when the PBGs–NH2/microRNA ratio was 40 or 80.  |  |  | human Hela cell line | >90% after 4 h incubation | The cells were not in a good state and many of them became irregular when ≥100 μg mL−1, although the positive expression rate was still >95%. | miR-7 | (Li et al., 2017c) |
| zinc(II) quercetin complexes (Zn+Q(PHt)) (BMC) |  |  |  | MG-63 cells |  | no declined cytocompatibility till >60μM | pre-miR-15b | (Raj Preeth et al., 2021) |
| Ascorbic Acid-PEI Carbon Dots (CD) | electrostatic interactions, miR-2861 fully complexed with the CD at a 4:1 weight ratio of the CD and miR |  |  | BMSCs | Cellular uptake had started before 5 min and exponentially boosted with time before 4 h and then became slow and plateau phases/saturation; transfection efficiency: 47.44% | With the increase of PEI concentration, the viability of BMSCs reduced remarkably, only 35.00% at 50 μg/mL; no significant cytotoxicity of CD | miR-2861 | (Bu et al., 2020) |
| PEI |  |  |  | hMSCs | endocytosis, transfection efficiency: 79% (4 kDa PEI) and 77% (40 kDa PEI) | no significant cytotoxicity | miR-100-5p, miR-143-3p, miR-20a | (Carthew et al., 2020) (Huynh et al., 2016) (Nguyen et al., 2014) |
| PEI-functionalized graphene oxide (GO) complex | electrostatic interactions; At the N/P ratio of 30 (Figure 3D), GPM complex would wrap miR-inhibitor inside and prevent it being degraded. |  | RNase A have no influence on the GFP RNA delivered into cells | mouse osteoblastic cells (MC3T3-E1) | cell uptake and transfection efficacy for 24h greater than naked miR-inhibitor or Lipofectamine 2000 groups | no significant toxicity | miR-214 inhibitor | (Ou et al., 2019) |
| silk-based orthopedic devices, Xfect RNA Transfection Reagent  | electrostatic interactions | initial release of ∼20% in the first 24 h, the continued release of an additional 42% in the next 48 h, an additional 36% after 168 h |  | hMSCs | Cells are typically transfected within 24 h and have bioactivity up to 2 or 3 weeks. | significantly higher cell viability after 72 h and 7 days, no clear difference in morphology, no apoptotic | antimiR-214 | (James et al., 2019) |
| Low molecular weight protamine (LMWP) | electrostatic interactions |  | no degradation in serum for up to 24 h | hMSCs | 6.5-fold transfection efficacy than the cationic lipids in 5h | no significant change in the viability of hMSCs, mild toxicity (around 14%) in liposomal group | miR-29b | (Suh et al., 2013) |
| Electrospinning of gelatin | The miRNA was loaded uniformly throughout the fibers. | an initial burst followed by sustained release for up to 72 h |  | MC3T3-E1 osteoblast-like cells |  | no significant differences in cell viability after 24h | miR-29a inhibitor | (James et al., 2014) |

References

Abu-Laban, M., Hamal, P., Arrizabalaga, J.H., Forghani, A., Dikkumbura, A.S., Kumal, R.R., et al. (2019). Combinatorial Delivery of miRNA-Nanoparticle Conjugates in Human Adipose Stem Cells for Amplified Osteogenesis. *Small* 15(50)**,** e1902864. doi: 10.1002/smll.201902864.

Akkouch, A., Eliason, S., Sweat, M.E., Romero-Bustillos, M., Zhu, M., Qian, F., et al. (2019). Enhancement of MicroRNA-200c on Osteogenic Differentiation and Bone Regeneration by Targeting Sox2-Mediated Wnt Signaling and Klf4. *Hum Gene Ther* 30(11)**,** 1405-1418. doi: 10.1089/hum.2019.019.

Balagangadharan, K., Viji Chandran, S., Arumugam, B., Saravanan, S., Devanand Venkatasubbu, G., and Selvamurugan, N. (2018). Chitosan/nano-hydroxyapatite/nano-zirconium dioxide scaffolds with miR-590-5p for bone regeneration. *Int J Biol Macromol* 111**,** 953-958. doi: 10.1016/j.ijbiomac.2018.01.122.

Bose, S., and Tarafder, S. (2012). Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review. *Acta Biomater* 8(4)**,** 1401-1421. doi: 10.1016/j.actbio.2011.11.017.

Brenner, T.K., Posa-Markaryan, K., Hercher, D., Sperger, S., Heimel, P., Keibl, C., et al. (2021). Evaluation of BMP2/miRNA co-expression systems for potent therapeutic efficacy in bone-tissue regeneration. *Eur Cell Mater* 41**,** 245-268. doi: 10.22203/eCM.v041a18.

Bu, W., Xu, X., Wang, Z., Jin, N., Liu, L., Liu, J., et al. (2020). Ascorbic Acid-PEI Carbon Dots with Osteogenic Effects as miR-2861 Carriers to Effectively Enhance Bone Regeneration. *ACS Appl Mater Interfaces* 12(45)**,** 50287-50302. doi: 10.1021/acsami.0c15425.

Carthew, J., Donderwinkel, I., Shrestha, S., Truong, V.X., Forsythe, J.S., and Frith, J.E. (2020). In situ miRNA delivery from a hydrogel promotes osteogenesis of encapsulated mesenchymal stromal cells. *Acta Biomater* 101**,** 249-261. doi: 10.1016/j.actbio.2019.11.016.

Castaño, I.M., Raftery, R.M., Chen, G., Cavanagh, B., Quinn, B., Duffy, G.P., et al. (2020). Rapid bone repair with the recruitment of CD206(+)M2-like macrophages using non-viral scaffold-mediated miR-133a inhibition of host cells. *Acta Biomater* 109**,** 267-279. doi: 10.1016/j.actbio.2020.03.042.

Chang, C.C., Venø, M.T., Chen, L., Ditzel, N., Le, D.Q.S., Dillschneider, P., et al. (2018). Global MicroRNA Profiling in Human Bone Marrow Skeletal-Stromal or Mesenchymal-Stem Cells Identified Candidates for Bone Regeneration. *Mol Ther* 26(2)**,** 593-605. doi: 10.1016/j.ymthe.2017.11.018.

Chen, M., Zhou, M., Fu, Y., Li, J., and Wang, Z. (2021). Effects of miR-672 on the angiogenesis of adipose-derived mesenchymal stem cells during bone regeneration. *Stem Cell Res Ther* 12(1)**,** 85. doi: 10.1186/s13287-021-02154-7.

Chen, X., Gu, S., Chen, B.F., Shen, W.L., Yin, Z., Xu, G.W., et al. (2015). Nanoparticle delivery of stable miR-199a-5p agomir improves the osteogenesis of human mesenchymal stem cells via the HIF1a pathway. *Biomaterials* 53**,** 239-250. doi: 10.1016/j.biomaterials.2015.02.071.

Delyagina, E., Li, W., Ma, N., and Steinhoff, G. (2011). Magnetic targeting strategies in gene delivery. *Nanomedicine (Lond)* 6(9)**,** 1593-1604. doi: 10.2217/nnm.11.143.

Deng, Y., Bi, X., Zhou, H., You, Z., Wang, Y., Gu, P., et al. (2014). Repair of critical-sized bone defects with anti-miR-31-expressing bone marrow stromal stem cells and poly(glycerol sebacate) scaffolds. *Eur Cell Mater* 27**,** 13-24; discussion 24-15. doi: 10.22203/ecm.v027a02.

Deng, Y., Zhou, H., Zou, D., Xie, Q., Bi, X., Gu, P., et al. (2013). The role of miR-31-modified adipose tissue-derived stem cells in repairing rat critical-sized calvarial defects. *Biomaterials* 34(28)**,** 6717-6728. doi: 10.1016/j.biomaterials.2013.05.042.

Ding, R., Liu, X., Zhang, J., Yuan, J., Zheng, S., Cheng, X., et al. (2021). Downregulation of miR-1-3p expression inhibits the hypertrophy and mineralization of chondrocytes in DDH. *J Orthop Surg Res* 16(1)**,** 512. doi: 10.1186/s13018-021-02666-1.

Fang, T., Wu, Q., Zhou, L., Mu, S., and Fu, Q. (2016). miR-106b-5p and miR-17-5p suppress osteogenic differentiation by targeting Smad5 and inhibit bone formation. *Exp Cell Res* 347(1)**,** 74-82. doi: 10.1016/j.yexcr.2016.07.010.

Gan, M., Zhou, Q., Ge, J., Zhao, J., Wang, Y., Yan, Q., et al. (2021). Precise in-situ release of microRNA from an injectable hydrogel induces bone regeneration. *Acta Biomater* 135**,** 289-303. doi: 10.1016/j.actbio.2021.08.041.

Geng, Z., Wang, X., Zhao, J., Li, Z., Ma, L., Zhu, S., et al. (2018). The synergistic effect of strontium-substituted hydroxyapatite and microRNA-21 on improving bone remodeling and osseointegration. *Biomater Sci* 6(10)**,** 2694-2703. doi: 10.1039/c8bm00716k.

Geng, Z., Yu, Y., Li, Z., Ma, L., Zhu, S., Liang, Y., et al. (2020). miR-21 promotes osseointegration and mineralization through enhancing both osteogenic and osteoclastic expression. *Mater Sci Eng C Mater Biol Appl* 111**,** 110785. doi: 10.1016/j.msec.2020.110785.

Hong, L., Sharp, T., Khorsand, B., Fischer, C., Eliason, S., Salem, A., et al. (2016). MicroRNA-200c Represses IL-6, IL-8, and CCL-5 Expression and Enhances Osteogenic Differentiation. *PLoS One* 11(8)**,** e0160915. doi: 10.1371/journal.pone.0160915.

Hoseinzadeh, S., Atashi, A., Soleimani, M., Alizadeh, E., and Zarghami, N. (2016). MiR-221-inhibited adipose tissue-derived mesenchymal stem cells bioengineered in a nano-hydroxy apatite scaffold. *In Vitro Cell Dev Biol Anim* 52(4)**,** 479-487. doi: 10.1007/s11626-015-9992-x.

Hosseinpour, S., Cao, Y., Liu, J., Xu, C., and Walsh, L.J. (2021). Efficient transfection and long-term stability of rno-miRNA-26a-5p for osteogenic differentiation by large pore sized mesoporous silica nanoparticles. *J Mater Chem B* 9(9)**,** 2275-2284. doi: 10.1039/d0tb02756a.

Hou, Y., Lin, W., Li, Y., Sun, Y., Liu, Y., Chen, C., et al. (2021). De-osteogenic-differentiated mesenchymal stem cells accelerate fracture healing by mir-92b. *J Orthop Translat* 27**,** 25-32. doi: 10.1016/j.jot.2020.10.009.

Huynh, C.T., Nguyen, M.K., Naris, M., Tonga, G.Y., Rotello, V.M., and Alsberg, E. (2016). Light-triggered RNA release and induction of hMSC osteogenesis via photodegradable, dual-crosslinked hydrogels. *Nanomedicine (Lond)* 11(12)**,** 1535-1550. doi: 10.2217/nnm-2016-0088.

James, E.N., Delany, A.M., and Nair, L.S. (2014). Post-transcriptional regulation in osteoblasts using localized delivery of miR-29a inhibitor from nanofibers to enhance extracellular matrix deposition. *Acta Biomater* 10(8)**,** 3571-3580. doi: 10.1016/j.actbio.2014.04.026.

James, E.N., Van Doren, E., Li, C., and Kaplan, D.L. (2019). Silk Biomaterials-Mediated miRNA Functionalized Orthopedic Devices. *Tissue Eng Part A* 25(1-2)**,** 12-23. doi: 10.1089/ten.TEA.2017.0455.

Jiang, F., Yin, F., Lin, Y., Xia, W., Zhou, L., Pan, C., et al. (2020). The promotion of bone regeneration through CS/GP-CTH/antagomir-133a/b sustained release system. *Nanomedicine* 24**,** 102116. doi: 10.1016/j.nano.2019.102116.

Jiang, W., Zhu, P., Zhang, T., Liao, F., Yu, Y., Liu, Y., et al. (2021). MicroRNA-205 mediates endothelial progenitor functions in distraction osteogenesis by targeting the transcription regulator NOTCH2. *Stem Cell Res Ther* 12(1)**,** 101. doi: 10.1186/s13287-021-02150-x.

Kureel, J., John, A.A., Dixit, M., and Singh, D. (2017). MicroRNA-467g inhibits new bone regeneration by targeting Ihh/Runx-2 signaling. *Int J Biochem Cell Biol* 85**,** 35-43. doi: 10.1016/j.biocel.2017.01.018.

Lee, W.Y., Li, N., Lin, S., Wang, B., Lan, H.Y., and Li, G. (2016). miRNA-29b improves bone healing in mouse fracture model. *Mol Cell Endocrinol* 430**,** 97-107. doi: 10.1016/j.mce.2016.04.014.

Lei, L., Liu, Z., Yuan, P., Jin, R., Wang, X., Jiang, T., et al. (2019). Injectable colloidal hydrogel with mesoporous silica nanoparticles for sustained co-release of microRNA-222 and aspirin to achieve innervated bone regeneration in rat mandibular defects. *J Mater Chem B* 7(16)**,** 2722-2735. doi: 10.1039/c9tb00025a.

Li, D., Yang, Z., Luo, Y., Zhao, X., Tian, M., and Kang, P. (2022). Delivery of MiR335-5p-Pendant Tetrahedron DNA Nanostructures Using an Injectable Heparin Lithium Hydrogel for Challenging Bone Defects in Steroid-Associated Osteonecrosis. *Adv Healthc Mater* 11(1)**,** e2101412. doi: 10.1002/adhm.202101412.

Li, H., Liu, P., Xu, S., Li, Y., Dekker, J.D., Li, B., et al. (2017a). FOXP1 controls mesenchymal stem cell commitment and senescence during skeletal aging. *J Clin Invest* 127(4)**,** 1241-1253. doi: 10.1172/jci89511.

Li, K.C., Lo, S.C., Sung, L.Y., Liao, Y.H., Chang, Y.H., and Hu, Y.C. (2017b). Improved calvarial bone repair by hASCs engineered with Cre/loxP-based baculovirus conferring prolonged BMP-2 and MiR-148b co-expression. *J Tissue Eng Regen Med* 11(11)**,** 3068-3077. doi: 10.1002/term.2208.

Li, R., Wang, H., John, J.V., Song, H., Teusink, M.J., and Xie, J. (2020). 3D Hybrid Nanofiber Aerogels Combining with Nanoparticles Made of a Biocleavable and Targeting Polycation and MiR-26a for Bone Repair. *Adv Funct Mater* 30(49). doi: 10.1002/adfm.202005531.

Li, S., Liu, Y., Tian, T., Zhang, T., Lin, S., Zhou, M., et al. (2021). Bioswitchable Delivery of microRNA by Framework Nucleic Acids: Application to Bone Regeneration. *Small* 17(47)**,** e2104359. doi: 10.1002/smll.202104359.

Li, X., Liang, Q., Zhang, W., Li, Y., Ye, J., Zhao, F., et al. (2017c). Bio-inspired bioactive glasses for efficient microRNA and drug delivery. *J Mater Chem B* 5(31)**,** 6376-6384. doi: 10.1039/c7tb01021d.

Li, Y., Fan, L., Liu, S., Liu, W., Zhang, H., Zhou, T., et al. (2013). The promotion of bone regeneration through positive regulation of angiogenic-osteogenic coupling using microRNA-26a. *Biomaterials* 34(21)**,** 5048-5058. doi: 10.1016/j.biomaterials.2013.03.052.

Liao, Y.H., Chang, Y.H., Sung, L.Y., Li, K.C., Yeh, C.L., Yen, T.C., et al. (2014). Osteogenic differentiation of adipose-derived stem cells and calvarial defect repair using baculovirus-mediated co-expression of BMP-2 and miR-148b. *Biomaterials* 35(18)**,** 4901-4910. doi: 10.1016/j.biomaterials.2014.02.055.

Liu, C., Wen, J., Meng, Y., Zhang, K., Zhu, J., Ren, Y., et al. (2015). Efficient delivery of therapeutic miRNA nanocapsules for tumor suppression. *Adv Mater* 27(2)**,** 292-297. doi: 10.1002/adma.201403387.

Liu, H., Dong, Y., Feng, X., Li, L., Jiao, Y., Bai, S., et al. (2019a). miR-34a promotes bone regeneration in irradiated bone defects by enhancing osteoblastic differentiation of mesenchymal stromal cells in rats. *Stem Cell Res Ther* 10(1)**,** 180. doi: 10.1186/s13287-019-1285-y.

Liu, J., Cui, Y., Kuang, Y., Xu, S., Lu, Q., Diao, J., et al. (2021). Hierarchically porous calcium-silicon nanosphere-enabled co-delivery of microRNA-210 and simvastatin for bone regeneration. *J Mater Chem B* 9(16)**,** 3573-3583. doi: 10.1039/d1tb00063b.

Liu, N., and Sun, Y. (2021). microRNA-148a-3p-targeting p300 protects against osteoblast differentiation and osteoporotic bone reconstruction. *Regen Med* 16(5)**,** 435-449. doi: 10.2217/rme-2020-0006.

Liu, Q., Lin, Z., Liu, Y., Du, J., Lin, H., and Wang, J. (2019b). Delivery of miRNA-29b Using R9-LK15, a Novel Cell-Penetrating Peptide, Promotes Osteogenic Differentiation of Bone Mesenchymal Stem Cells. *Biomed Res Int* 2019**,** 3032158. doi: 10.1155/2019/3032158.

Liu, Z., Chang, H., Hou, Y., Wang, Y., Zhou, Z., Wang, M., et al. (2018a). Lentivirus‑mediated microRNA‑26a overexpression in bone mesenchymal stem cells facilitates bone regeneration in bone defects of calvaria in mice. *Mol Med Rep* 18(6)**,** 5317-5326. doi: 10.3892/mmr.2018.9596.

Liu, Z., Chen, X., Zhang, Z., Zhang, X., Saunders, L., Zhou, Y., et al. (2018b). Nanofibrous Spongy Microspheres To Distinctly Release miRNA and Growth Factors To Enrich Regulatory T Cells and Rescue Periodontal Bone Loss. *ACS Nano* 12(10)**,** 9785-9799. doi: 10.1021/acsnano.7b08976.

Mazziotta, C., Lanzillotti, C., Iaquinta, M.R., Taraballi, F., Torreggiani, E., Rotondo, J.C., et al. (2021). MicroRNAs Modulate Signaling Pathways in Osteogenic Differentiation of Mesenchymal Stem Cells. *Int J Mol Sci* 22(5). doi: 10.3390/ijms22052362.

McCully, M., Conde, J., P, V.B., Mullin, M., Dalby, M.J., and Berry, C.C. (2018). Nanoparticle-antagomiR based targeting of miR-31 to induce osterix and osteocalcin expression in mesenchymal stem cells. *PLoS One* 13(2)**,** e0192562. doi: 10.1371/journal.pone.0192562.

Meech, R., Edelman, D.B., Jones, F.S., and Makarenkova, H.P. (2005). The homeobox transcription factor Barx2 regulates chondrogenesis during limb development. *Development* 132(9)**,** 2135-2146. doi: 10.1242/dev.01811.

Mencía Castaño, I., Curtin, C.M., Duffy, G.P., and O'Brien, F.J. (2016). Next generation bone tissue engineering: non-viral miR-133a inhibition using collagen-nanohydroxyapatite scaffolds rapidly enhances osteogenesis. *Sci Rep* 6**,** 27941. doi: 10.1038/srep27941.

Mencía Castaño, I., Curtin, C.M., Duffy, G.P., and O'Brien, F.J. (2019). Harnessing an Inhibitory Role of miR-16 in Osteogenesis by Human Mesenchymal Stem Cells for Advanced Scaffold-Based Bone Tissue Engineering. *Tissue Eng Part A* 25(1-2)**,** 24-33. doi: 10.1089/ten.TEA.2017.0460.

Mencía Castaño, I., Curtin, C.M., Shaw, G., Murphy, J.M., Duffy, G.P., and O'Brien, F.J. (2015). A novel collagen-nanohydroxyapatite microRNA-activated scaffold for tissue engineering applications capable of efficient delivery of both miR-mimics and antagomiRs to human mesenchymal stem cells. *J Control Release* 200**,** 42-51. doi: 10.1016/j.jconrel.2014.12.034.

Meng, Y., Li, X., Li, Z., Liu, C., Zhao, J., Wang, J., et al. (2016a). Surface Functionalization of Titanium Alloy with miR-29b Nanocapsules To Enhance Bone Regeneration. *ACS Appl Mater Interfaces* 8(9)**,** 5783-5793. doi: 10.1021/acsami.5b10650.

Meng, Y., Liu, C., Zhao, J., Li, X., Li, Z., Wang, J., et al. (2016b). An injectable miRNA-activated matrix for effective bone regeneration in vivo. *J Mater Chem B* 4(43)**,** 6942-6954. doi: 10.1039/c6tb01790h.

Meng, Y.B., Li, X., Li, Z.Y., Zhao, J., Yuan, X.B., Ren, Y., et al. (2015). microRNA-21 promotes osteogenic differentiation of mesenchymal stem cells by the PI3K/β-catenin pathway. *J Orthop Res* 33(7)**,** 957-964. doi: 10.1002/jor.22884.

Moncal, K.K., Aydin, R.S.T., Abu-Laban, M., Heo, D.N., Rizk, E., Tucker, S.M., et al. (2019). Collagen-infilled 3D printed scaffolds loaded with miR-148b-transfected bone marrow stem cells improve calvarial bone regeneration in rats. *Mater Sci Eng C Mater Biol Appl* 105**,** 110128. doi: 10.1016/j.msec.2019.110128.

Nguyen, M.K., Jeon, O., Dang, P.N., Huynh, C.T., Varghai, D., Riazi, H., et al. (2018). RNA interfering molecule delivery from in situ forming biodegradable hydrogels for enhancement of bone formation in rat calvarial bone defects. *Acta Biomater* 75**,** 105-114. doi: 10.1016/j.actbio.2018.06.007.

Nguyen, M.K., Jeon, O., Krebs, M.D., Schapira, D., and Alsberg, E. (2014). Sustained localized presentation of RNA interfering molecules from in situ forming hydrogels to guide stem cell osteogenic differentiation. *Biomaterials* 35(24)**,** 6278-6286. doi: 10.1016/j.biomaterials.2014.04.048.

Ou, L., Lan, Y., Feng, Z., Feng, L., Yang, J., Liu, Y., et al. (2019). Functionalization of SF/HAP Scaffold with GO-PEI-miRNA inhibitor Complexes to Enhance Bone Regeneration through Activating Transcription Factor 4. *Theranostics* 9(15)**,** 4525-4541. doi: 10.7150/thno.34676.

Pan, T., Song, W., Gao, H., Li, T., Cao, X., Zhong, S., et al. (2016). miR-29b-Loaded Gold Nanoparticles Targeting to the Endoplasmic Reticulum for Synergistic Promotion of Osteogenic Differentiation. *ACS Appl Mater Interfaces* 8(30)**,** 19217-19227. doi: 10.1021/acsami.6b02969.

Peng, H., Lu, S.L., Bai, Y., Fang, X., Huang, H., and Zhuang, X.Q. (2018). MiR-133a inhibits fracture healing via targeting RUNX2/BMP2. *Eur Rev Med Pharmacol Sci* 22(9)**,** 2519-2526. doi: 10.26355/eurrev\_201805\_14914.

Qadir, A.S., Lee, J., Lee, Y.S., Woo, K.M., Ryoo, H.M., and Baek, J.H. (2018). Distal-less homeobox 3, a negative regulator of myogenesis, is downregulated by microRNA-133. *J Cell Biochem*. doi: 10.1002/jcb.27533.

Qureshi, A.T., Monroe, W.T., Dasa, V., Gimble, J.M., and Hayes, D.J. (2013). miR-148b-nanoparticle conjugates for light mediated osteogenesis of human adipose stromal/stem cells. *Biomaterials* 34(31)**,** 7799-7810. doi: 10.1016/j.biomaterials.2013.07.004.

Raj Preeth, D., Saravanan, S., Shairam, M., Selvakumar, N., Selestin Raja, I., Dhanasekaran, A., et al. (2021). Bioactive Zinc(II) complex incorporated PCL/gelatin electrospun nanofiber enhanced bone tissue regeneration. *Eur J Pharm Sci* 160**,** 105768. doi: 10.1016/j.ejps.2021.105768.

Remy, M.T., Akkouch, A., He, L., Eliason, S., Sweat, M.E., Krongbaramee, T., et al. (2021). Rat Calvarial Bone Regeneration by 3D-Printed β-Tricalcium Phosphate Incorporating MicroRNA-200c. *ACS Biomater Sci Eng* 7(9)**,** 4521-4534. doi: 10.1021/acsbiomaterials.0c01756.

Rossi, M., Pitari, M.R., Amodio, N., Di Martino, M.T., Conforti, F., Leone, E., et al. (2013). miR-29b negatively regulates human osteoclastic cell differentiation and function: implications for the treatment of multiple myeloma-related bone disease. *J Cell Physiol* 228(7)**,** 1506-1515. doi: 10.1002/jcp.24306.

Schade, A., Müller, P., Delyagina, E., Voronina, N., Skorska, A., Lux, C., et al. (2014). Magnetic Nanoparticle Based Nonviral MicroRNA Delivery into Freshly Isolated CD105(+) hMSCs. *Stem Cells Int* 2014**,** 197154. doi: 10.1155/2014/197154.

Shen, W., Sun, B., Zhou, C., Ming, W., Zhang, S., and Wu, X. (2020). CircFOXP1/FOXP1 promotes osteogenic differentiation in adipose-derived mesenchymal stem cells and bone regeneration in osteoporosis via miR-33a-5p. *J Cell Mol Med* 24(21)**,** 12513-12524. doi: 10.1111/jcmm.15792.

Su, X., Liao, L., Shuai, Y., Jing, H., Liu, S., Zhou, H., et al. (2015). MiR-26a functions oppositely in osteogenic differentiation of BMSCs and ADSCs depending on distinct activation and roles of Wnt and BMP signaling pathway. *Cell Death Dis* 6(8)**,** e1851. doi: 10.1038/cddis.2015.221.

Suh, J.S., Lee, J.Y., Choi, Y.S., Chung, C.P., and Park, Y.J. (2013). Peptide-mediated intracellular delivery of miRNA-29b for osteogenic stem cell differentiation. *Biomaterials* 34(17)**,** 4347-4359. doi: 10.1016/j.biomaterials.2013.02.039.

Sui, L., Wang, M., Han, Q., Yu, L., Zhang, L., Zheng, L., et al. (2018). A novel Lipidoid-MicroRNA formulation promotes calvarial bone regeneration. *Biomaterials* 177**,** 88-97. doi: 10.1016/j.biomaterials.2018.05.038.

Sun, M.H., Wang, W.J., Li, Q., Yuan, T., and Weng, W.J. (2018). Autologous oxygen release nano bionic scaffold composite miR-106a induced BMSCs enhances osteoblast conversion and promotes bone repair through regulating BMP-2. *Eur Rev Med Pharmacol Sci* 22(21)**,** 7148-7155. doi: 10.26355/eurrev\_201811\_16246.

Sun, X., Li, X., Qi, H., Hou, X., Zhao, J., Yuan, X., et al. (2020). MiR-21 nanocapsules promote early bone repair of osteoporotic fractures by stimulating the osteogenic differentiation of bone marrow mesenchymal stem cells. *J Orthop Translat* 24**,** 76-87. doi: 10.1016/j.jot.2020.04.007.

Tahmasebi, A., Enderami, S.E., Saburi, E., Islami, M., Yaslianifard, S., Mahabadi, J.A., et al. (2020). Micro-RNA-incorporated electrospun nanofibers improve osteogenic differentiation of human-induced pluripotent stem cells. *J Biomed Mater Res A* 108(2)**,** 377-386. doi: 10.1002/jbm.a.36824.

Tu, M., Tang, J., He, H., Cheng, P., and Chen, C. (2017). MiR-142-5p promotes bone repair by maintaining osteoblast activity. *J Bone Miner Metab* 35(3)**,** 255-264. doi: 10.1007/s00774-016-0757-8.

Vimalraj, S., Saravanan, S., Vairamani, M., Gopalakrishnan, C., Sastry, T.P., and Selvamurugan, N. (2016). A Combinatorial effect of carboxymethyl cellulose based scaffold and microRNA-15b on osteoblast differentiation. *Int J Biol Macromol* 93(Pt B)**,** 1457-1464. doi: 10.1016/j.ijbiomac.2015.12.083.

Vishal, M., Vimalraj, S., Ajeetha, R., Gokulnath, M., Keerthana, R., He, Z., et al. (2017). MicroRNA-590-5p Stabilizes Runx2 by Targeting Smad7 During Osteoblast Differentiation. *J Cell Physiol* 232(2)**,** 371-380. doi: 10.1002/jcp.25434.

Wang, X., Guo, B., Li, Q., Peng, J., Yang, Z., Wang, A., et al. (2013). miR-214 targets ATF4 to inhibit bone formation. *Nat Med* 19(1)**,** 93-100. doi: 10.1038/nm.3026.

Wang, Y., He, R., Yang, A., Guo, R., Liu, J., Liang, G., et al. (2021). Role of miR-214 in biomaterial transplantation therapy for osteonecrosis. *Biomed Mater Eng*. doi: 10.3233/bme-211296.

Wang, Z., Wu, G., Wei, M., Liu, Q., Zhou, J., Qin, T., et al. (2016). Improving the osteogenesis of human bone marrow mesenchymal stem cell sheets by microRNA-21-loaded chitosan/hyaluronic acid nanoparticles via reverse transfection. *Int J Nanomedicine* 11**,** 2091-2105. doi: 10.2147/ijn.S104851.

Wei, J., Li, H., Wang, S., Li, T., Fan, J., Liang, X., et al. (2014). let-7 enhances osteogenesis and bone formation while repressing adipogenesis of human stromal/mesenchymal stem cells by regulating HMGA2. *Stem Cells Dev* 23(13)**,** 1452-1463. doi: 10.1089/scd.2013.0600.

Wu, G., Feng, C., Hui, G., Wang, Z., Tan, J., Luo, L., et al. (2016a). Improving the osteogenesis of rat mesenchymal stem cells by chitosan-based-microRNA nanoparticles. *Carbohydr Polym* 138**,** 49-58. doi: 10.1016/j.carbpol.2015.11.044.

Wu, G., Feng, C., Quan, J., Wang, Z., Wei, W., Zang, S., et al. (2018). In situ controlled release of stromal cell-derived factor-1α and antimiR-138 for on-demand cranial bone regeneration. *Carbohydr Polym* 182**,** 215-224. doi: 10.1016/j.carbpol.2017.10.090.

Wu, S., Liu, W., and Zhou, L. (2016b). MiR-590-3p regulates osteogenic differentiation of human mesenchymal stem cells by regulating APC gene. *Biochem Biophys Res Commun* 478(4)**,** 1582-1587. doi: 10.1016/j.bbrc.2016.08.160.

Xie, Q., Wang, Z., Zhou, H., Yu, Z., Huang, Y., Sun, H., et al. (2016). The role of miR-135-modified adipose-derived mesenchymal stem cells in bone regeneration. *Biomaterials* 75**,** 279-294. doi: 10.1016/j.biomaterials.2015.10.042.

Xie, Q., Wei, W., Ruan, J., Ding, Y., Zhuang, A., Bi, X., et al. (2017). Effects of miR-146a on the osteogenesis of adipose-derived mesenchymal stem cells and bone regeneration. *Sci Rep* 7**,** 42840. doi: 10.1038/srep42840.

Xiong, A., He, Y., Gao, L., Li, G., Weng, J., Kang, B., et al. (2020). Smurf1-targeting miR-19b-3p-modified BMSCs combined PLLA composite scaffold to enhance osteogenic activity and treat critical-sized bone defects. *Biomater Sci* 8(21)**,** 6069-6081. doi: 10.1039/d0bm01251c.

Xue, Y., Guo, Y., Yu, M., Wang, M., Ma, P.X., and Lei, B. (2017). Monodispersed Bioactive Glass Nanoclusters with Ultralarge Pores and Intrinsic Exceptionally High miRNA Loading for Efficiently Enhancing Bone Regeneration. *Adv Healthc Mater* 6(20). doi: 10.1002/adhm.201700630.

Yan, J., Lu, X., Zhu, X., Hu, X., Wang, L., Qian, J., et al. (2020). Effects of miR-26a on Osteogenic Differentiation of Bone Marrow Mesenchymal Stem Cells by a Mesoporous Silica Nanoparticle - PEI - Peptide System. *Int J Nanomedicine* 15**,** 497-511. doi: 10.2147/ijn.S228797.

Yan, J., Zhang, C., Zhao, Y., Cao, C., Wu, K., Zhao, L., et al. (2014). Non-viral oligonucleotide antimiR-138 delivery to mesenchymal stem cell sheets and the effect on osteogenesis. *Biomaterials* 35(27)**,** 7734-7749. doi: 10.1016/j.biomaterials.2014.05.089.

Yan, S.G., Zhang, J., Tu, Q.S., Ye, J.H., Luo, E., Schuler, M., et al. (2011). Enhanced osseointegration of titanium implant through the local delivery of transcription factor SATB2. *Biomaterials* 32(33)**,** 8676-8683. doi: 10.1016/j.biomaterials.2011.07.072.

Yang, C., Liu, X., Zhao, K., Zhu, Y., Hu, B., Zhou, Y., et al. (2019). miRNA-21 promotes osteogenesis via the PTEN/PI3K/Akt/HIF-1α pathway and enhances bone regeneration in critical size defects. *Stem Cell Res Ther* 10(1)**,** 65. doi: 10.1186/s13287-019-1168-2.

Yang, L., He, X., Jing, G., Wang, H., Niu, J., Qian, Y., et al. (2021). Layered Double Hydroxide Nanoparticles with Osteogenic Effects as miRNA Carriers to Synergistically Promote Osteogenesis of MSCs. *ACS Appl Mater Interfaces* 13(41)**,** 48386-48402. doi: 10.1021/acsami.1c14382.

Yoshizuka, M., Nakasa, T., Kawanishi, Y., Hachisuka, S., Furuta, T., Miyaki, S., et al. (2016). Inhibition of microRNA-222 expression accelerates bone healing with enhancement of osteogenesis, chondrogenesis, and angiogenesis in a rat refractory fracture model. *J Orthop Sci* 21(6)**,** 852-858. doi: 10.1016/j.jos.2016.07.021.

Yu, M., Xue, Y., Ma, P.X., Mao, C., and Lei, B. (2017). Intrinsic Ultrahigh Drug/miRNA Loading Capacity of Biodegradable Bioactive Glass Nanoparticles toward Highly Efficient Pharmaceutical Delivery. *ACS Appl Mater Interfaces* 9(10)**,** 8460-8470. doi: 10.1021/acsami.6b13874.

Yuan, X., Han, L., Lin, H., Guo, Z., Huang, Y., Li, S., et al. (2019). The role of antimiR-26a-5p/biphasic calcium phosphate in repairing rat femoral defects. *Int J Mol Med* 44(3)**,** 857-870. doi: 10.3892/ijmm.2019.4249.

Zhang, J., Zhang, T., Tang, B., Li, J., and Zha, Z. (2021a). The miR-187 induced bone reconstruction and healing in a mouse model of osteoporosis, and accelerated osteoblastic differentiation of human multipotent stromal cells by targeting BARX2. *Pathol Res Pract* 219**,** 153340. doi: 10.1016/j.prp.2021.153340.

Zhang, Y., Zhuang, Z., Wei, Q., Li, P., Li, J., Fan, Y., et al. (2021b). Inhibition of miR-93-5p promotes osteogenic differentiation in a rabbit model of trauma-induced osteonecrosis of the femoral head. *FEBS Open Bio*. doi: 10.1002/2211-5463.13218.

Zhao, C., Gu, Y., Wang, Y., Qin, Q., Wang, T., Huang, M., et al. (2021). miR-129-5p Promotes Osteogenic Differentiation of BMSCs and Bone Regeneration via Repressing Dkk3. *Stem Cells Int* 2021**,** 7435605. doi: 10.1155/2021/7435605.

Zhao, C., Sun, W., Zhang, P., Ling, S., Li, Y., Zhao, D., et al. (2015). miR-214 promotes osteoclastogenesis by targeting Pten/PI3k/Akt pathway. *RNA Biol* 12(3)**,** 343-353. doi: 10.1080/15476286.2015.1017205.

Zhou, Y., Chen, X., Zhu, Z., Bi, D., and Ma, S. (2021). MiR-133a delivery to osteoblasts ameliorates mechanical unloading-triggered osteopenia progression in vitro and in vivo. *Int Immunopharmacol* 97**,** 107613. doi: 10.1016/j.intimp.2021.107613.