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Combination of Filtered Bone Marrow Aspirate and Biomimetic Scaffold for the Treatment of Knee Osteochondral Lesions: Cellular and Early Clinical Results of a Single Centre Case Series.

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Author information

Abstract

BACKGROUND:

Osteochondral injury is a very common orthopaedic pathology, mainly affecting young, active population, with limited current treatment options. Herein we are presenting cellular and early clinical data of a patient series treated for chronic osteochondral lesions in the knee with a filter-based intra-operative bone marrow aspirate (BMA) separation device.

METHODS:

Fifteen patients with chronic knee osteochondral lesions (60% females, 19-59 years) were included in this prospective case series. Filtered BMA (f-BMA), containing mesenchymal stem/stromal cells (MSCs), was combined with a biomimetic collagen-hydroxyapatite scaffold (CHAS) and implanted into the site of the lesion. Harvested BMA and post-separation f-BMA were analysed for blood cell counts, flow cytometry, and fibroblast colony forming units (CFU-Fs). Patients were followed for serious adverse events and graft failures. Clinical evaluation was assessed using the knee injury and osteoarthritis outcome score (KOOS). In 8 patients a magnetic resonance imaging (MRI)/arthroscopy were performed.

RESULTS:

Cell suspension contained 0.027% $CD271^+$ $CD45^-$ $7\text{-}AAD^-$ cells, 0.15% $CD73^+$ $CD90^+$ $CD105^+$ cells and 0.0012% CFU-Fs of all nucleated cells with 86% viability. Filtration process resulted in 12.8 (4.0-40.8) fold enrichment in terms of CFU-F content in comparison to initial BMA. No serious adverse events related directly to the osteochondral treatment were reported. After an average follow-up of 20 months (14-25) all KOOS subscales (Symptoms/Pain/Daily activities/Sport and recreation/Quality of life) increased significantly from pre-operative 55/56/67/30/30 to post-operative 73/76/79/51/52 (p values < 0.05), respectively. MRI or arthroscopic evaluation revealed nearly normal to normal overall International Cartilage Repair Society assessment in 7/8 patients.

CONCLUSION:

The filter-based BMA separation procedure significantly increased the frequency of mesenchymal stem/stromal cells (MSCs), however their concentration was not increased. The clinical evaluation revealed high safety profile of the treatment and resulted in improved clinical status of the patients.

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Adipose-Derived Mesenchymal Stem Cell Treatments and Available Formulations.

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Abstract

PURPOSE OF REVIEW:

The use of human adipose-derived mesenchymal stem cells (ADSCs) has gained attention due to its potential to expedite healing and the ease of harvesting; however, clinical evidence is limited, and questions concerning optimal method of delivery and long-term outcomes remain unanswered.

RECENT FINDINGS:

Administration of ADSCs in animal models has been reported to aid in improved healing benefits with enhanced repair biomechanics, superior gross histological appearance of injury sites, and higher concentrations of growth factors associated with healing compared to controls. Recently, an increasing body of research has sought to examine the effects of ADSCs in humans. Several available processing techniques and formulations for ADSCs exist with evidence to suggest benefits with the use of ADSCs, but the superiority of any one method is not clear. Evidence from the most recent clinical studies available demonstrates promising outcomes following treatment of select musculoskeletal pathologies with ADSCs despite reporting variability among ADSCs harvesting and processing; these include (1) healing benefits and pain improvement for rotator cuff and Achilles tendinopathies, (2) improvements in pain and function in those with knee and hip osteoarthritis, and (3) improved cartilage regeneration for osteochondral focal defects of the knee and talus. The limitation to most of this literature is the use of other therapeutic biologics in combination with ADSCs. Additionally, many studies lack control groups, making establishment of causation inappropriate. It is imperative to perform higher-quality studies using consistent, predictable control populations and to standardize formulations of ADSCs in these trials

Int J Mol Sci. 2020 Apr 15;21(8). pii: E2753. doi: 10.3390/ijms21082753.

The Expression Profile of Dental Pulp-Derived Stromal Cells Supports Their Limited Capacity to Differentiate into Adipogenic Cells.

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Abstract

Mesenchymal stromal cells (MSCs) can self-renew, differentiate into specialised cells and have different embryonic origins-ectodermal for dental pulp-derived MSCs (DPSCs) and mesodermal for adipose tissue-derived MSCs (ADSCs). Data on DPSCs adipogenic differentiation potential and timing vary, and the lack of molecular and genetic information prompted us to gain a better understanding of DPSCs adipogenic differentiation potential and gene expression profile. While DPSCs differentiated

readily along osteogenic and chondrogenic pathways, after 21 days in two different types of adipogenic induction media, DPSCs cultures did not contain lipid vacuoles and had low expression levels of the adipogenic genes proliferator-activated receptor gamma (PPARG), lipoprotein lipase (LPL) and CCAAT/enhancer-binding protein alpha (CEBPA). To better understand this limitation in adipogenesis, transcriptome analysis in undifferentiated DPSCs was carried out, with the ADSC transcriptome used as a positive control. In total, 14,871 transcripts were common to DPSCs and ADSCs, some were unique (DPSCs: 471, ADSCs: 1032), and 510 were differentially expressed genes. Detailed analyses of overrepresented transcripts showed that DPSCs express genes that inhibit adipogenic differentiation, revealing the possible mechanism for their limited adipogenesis.

Cells. 2020 Apr 21;9(4). pii: E1034. doi: 10.3390/cells9041034.

Citrate Mediates Crosstalk between Mitochondria and the Nucleus to Promote Human Mesenchymal Stem Cell In Vitro Osteogenesis.

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Abstract

Citrate, generated in the mitochondria, is a key metabolite that might link metabolism with signaling, chromatin structure and transcription to orchestrate mesenchymal stem cells (MSCs) fate determination. Based on a detailed morphological analysis of 3D reconstruction of mitochondria and nuclei in single cells, we identified contact sites between these organelles that drastically increase in volume and number during the early stage of mesenchymal stem cell differentiation. These contact sites create a microdomain that facilitates exchange of signals from mitochondria to the nucleus. Interestingly, we found that the citrate derived from mitochondria is necessary for osteogenic lineage determination. Indeed, inhibition of the citrate transporter system dramatically affected osteogenesis, reduced citrate levels that could be converted in α-ketoglutarate, and consequently affected epigenetic marker H3K9me3 associated with the osteogenesis differentiation process. These findings highlight that mitochondrial metabolites play key regulatory roles in the MSCs differentiation process. Further in-depth investigation is needed to provide novel therapeutic strategies in the field of regenerative medicine**.**

Pharmaceutics. 2020 Apr 21;12(4). pii: E381. doi: 10.3390/pharmaceutics12040381.

Polyphenols-Loaded Sericin Self-Assembling Nanoparticles: A Slow-Release for Regeneration by Tissue-Resident Mesenchymal Stem/Stromal Cells.

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Abstract

Mesenchymal stem/stromal cells (MSCs) are a therapeutic target to promote tissue regeneration, mainly when oxidative stress-mediated damage is involved in disease pathogenesis. Here, slow-release silk sericin nanoparticles (SNPs) loaded with natural antioxidant polyphenols were developed to sustain regeneration by tissue-resident MSCs. SNPs were prepared by exploiting a self-assembly method with poloxamer and were loaded with proanthocyanidins (P), quercetin (Q) or epigallocatechin gallate (E). SNPs, with a diameter less than 150 nm, were able to encapsulate both hydrophilic (P and E) and hydrophobic (Q) drugs. A slow and controlled release was obtained from SNPs for all the actives in PBS, while in EtOH, Q and E showed a burst release but P did not. Kinetic models revealed lower diffusion of P than other biomolecules, probably due to the higher steric hindrance of P. The in vitro anti-oxidant, anti-elastase and anti-tyrosinase properties of SNPs were assessed: loading the P and E into SNPs preserved the in vitro biological activities whereas for Q, the anti-elastase activity was strongly improved. Moreover, all formulations promoted MSC metabolic activity over 72 h. Finally, SNPs exhibited a strong ability to protect MSCs from oxidative stress, which supports their potential use for regenerative purposes mediated by tissue-resident MSCs.

ACS Appl Mater Interfaces. 2020 Apr 23. doi: 10.1021/acsami.9b20939. [Epub ahead of print]

Cell-Cell Adhesion Driven Contact Guidance and its Effect on hMSC Differentiation.

Le Saux G, Wu MC, Toledo E, Chen YQ, Fan YJ, Kuo JC, Schvartzman M.

Abstract

Contact guidance has been extensively explored using patterned adhesion functionalities that predominantly mimic cell-matrix interactions. Whether similar contact guidance can be driven by other types of interactions, such as cell-cell adhesion, still remains a question. Herein, we address this query by engineering a set of microstrip patterns of (i) cell-cell adhesion ligands and (ii) segregated cell-cell and cell-matrix ligands, as a simple yet versatile set of platforms for the guidance of spreading, adhesion, and differentiation of mesenchymal stem cells. We found, for the first time to the best of our knowledge, that micropatterns of cell-cell adhesion ligands can induce contact guidance. Surprisingly, we found that patterns of alternating cell-matrix and cell-cell strips also induce contact guidance despite providing a spatial continuum for cell adhesion. We believe that this guidance is due to the difference between the potencies of the two adhesions. Furthermore, we found that patterns that combine the two segregated adhesion functionalities induce more human mesenchymal stem cell osteogenic differentiation than monofunctional patterns. This work provides a new insight into the functional crosstalk between cell-cell and cell-matrix adhesions and overall, further highlights the ubiquitous impact of the biochemical anisotropy of the extracellular environment on cell function.

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A bone matrix-simulating scaffold to alleviate replicative senescence of mesenchymal stem cells during long-term expansion.

Su X[1,2], Jing H[3], Yu W[2], Lei F[1,2], Wang R[1,2], Hu C[2], Li M[1,2], Lin T[1,2], Zhou H[2], Wang F[2], Liao L[1,4].
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Abstract

Replicative senescence during in vitro augmentation, which is mostly induced by the loss of physiological microenvironment, hinders the application of mesenchymal stem cells (MSCs) in the clinic. Here, we investigated whether MSCs senescence could be prevented by bio-scaffold mimicking the natural tissue matrix. Human umbilical cord mesenchymal stem cells (hUCMSCs) exhibited a senescent phenotype during a long-term passage in the conventional culture dish. To fabricate the bone matrix, a naturally-based matrix composed of nano-hydroxyapatite/chitosan/poly lactide-co-glycolide (nHA/CS/PLGA) was produced. Long-term passage resulted in an obvious increase in the expression of senescence markers and a reduction in the expression of master genes involved in tissue regeneration. Functional assay confirmed that nHA/CS/PLGA scaffold preserved the proliferation and differentiation of hUCMSCs even after being passaged 27 times. Moreover, in vivo ectopic bone formation assay revealed the bone formation of hUCMSCs cultured on the nano-scaffolds for the long term was as robust as the cells in the early passage. In summary, our results demonstrate that nHA/CS/PLGA scaffold effectively preserves the stemness and youth of hUCMSCs in the long-term passage. Taken advantage of its compatibility and bioactivity, nHA/CS/PLGA scaffold is of great potential in large-scale expansion of MSCs for stem cell therapy and tissue engineering.

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Human bone marrow mesenchymal stem cell injection in subchondral lesions of knee osteoarthritis: a prospective randomized study versus contralateral arthroplasty at a mean fifteen year follow-up.

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Abstract

PURPOSE:

Recently, mesenchymal stem cells (MSCs) have been proposed as potential treatment modalities for knee osteoarthritis. However, indications and long-term results have not been frequently reported. The purpose of this study was to determine whether bone marrow lesion on MRI are predictive of risk progression to total knee arthroplasty during the first ten years after subchondral cell therapy.

METHODS:

This study included 140 adults aged 65 to 90 years. These 140 patients (mean age 75.4 ± 14.2 years) planned to undergo staged-bilateral total knee arthroplasty (TKA) for medial osteoarthritis, had "comparable" pain in both knees, and accepted randomization of the knees for surgery. They received TKA on one side and a subchondral injection of MSCs (from iliac bone marrow concentrate) on the contralateral knee during the same anaesthetic. The bone marrow graft of 20 cm^3 volume (10 cc in the tibia and 10 cc in the femur) contained average 7800 MSCs/mL (range 3120 to 11,560). The baseline

volume of bone marrow lesions (BMLs) on the tibia and on the femoral condyle determined on MRI was average 3.4 cm^3 (range 0.4 to 6.4 cm^3). The risk of subsequent knee arthroplasty due to absence of bone marrow lesions regression as well as osteoarthritis (OA) grade was evaluated with Cox proportional-hazards ratio after control of baseline variables (number of cells injected, age, knee alignment).

RESULTS:

After treatment with MSCs injection in bone marrow lesions of the subchondral bone, medial femorotibial compartment BML volume experienced regression over 24 months (mean regression 1.5 cm^3, range 0.8 to 3.2 cm^3). At the most recent follow up (average of 15 years, range 10 to 20 years), a total of 25 (18%) of the 140 patients underwent total knee arthroplasty performed at a mean of ten years (range, 5 to 15 years) after the date of the cell therapy. The overall incidence of knee arthroplasty after cell therapy was 1.19% per person-year which was equivalent to the risk of a revision for a primary TKA in the contralateral knees of the same patient population (21 revisions, corresponding to 1.00% revision per person-year; $p = 0.34$). After adjusting for confounders, persistent BMLs larger than 3 cm^3 after cell therapy was a strong independent risk factor for total knee arthroplasty (hazard ratio HR = 4.42 [95% CI = 2.34 to 7.21]; $p < 0.001$), regardless of OA grade, with higher risks demonstrated for larger BMLs. Incidence rates of arthroplasty were also higher for young patients and for knees presenting severe malalignment.

CONCLUSIONS:

This study showed that subchondral bone marrow concentrate (as compared with TKA) had a sufficient effect on pain to postpone or avoid the TKA in the contra lateral joint of patients with bilateral osteoarthritis. Bone marrow lesions were predictive factors for future knee arthroplasty in the knee with subchondral cell therapy at ten years follow-up.

KEYWORDS:

Bone marrow; Knee osteoarthritis; Mesenchymal stem cells; Subchondral bone injectio

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Therapeutic potential of stem cell-derived extracellular vesicles in osteoarthritis: preclinical study findings.

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Author information

Abstract

Extracellular vesicles (EVs) are nano-sized particles secreted by almost all cell types, and they mediate various biological processes via cell-to-cell communication. Compared with parental cells for therapeutic purposes, stem cell-derived EVs have several advantages such as reduced risk of rejection, less oncogenic potential, ease of long-term storage, lower chance of thromboembolism, and readiness for immediate use. Recent studies have demonstrated that EVs from stem cells, mostly from mesenchymal stem cells (MSCs) from various tissues, have anti-inflammatory, anti-oxidative, anti-

apoptotic, and proliferative role in injured organs including osteoarthritic lesions. Herein, we provide a review about the up-to-date studies in preclinical application of stem cell-derived EVs in osteoarthritis animal arthritis models.

Stem Cells Int. 2020 Apr 3;2020:3150716. doi: 10.1155/2020/3150716. eCollection 2020.

Vitamin C Treatment Rescues Prelamin A-Induced Premature Senescence of Subchondral Bone Mesenchymal Stem Cells.

Qu YN[1], Zhang L[2], Wang T[2], Zhang HY[2], Yang ZJ[2], Yuan FF[2], Wang Y[2], Li SW[2], Jiang XX[2], Xie XH[1].
Author information

Abstract

Aging is a predominant risk factor for many chronic conditions. Stem cell dysfunction plays a pivotal role in the aging process. Prelamin A, an abnormal processed form of the nuclear lamina protein lamin A, has been reported to trigger premature senescence. However, the mechanism driving stem cell dysfunction is still unclear. In this study, we found that while passaging subchondral bone mesenchymal stem cells (SCB-MSCs) *in vitro*, prelamin A accumulation occurred concomitantly with an increase in senescence-associated *β*-galactosidase (SA-*β*-Gal) expression. Unlike their counterparts, SCB-MSCs with prelamin A overexpression (MSC/PLA) demonstrated decreased proliferation, osteogenesis, and adipogenesis but increased production of inflammatory factors. In a hind-limb ischemia model, MSC/PLA also exhibited compromised therapy effect. Further investigation showed that exogenous prelamin A triggered abnormal nuclear morphology, DNA and shelterin complex damage, cell cycle retardation, and eventually cell senescence. Changes in gene expression profile were also verified by microarray assay. Interestingly, we found that ascorbic acid or vitamin C (VC) treatment could inhibit prelamin A expression in MSC/PLA and partially reverse the premature aging in MSC/PLA, with reduced secretion of inflammatory factors and cell cycle arrest and resistance to apoptosis. Importantly, after VC treatment, MSC/PLA showed enhanced therapy effect in the hind-limb ischemia model. In conclusion, prelamin A can accelerate SCB-MSC premature senescence by inducing DNA damage. VC can be a potential therapeutic reagent for prelamin A-induced aging defects in MSCs.

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Cancer stem cell and mesenchymal cell cooperative actions in metastasis progression and hormone resistance in prostate cancer: Potential role of androgen and gonadotropin-releasing hormone receptors (Review).

Contreras HR[1], López-Moncada F[1], Castellón EA[1].
Author information

Abstract

Prostate cancer (PCa) is the leading cause of male cancer-associated mortality worldwide. Mortality is associated with metastasis and hormone resistance. Cellular, genetic and molecular mechanisms

underlying metastatic progression and hormone resistance are poorly understood. Studies have investigated the local effects of gonadotropin-releasing hormone (GnRH) analogs (used for androgen deprivation treatments) and the presence of the GnRH receptor (GnRH-R) on PCa cells. Furthermore, cell subpopulations with stem-like properties, or cancer stem cells, have been isolated and characterized using a cell culture system derived from explants of human prostate tumors. In addition, the development of preclinical orthotopic models of human PCa in a nonobese diabetic/severe combined immunodeficiency mouse model of compromised immunity has enabled the establishment of a reproducible system of metastatic progression in vivo. There is increasing evidence that metastasis is a complex process involving the cooperative actions of different cancer cell subpopulations, in which cancer stem-like cells would be responsible for the final step of colonizing premetastatic niches. It has been hypothesized that PCa cells with stemness and mesenchymal signatures act cooperatively in metastatic progression and the inhibition of stemness genes, and that overexpression of androgen receptor (AR) and GnRH-R decreases the rate the metastasis and sensitizes tumors to hormone therapy. The aim of the present review is to analyze the evidence regarding this cooperative process and the possible influence of stem-like cell phenotypes, AR and GnRH-R in metastatic progression and hormone resistance. These aspects may represent an important contribution in the understanding of the mechanisms underlying metastasis and hormone resistance in PCa, and potential routes to blocking these processes, enabling the development of novel therapies that would be particularly relevant for patients with metastatic and castration-resistant PCa.

J Biomed Mater Res A. 2020 Apr 21. doi: 10.1002/jbm.a.36967. [Epub ahead of print]

Spreading Area and Shape Regulate the Apoptosis and Osteogenesis of Mesenchymal Stem Cells on Circular and Branched Micropatterned Islands.

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Abstract

The topography of extracellular matrix regulates the differentiation of mesenchymal stem cells (MSCs). In particular, the effect of spreading shape or area on cellular differentiation and viability of individual MSCs cultured in the confined adhesive regions is an interesting fundamental issue. In this study, the adhesive patterns with the circularity of 0.1 or 1 and the areas of 314, 628, 1256 or 2512 μm^2 were constructed using micropatterning technology. The expression of osteogenesis marker alkaline phosphatase and the apoptosis level of individual MSCs were measured using double fluorescent staining. Results indicated that individual MSCs confined in the small area showed an apoptotic tendency, and those in the large area might enter into osteogenesis. The branched shape with small circularity increased MSC viability but reduced their pluripotency compared with the circular shape. The expression of other osteogenesis. markers, such as osteocalcin and collagen I, confirmed that large and branched pattern promoted MSC osteogenesis. In addition, the transcriptional coactivator yes-associated protein (YAP) was transfered higher in the nuclei of the large and branched cells than other

micropatterned groups. This study suggested that the spreading area and shape of individual MSCs regulate their viability and osteogenesis through the YAP pathway.

Biomed Res Int. 2020 Mar 27;2020:9847579. doi: 10.1155/2020/9847579. eCollection 2020.

Effects of Mesenchymal Stem Cell Coculture on Human Lung Small Airway Epithelial Cells.

Schmelzer E[1], Miceli V[2], Chinnici CM[3,4], Bertani A[5], Gerlach JC[1,6].
Author information

Abstract

Mesenchymal stem cells (MSCs) and their secreted extracellular vesicles have been used effectively in different lung disease animal models and clinical trials. Their specific beneficial effects, the potential differences between MSCs derived from different organs, and interactions between MSC products and target cells still need to be studied further. Therefore, we investigated the effects of secreted products of human MSCs derived from the bone marrow and adipose tissue on human lung small airway epithelial (AE) cells *in vitro*. AE cells were cocultured with MSCs in inserts that allowed the free exchange of medium but did not allow direct cell-to-cell contact. We examined the effects on AE cell viability, proliferation, cell numbers, expression of AE cell-specific genes, and CD54 (intercellular adhesion molecule 1 (ICAM1)) surface positivity, as well as the secretion/uptake of growth factors relevant for AE cell. We found that coculture increased the viability of AE cells. The majority of AE cells expressed CD54 on their surface, but the percentage of cells being positive for CD54 did not increase in coculture. However, ICAM1 gene expression was increased in coculture. Also, we observed increased gene expression of mucin (MUC1), a lung-enriched cell surface glycoprotein. These observed effects were the same between bone marrow and adipose tissue MSCs. However, MSCs derived from adipose tissue reduced angiopoietin concentrations in coculture, whereas those from the bone marrow did not. Conclusively, MSCs influenced AE cells positively by increasing their viability and affecting gene expression, with some effects being specific for the tissue origin of MSCs.

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Functionally engineered extracellular vesicles improve bone regeneration.

Huang CC[1], Kang M[1], Lu Y[1], Shirazi S[1], Diaz JI[1], Cooper LF[1], Gajendrareddy P[2], Ravindran S[3].
Author information

Abstract

Lineage specific differentiation of host mesenchymal stem cells (MSCs) is a necessary step for bone repair/regeneration. Clinically, growth factors such as bone morphogenetic protein 2 (BMP2) are used to enhance/hasten this process to heal critical sized defects. However, the clinical application of such growth factors is fraught with dosage challenges as well as immunological and ectopic complications. The identification of extracellular vesicles (EVs) as active components of the MSC secretome suggest alternative approaches to enhancing bone regeneration. Based on our earlier studies on the properties

of EVs from lineage specified MSCs, this study sought to engineer EVs to enhance osteogenic differentiation. To generate MSC EVs with enhanced osteoinductive abilities, genetically modified human bone marrow derived MSCs (HMSCs) were generated by constitutively expressing BMP2. We hypothesized that these cells would generate functionally engineered EVs (FEEs) with enhanced osteoinductive properties. Our results show that these FEEs maintained the general physical and biochemical characteristics of naïve HMSC EVs in the form of size distribution, EV marker expression and endocytic properties but show increased bone regenerative potential compared to MSC EVs in a rat calvarial defect model in vivo. Mechanistic studies revealed that although BMP2 was constitutively expressed in the parental cells, the corresponding EVs (FEEs) do not contain BMP2 protein as an EV constituent. Further investigations revealed that the FEEs potentiate the BMP2 signaling cascade possibly due to an altered miRNA composition. Collectively, these studies indicate that EVs' functionality may be engineered by genetic modification of the parental MSCs to induce osteoinduction and bone regeneration. SIGNIFICANCE STATEMENT: With mounting evidence for the potential of MSC EVs in treatment of diseases and regeneration of tissues, it is imperative to evaluate if they can be modified for application specificity. The results presented here indicate the possibility for generating Functionally Engineered EVs (FEEs) from MSC sources. As a proof of concept approach, we have shown that EVs derived from genetically modified MSCs (BMP2 overexpression) can be effective as biomimetic substitutes for growth factors for enhanced tissue-specific regeneration (bone regeneration) in vivo. Mechanistic studies highlight the role of EV miRNAs in inducing pathway-specific changes. We believe that this study will be useful to researchers evaluating EVs for regenerative medicine applications.