Stem Cells and Cloning: Advances and Applications

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ORIGINAL RESEARCH

Stem-cell therapy for ovariectomy-induced osteoporosis in rats: a comparison of three treatment modalities

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Background: Recent studies have shown that ovariectomy-induced osteoporosis in rats can be reversed by infusion of osteoblasts cultured from mesenchymal stem cells (MSCs). This study compares the influence of MSCs, osteoblasts, and exosomes derived from osteoblasts for the treatment of osteoporosis.

Methods: Osteoporosis was induced in 40 female Sprague Dawley rats by performing ovariectomy. After 12 weeks, bone marrow was harvested and MSCs separated from bone-marrow aspirate as described by Piao et al. After 15 days, autologous osteogenically differentiated cells from the MSCs were available. Exosomes were isolated from osteoblasts by modification of the technique described by Ge et al. MSCs and osteoblasts (10^6 cells in 0.5 mL normal saline) and exosomes (100 µg protein) were injected into the tail veins of the animals. Animals were euthanized after 12 weeks and femurs and lumbar spines dissected and analyzed using high-resolution peripheral quantitative computed tomography.

Results: When compared to the control group, osteoblast-treated animals showed significant differences in all parameters compared, with *P*-values ranging between <0.002 and <0.0001. Comparison among osteoblasts, MSCs, and exosomes, showed that osteoblasts had positive and statistically significant new-bone formation. The comparison for the spine was similar to the distal femur for osteoblasts.

Conclusion: This study showed robust positive bone-forming changes after osteoblast injection in the distal femur and the spine when compared to controls, MSCs, and exosomes.

Keywords: osteoporosis, ovariectomy, osteoblasts, mesenchymal stem cells (MSCs), exosomes

Introduction

Osteoporosis is a chronic and debilitating disease of aging. Postmenopausal osteoporosis has come close to becoming an epidemic in the developed and developing world, with increased morbidity and mortality. The “silent disease", as it is rightfully termed, presents with a fracture due to minimal trauma its first indication.[5] The true cost of managing osteoporosis-related fractures in Saudi Arabia is still unknown, but a recent study indicated that by 2050, the lifetime cost of treating osteoporosis-related femoral fractures in Saudi Arabia may reach over SR35 billion annually (US$9.33 billion).[6] The cost of managing osteoporosis is increasing exponentially, but new modalities of treatment are lagging behind. We still are dependent on antiresorptives, such as bisphosphonates, anabolic agents like synthetic parathyroid hormone, and the newer

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monoclonal antibody denosumab, and all have their limitations and complications of use.[7–12]

Preclinical studies have shown promising results in the management of ovariectomy-induced osteoporosis. Kiernan et al[2] showed that mesenchymal stem cells (MSCs) injected into a mouse model led to improved bone formation and reversed microarchitecture, suggesting that MSC injections may be used to combat osteoporosis, whereas Sadat-Ali et al[1] reported that culture-expanded osteoblasts, when injected in ovariectomized (Ovx) rats, were effective in significantly increasing bone formation.

Exosomes, which are bioactive microvesicles, are secreted by MSCs, and osteoblasts are nanoparticles of 30–100 nm in size and carry proteins and RNAs.[13] MSC-derived exosomes have been found to mediate in promoting healing of tissue and repair of acute and chronic injuries.[14]

With the increasing number of aged people around the world and increase in the longevity of the human race, osteoporosis is bound to trouble health-care economics, added to the fact that research and development of new osteoporosis drugs are at a standstill. Stem-cell therapy opens a new avenue in the treatment of osteoporosis if the reversal of osteoporosis in animal studies is confirmed.

The objective of this study was to compare the efficacy of culture expanded MSCs, osteoblasts, and osteoblast-derived exosomes on the effect of Ovx-induced osteoporosis in rats.

Methods

Ethical approval was obtained from Imam AbdulRahman Bin Faisal University, Dammam, Saudi Arabia (vide number 2015116/2017), 40 Sprague Dawley female rats were used as our model to study reversal of osteoporosis. The rats were housed and handled in accordance with the National Advisory Committee for Laboratory Animal Research guidelines. Animals were accommodated with total mobility, fed a standard diet, and the room maintained at 26°C. Ovariectomy was done at 1 month of age to cause osteoporosis. At 12 weeks, a bone biopsy was performed to look at the quality of bone. Bone marrow was aspirated at the time of the biopsy. From the bone-marrow aspirate of the individual rats, using the technique described by Piao et al,[3] MSCs were separated. In this study, no MSCs from other species, such as human or BALB/c mouse were used. For each individual rat, MSCs obtained from the bone-marrow aspirate and osteoblasts cultured from the cell suspension, as descrbed earlier.[2] After 2 weeks, osteoblasts were ready to be injected.

Isolation of exosomes from rat bone-marrow MSC–derived osteoblasts

MSC-derived osteoblasts were seeded onto T75 cell-culture flasks (Nalge Nunc International, New York, NY, USA) at a rate of 10^3 cells/cm^2 alongside 20 mL basal DMEM (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% exosome-depleted FBS (Thermo Fisher Scientific), 5 mM L-glutamine (Thermo Fisher Scientific) , 100 IU/mL penicillin (Sigma-Aldrich, St Louis, MO, USA) and 100 µg/mL streptomycin (Sigma-Aldrich). Cells were allowed to attach and grow up to 4 days in the same medium at 37°C at 5% CO_2 and 95% humidity in a CO_2 incubator. Culture supernatant containing osteoblast exosomes was aseptically collected from five of such flasks into two 5 mL cenTrifuge tubes. Adhered osteoblast cells were harvested and frozen for further use. The culture supernatant was further subjected to ultracentrifugation to pelletize exosomes. If present, any cells were removed by spinning the supernatant at 300 *g* for 10 minutes at 4°C. Dead cells in the supernatant were removed by centrifugation at 2,000 *g* for 10 minutes at 4°C. Further cenTrifugation at 10,000 *g* for 30 minutes at 4°C was done to pelletize and remove cell debris. Finally, the supernatant was ultracentrifuged (Sorvall WX) at 100,000 *g* for 70 minutes at 4°C under vacuum to pelletize exosomes. Contaminated protein in the pelletized exosomes was removed by repeated washing in normal saline solution (0.85% NaCl) before final pelletization at the same speed. Pelletized exosomes were resuspended in normal saline solution and concentration measured (as 100 µg/mLprotein) and aliquoted for further use.

Animals were divided into four groups: group 1 (control) received injected normal saline, group 2 MSCs, group 3 osteoblasts, and group 4 exosomes. MSCs and osteoblasts of 10^6 cells in 0.5 mL normal saline and (for exosomes) 100 µg protein were injected into the tail veins of the animals.

Rats were killed after 8 weeks of MSC, osteoblast, and exosome infusion by overdose of ketamine mixed with xylazine. The study bones were dissected, stored in 1% formalin, and grouped. The specimens were shipped to B-Cube , Brüttisellen, Switzerland. New-bone formation and bone-strength parameters were measured and calculated as reported earlier.[2]

Table 1 Structural indices of distal femur in the four groups analyzed by high-resolution peripheral quantitative computed tomography

Parameter	Group 1 (control)	Group 2 (MSCs)	Group 3 (osteoblasts)	Group 4 (exosomes)
TV, mm^3 (voxels)	42.178±4.727	38.894±5.528	31.615±1.331	42.836±4.563
BV, mm^3 (voxels)	3.933±1.540	3.954±0.967	5.959±1.953	4.274±0.930
BV/TV, % (voxels)	0.091±0.027	0.102±0.021	0.191±0.071	0.099±0.015
Trabecular number, 1/mm(voxels)	0.839±0.268	0.850±0.315	2.813±2.324	0.869±0.104
Trabecular thickness, 1/mm (voxels)	0.081±0.002	0.082±0.004	0.064±0.001	0.076±0.004
Connectivity density (normed by TV), $1/mm^3$ (voxels)	27.765±12.122	32.061±7.396	167.767±81.358	33.741±5.493
Trabecular separation (marrow thickness), mm (voxels)	1.348±0.441	1.364±0.453	0.552±0.327	1.245±0.178
TV, mm^3 (Tri)	41.753±4.696	38.483±5.499	31.234±1.315	42.401±4.550
BV, mm^3 (Tri)	3.933±1.540	3.892±0.962	5.810±1.972	4.212±0.928
BS (Tri)	107.696±27.559	120.488±30.732	228.553±69.641	137.280±27.154
BS/BV (Tri)	32.061±0.886	30.951±2.123	39.622±1.512	32.750±1.582
Trabecular number, 1/mm (Tri)	1.440±0.396	1.562±0.297	3.695±1.300	1.610±0.184
Trabecular thickness, 1/mm (Tri)	0.062±0.002	0.065±0.004	0.071±0.002	0.061±0.003
Trabecular spacing	0.671±0.215	0.590±0.119	0.240±0.087	0.566±0.079

Note: Tri, based on Triangularization of surface. Data shown as mean ± SD.
Abbreviations: TV, total volume; BV, bone volume; MSCs, mesenchymal stem cells; BS, bone surface.

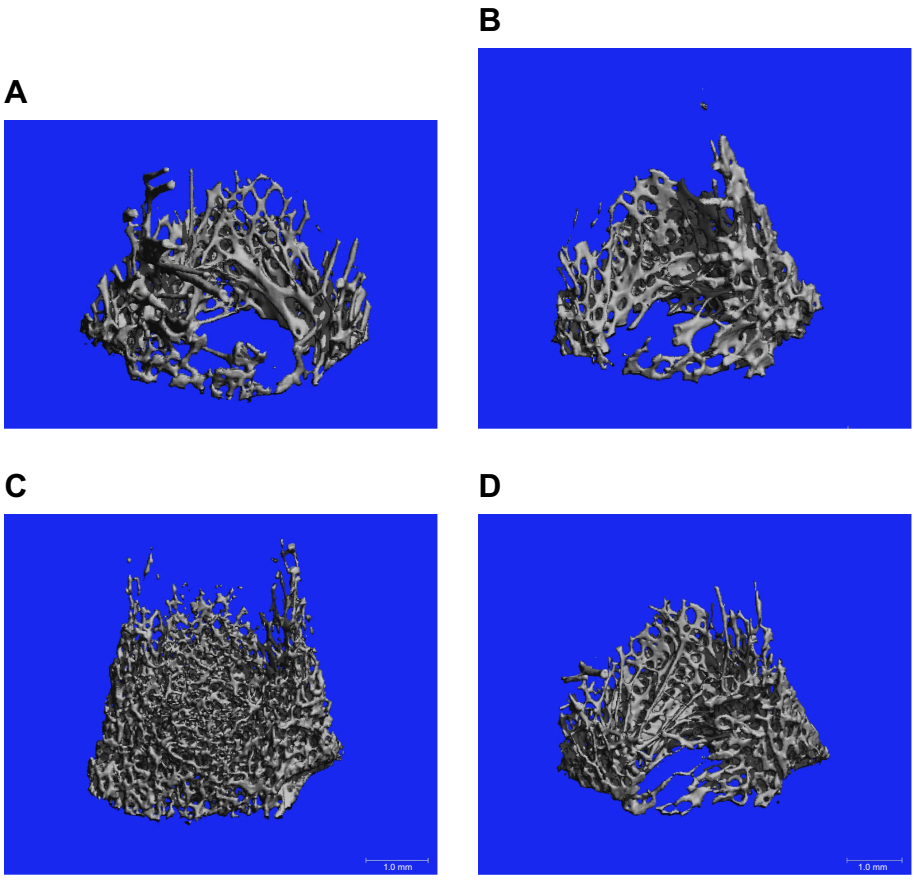

Figure 1 Distal femur analyzed by high-resolution peripheral quantitative computed tomography compariing control (**A**) and treated groups of mesenchymal stem cells (**B**), osteoblasts (**C**), and exosomes (**D**).

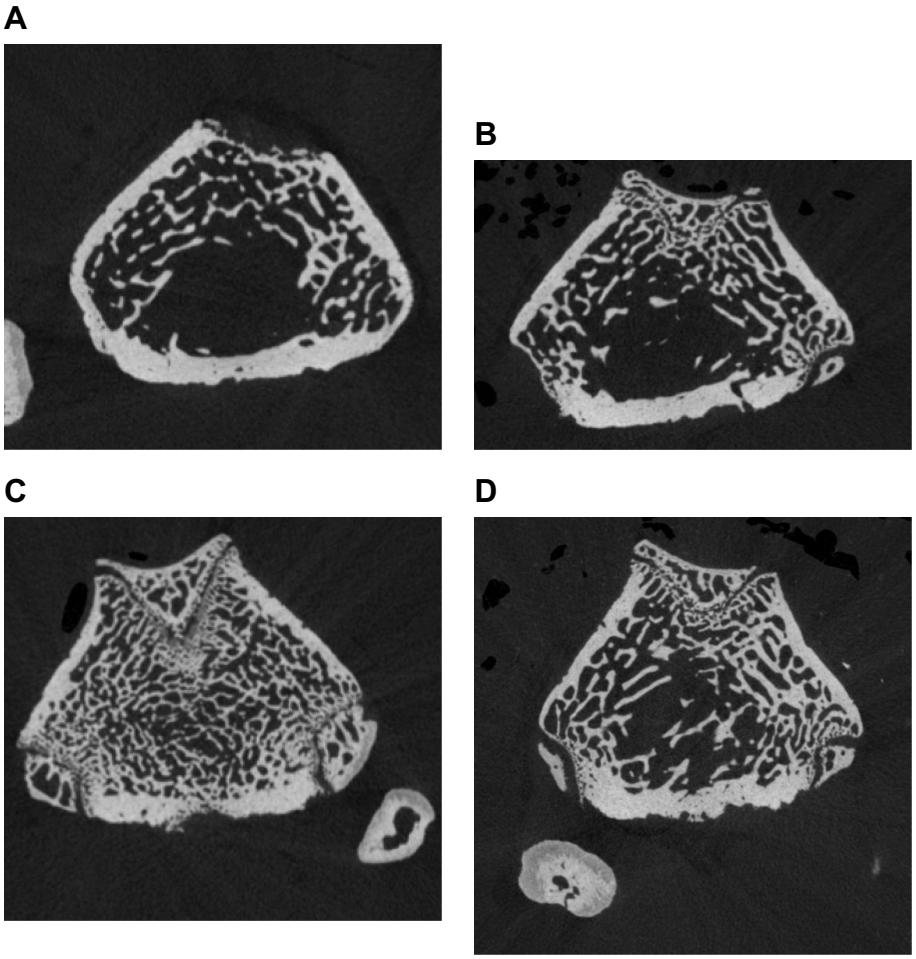

Figure 2 Sagittal sections of distal femur analyzed by high-resolution peripheral quantitative computed tomography compairing control (**A**) and treated groups of mesenchymal stem cells (**B**), osteoblasts (**C**), and exosomes (**D**).

Table 2 Comparison between control versus MSCs for distal femur

Parameter	Group 1 (control)	Group 2 (MSCs)	*P*-value
TV, mm^3 (voxels)	42.178±4.727	38.894±5.528	0.093
BV, mm^3 (voxels)	3.933±1.540	3.954±0.967	0.9
BV/TV, % (voxels)	0.091±0.027	0.102±0.021	0.06
Trabecular number, 1/mm (voxels)	0.839±0.268	0.850±0.315	0.9
Trabecular thickness, 1/mm (voxels)	0.081±0.002	0.082±0.004	0.5
Connectivity density, normed by TV, 1/mm^3 (voxels)	27.765±12.122	32.061±7.396	0.09
Trabecular separation = marrow thickness, mm (voxels)	1.348±0.441	1.364±0.453	0.53
TV, Tmm^3 (TRI)	41.753±4.696	38.483±5.499	0.09
BV, TV mm^3 (TRI)	3.933±1.540	3.892±0.962	0.89
TRI- BS	107.696±27.559	120.488±30.732	0.22
TRI- BS/BV	32.061±0.886	30.951±2.123	0.13
Trabecular number, 1/mm (TRI)	1.440±0.396	1.562±0.297	0.22
Trabecular thickness, 1/mm (TRI)	0.062±0.002	0.065±0.004	0.04
Trabecular spacing	0.671±0.215	0.590±0.119	0.05

Note: Tri, based on Triangularization of surface. Data shown as mean ± SD.
Abbreviations: TV, total volume; BV, bone volume; BS, bone surface.

Table 3 Comparison between control versus osteoblasts for distal femur

Parameter	Group 1 (control)	Group 3 (osteoblasts)	*P*-value
TV, mm^3 (voxels)	42.178±4.727	31.615±1.331	
BV, mm^3 (voxels)	3.933±1.540	5.959±1.953	0.009
BV/TV, % (voxels)	0.091±0.027	0.191±0.071	0.001
Trabecular number, 1/mm (voxels)	0.839±0.268	2.813±2.324	0.025
Trabecular thickness, 1/mm (voxels)	0.081±0.002	0.064±0.001	0.01
Connectivity density, normed by TV, 1/mm^3 (voxels)	27.765±12.122	167.767±81.358	0.0004
Trabecular separation = marrow thickness, mm (voxels)	1.348±0.441	0.552±0.327	0.0001
TV, Tmm^3 (TRI)	41.753±4.696	31.234±1.315	0.001
BV, mm^3 (TRI)	3.933±1.540	5.810±1.972	0.01
TRI- BS	107.696±27.559	228.553±69.641	0.0004
TRI- BS/BV	32.061±0.886	39.622±1.512	0.0001
Trabecular number, 1/mm (TRI)	1.440±0.396	3.695±1.300	0.0004
Trabecular thickness, 1/mm (TRI)	0.062±0.002	0.071±0.002	0.001
Trabecular spacing	0.671±0.215	0.240±0.087	0.0001

Note: Tri, based on Triangularization of surface. Data shown as mean ± SD.
Abbreviations: TV, total volume; BV, bone volume; MSCs, mesenchymal stem cells; BS, bone surface.

Table 4 Comparison between control versus exosomes for distal femur

Parameter	Group 1 (control)	Group 4 (exosomes)	*P*-value
TV, mm^3 (voxels)	42.178±4.727	42.836±4.563	0.2
BV, mm^3 (voxels)	3.933±1.540	4.274±0.930	0.2
BV/TV, % (voxels)	0.091±0.027	0.099±0.015	0.06
Trabecular number, 1/mm (voxels)	0.839±0.268	0.869±0.104	0.3
Trabecular thickness, 1/mm (voxels)	0.081±0.002	0.076±0.004	0.02
Connectivity density, normed by TV, 1/mm^3 (voxels)	27.765±12.122	33.741±5.493	0.007
Trabecular separation = marrow thickness, mm (voxels)	1.348±0.441	1.245±0.178	0.3
TV, Tmm^3 (TRI)	41.753±4.696	42.401±4.550	0.4
BV, mm^3 (TRI)	3.933±1.540	4.212±0.928	0.3
TRI- BS	107.696±27.559	137.280±27.154	0.06
TRI- BS/BV	32.061±0.886	32.750±1.582	0.3
Trabecular number, 1/mm (TRI)	1.440±0.396	1.610±0.184	0.01
Trabecular thickness, 1/mm (TRI)	0.062±0.002	0.061±0.003	0.2
Trabecular spacing	0.671±0.215	0.566±0.079	0.002

Note: Tri, based on Triangularization of surface. Data shown as mean ± SD.
Abbreviations: TV, total volume; BV, bone volume; BS, bone surface.

Statistical analysis

Each sample was measured three times and an average taken to have acceptable precision.[2] Data were analyzed using SPSS version 21 with the level of statistical significance set at <0.05.

Results

There were no deaths in any of the groups. When compared to the control group for distal femur, osteoblast-treated animals had significant differences in most of the parameters compared, with *P*-values ranging between <0.002 and <0.0001 (Table 1). Trabecular number and thickness were significantly higher in the osteoblast group than the other groups: 3.695±1.300–1.562±0.297 (MSC group) and 1.610±0.184 (exosome group). Trabecular spacing was 0.240±0.087 in the osteoblast group, 0.590±0.119 in the MSCs group, 0.566±0.079 in the exosome group, and 0.062±0.002 in the control group.

Figure 1 shows high-resolution peripheral quantitative computed tomography scans of the distal femur in the four groups. The osteoblast group showed more bone formation than the other two groups (MSC and exosomes). Figure 2, A shows saggital sections of the distal femur, showing a dense trabecular pattern in the osteoblast group compared to the other groups.

Table 5 Structural indices of spine in the four groups as analyzed by HRpQCT

Parameter	Group 1 (control)	Group 2 (MSCs)	Group 3 (osteoblasts)	Group 4 (exosomes)
TV, mm^3 (voxels)	21.854±5.980	19.173±2.450	15.592±5.397	23.004±6.644
BV, mm^3 (voxels)	3.238±0.534	3.325±0.549	4.677±0.611	4.549±1.787
BV/TV, % (voxels)	0.152±0.023	0.173±0.007	0.315±0.067	0.194±0.033
Trabecular number, 1/mm (voxels)	2.122±0.549	2.180±0.341	4.100±0.307	2.648±0.100
Trabecular thickness, 1/mm (voxels)	0.072±0.002	0.077±0.006	0.075±0.006	0.075±0.006
Connectivity density, normed by TV, 1/mm^3 (voxels)	38.553±3.724	37.310±3.904	87.915±12.768	46.901±4.202
Trabecular separation = marrow thickness, mm (voxels)	0.502±0.142	0.472±0.093	0.235±0.20	0.368±0.012
TV, Tmm^3 (TRI)	21.539±5.911	18.865±2.435	15.354±5.314	22.678±6.591
BV, mm^3 (TRI)	3.202±0.517	3.301±0.554	4.717±0.593	4.540±1.811
TRI- BS	108.232±23.101	100.067±19.317	139.988±35.988	137.803±44.209
TRI- BS/BV	33.622±1.696	30.263±1.662	29.454±4.809	31.208±3.336
Trabecular number, 1/mm (TRI)	2.556±0.318	2.643±0.237	4.644±0.378	3.018±0.279
Trabecular thickness, 1/mm (TRI)	0.063±0.003	0.066±0.004	0.069±0.010	0.065±0.007
Trabecular spacing	0.336±0.048	0.314±0.032	0.147±0.029	0.269±0.036

Note: Tri, based on Triangularization of surface. Data shown as mean ± SD.
Abbreviations: TV, total volume; BV, bone volume; MSCs, mesenchymal stem cells; BS, bone surface; HRpQCT, high resolution peripheral quantitative computed tomography.

Table 6 Comparison between control versus MSCs for spinal indices

	Group 1 (control)	Group 2 (MSCs)	*P*-value
TV, mm^3 (voxels)	21.854±5.980	19.173±2.450	0.007
BV, mm^3 (voxels)	3.238±0.534	3.325±0.549	0.6
BV/TV, % (voxels)	0.152±0.023	0.173±0.007	0.001
Trabecular number, 1/mm (voxels)	2.122±0.549	2.180±0.341	0.603
Trabecular thickness, 1/mm (voxels)	0.072±0.002	0.077±0.006	0.027
Connectivity density, normed by TV, 1/mm^3 (voxels)	38.553±3.724	37.310±3.904	0.340
Trabecular separation = marrow thickness, mm (voxels)	0.502±0.142	0.472±0.093	0.334
TV, Tmm^3 (TRI)	21.539±5.911	18.865±2.435	0.007
BV, mm^3 (TRI)	3.202±0.517	3.301±0.554	0.3
TRI- BS	108.232±23.101	100.067±19.317	0.2
TRI- BS/BV	33.622±1.696	30.263±1.662	0.002
Trabecular number, 1/mm (TRI)	2.556±0.318	2.643±0.237	0.4
Trabecular thickness, 1/mm (TRI)	0.063±0.003	0.066±0.004	0.041
Trabecular spacing	0.336±0.048	0.314±0.032	0.057

Note: Tri, based on Triangularization of surface.
Abbreviations: TV, total volume; BV, bone volume; BS, bone surface.

For control versus MSC groups, the later was significant only in total volume, thickness of trabeculae, and connectivity density (P<0.09, 0.04, and 0.05, respectively; Table 2), whereas in the exosome group significant parameters were trabecular thickness (P<0.002), trabecular number (P<0.01), connective density (P<0.007), and trabecular spacing (P<0.002; Table 3). Table 4 compares the control and exosome groups, showing better index values for the latter.

Table 5 shows ththat the four groups of spine showed similarity with regard to the distal femur. Trabecular numbers (1/mm) in the osteoblast group were 4.644 ±0.378, 2.643±0.237 (MSC group), and 3.018±0.279 (exosome group), while trabecular spacing was the lowest in the osteoblast group (0.147±0.029), with 0.269±0.036 in the exosome group and 0.314±0.032 in the the MSC group. Tables 6–8 highlight comparisons among the groups. Figure 3A shows scans of the fourth lumbar vertebra in the four groups, depicting wide spaces in the control and MSC groups when compared to the osteoblast and exosome groups.

Table 7 Comparison between control versus osteoblasts for spinal indices

Parameter	Group 1 (control)	Group 3 (osteoblasts)	*P*-value
TV, mm^3 (voxels)	21.854±5.980	15.592±5.397	0.001
BV, mm^3 (voxels)	3.238±0.534	4.677±0.611	0.001
BV/TV, % (voxels)	0.152±0.023	0.315±0.067	0.001
Trabecular number, 1/mm (voxels)	2.122±0.549	4.100±0.307	0.001
Trabecular thickness, 1/mm (voxels)	0.072±0.002	0.075±0.006	0.01
Connectivity density, normed by TV, 1/mm^3 (voxels)	38.553±3.724	87.915±12.768	0.001
Trabecular separation = marrow thickness, mm (voxels)	0.502±0.142	0.235±0.20	0.002
TV, Tmm^3 (TRI)	21.539±5.911	15.354±5.314	0.001
BV, mm^3 (TRI)	3.202±0.517	4.717±0.593	0.001
TRI- BS	108.232±23.101	139.988±35.988	0.001
TRI- BS/BV	33.622±1.696	29.454±4.809	0.022
Trabecular number, 1/mm (TRI)	2.556±0.318	4.644±0.378	0.001
Trabecular thickness, 1/mm (TRI)	0.063±0.003	0.069±0.010	0.002
Trabecular spacing	0.336±0.048	0.147±0.029	0.001

Note: Tri, based on Triangularization of surface. Datashown as mean ± SD.
Abbreviations: TV, total volume; BV, bone volume; BS, bone surface.

Table 8 Comparison between control versus exosomes for spinal indices

Parameter	Group 1 (control)	Group 4 (exosomes)	*P*-value
TV, mm^3 (voxels)	21.854±5.980	23.004±6.644	0.590
BV, mm^3 (voxels)	3.238±0.534	4.549±1.787	0.04
BV/TV, % (voxels)	0.152±0.023	0.194±0.033	0.003
Trabecular number, 1/mm (voxels)	2.122±0.549	2.648±0.100	0.001
Trabecular thickness, 1/mm (voxels)	0.072±0.002	0.075±0.006	0.001
Connectivity density, normed by TV, 1/mm^3 (voxels)	38.553±3.724	46.901±4.202	0.0002
Trabecular separation = marrow thickness, mm (voxels)	0.502±0.142	0.368±0.012	0.001
TV, Tmm^3 (TRI)	21.539±5.911	22.678±6.591	0.598
BV, mm^3 (TRI)	3.202±0.517	4.540±1.811	0.044
TRI- BS	108.232±23.101	137.803±44.209	0.06
TRI- BS/BV	33.622±1.696	31.208±3.336	0.048
Trabecular number, 1/mm (TRI)	2.556±0.318	3.018±0.279	0.006
Trabecular thickness, 1/mm (TRI)	0.063±0.003	0.065±0.007	0.389
Trabecular spacing	0.336±0.048	0.269±0.036	0.002

Note: Tri, based on Triangularization of surface. Data shown as mean ± SD.
Abbreviations: TV, total volume; BV, bone volume; BS, bone surface.

Discussion

Our study shows that Ovx-induced osteoporosis in rats can be reversed and the efficacy of osteoblasts is far superior to MSCs and osteoblast-extracted exosomes. Earlier studies have shown that MSCs influence bone formation in Ovx-induced osteoporosis, but this comparative study shows that MSCs does have positive effects, but not to the extent of osteoblasts. Osteoblast-derived exosomes also had a significant impact on some analyzed parameters, such as MSCs and osteoblasts.

Bone strength comes from bone volume, trabecular number, thickness, and connectivity, density and lack of any of these deprives the bone of mechanical strength.[13] Both MSCs and exosomes increased number and thickness with connectivity density, but showed no significant increase in bone volume. The increase in trabecular number and thickness without increasing actual bone volume does not help in reversing the low bone mass related to osteoporosis.

It has been reported that 200 million people suffer from osteoporosis and that it causes 8.9 million fractures,[14] with expected cost of treatment of osteoporosis fractures in the

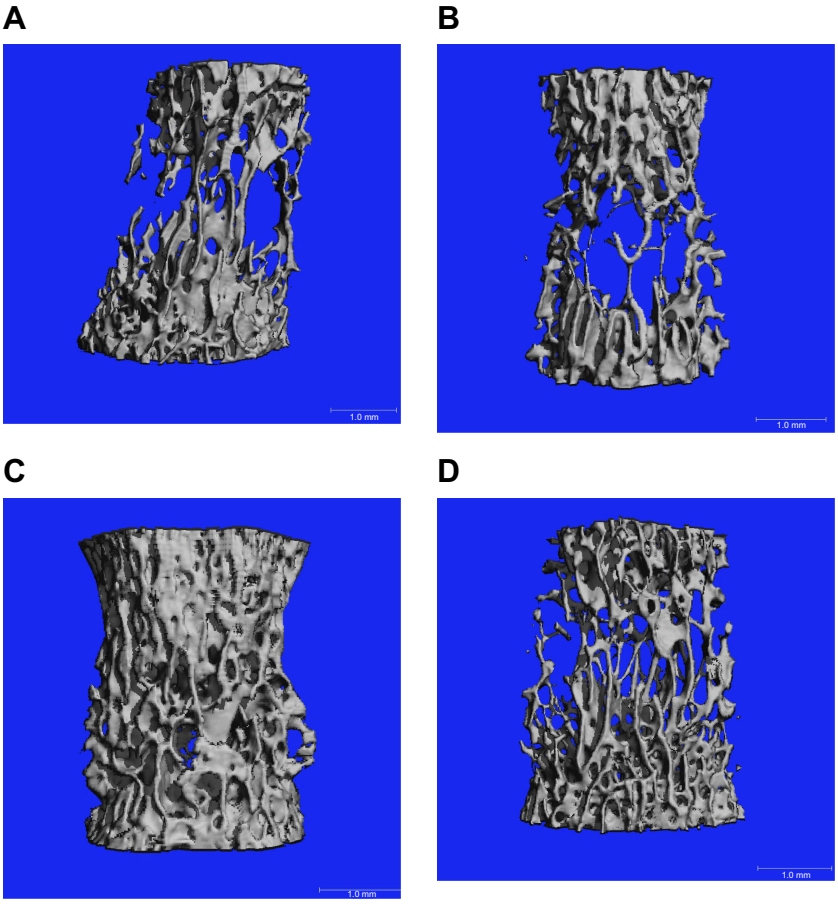

Figure 3 Lumbar fourth vertebral body analyzed by high-resolution peripheral quantitative computed tomography compariing control (**A**) and treated groups of mesenchymal stem cells (**B**), osteoblasts (**C**), and exosomes (**D**).

US to surpass $25 billion.[15] Assessment from China reported that by 2050, the cost of fractures due to osteoporosis will reach 1 million fractures, costing $25.43 billion.[16] These high figures are present despite the preventive measures and awareness of osteoporosis and its complications. There are a couple of reasons for this. As the population is aging and people are living longer, they suffer from osteoporosis for longer periods.

Physicians are looking for new therapies because of complications in presently available potent drugs. However, emerging therapies for the management of osteoporosis got halted when two promising drugs were discontinued due to a higher risk of complications. Romosozumab was reported to have more cardiovascular events compared with alendronate.[17] Odanacatib, a selective inhibitor of cathespin K to decrease bone resorption, was discontinued due to an increased risk of stroke.[18] Many researchers believe that medications with novel mechanisms to treat osteoporosis can be expected in the future, but they do not know when.[19–21] Our preclinical study has shown that stem-cell therapy could be one of the novel treatment modalities. In line with this, we have decided to replicate the study in a larger animal.

In conclusion, this study shows that osteoblasts are potent terminal cells that could be used in the reversal of osteoporosis, while MSCs and exosomes showed positive changes that were partial. The efficacy of exosomes needs to be further evaluated by giving a higher µg/mL protein to see the effect on bone formation.

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Disclosure

The authors report no conflicts of interest in this work.

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