Nell-1 Is a Key Functional Modulator in Osteochondrogenesis and Beyond

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Appendix

Appendix Table. Animal models used for evaluating Nell-1 in bone regeneration

	Defect site	Defect size	Species	Nell-1 Dose	Scaffold	Seeding Cells	Other Growth Factor/ Medication	Conclusion	Reference
Craniofacial	Intermaxillary suture	Distracted 4 mm wide perpendicular to the suture	4-week-old male rats	100 ng/ml NELL-1 protein	-	-	200 ng/ml BMP-7	1) BMP-7 and NELL-1 induced similar bone formation in the distracted suture; 2) BMP-7 induced both chondrocyte proliferation and differentiation; 3) NELL-1 accelerated chondrocyte hypertrophy and endochondral bone formation.	Cowan et al., 2006
	Parietal bone	3-mm full-thickness craniotomy defects	3-month-old male SD rats	200 ng/scaffold of NELL-1 protein was diluted in 0.025% type I collagen solution	500-μm-thick 85/15 poly(lactic-co-glycolic acid) (PLGA)	-	200 ng/scaffold of BMP-2	The osteogenic potential of NELL-1 to induce bone regeneration equivalent to BMP-2.	Aghaloo et al., 2006
	Calvarial bone	5-mm critical sized	3-month-old male SD rats	589 ng of NELL-1 + 589 ng of BMP-2; 1,178 ng of NELL-1 + 1,178 ng of BMP-2	500-mm-thick 85/15 PLGA	-	589 and 1,178 ng BMP-2	More mature and complete defect healing when the combination of NELL-1 + BMP-2 was compared with BMP-2 alone at lower doses.	Aghaloo et al., 2010
	Calvarial bone	Polyehylene (PE) particle-induced osteolysis	6- to 8-week old female Balb/c mice	$1X10^9$ pfu Ad*Nell-1*	-	-	-	The new bone promoted with the Ad-GFP-*NELL-1* injection could almost compensate the PE-induced osteolysis.	Guo et al., 2012
	Parietal bone	4-mm	10-week-old mixed gender CD-1 mice	600 μg/ml NELL-1 protein	custom fabricated PLGA disc with hydroxyapatite coating	-	1.0 mM Smoothened agonist (SAG)	Increased bone formation by microCT analyses with either SAG or NELL-1 alone, but significantly greater bone formation with SAG + NELL-1, accompanied by increased defect vascularization at both 4 and 8 weeks.	Lee et al., 2017

Maxillary sinus floor elevation	-	New Zealand white male rabbits	50 pfu/cell Ad*Nell-1*; 25 pfu/cell Ad*Nell-1* + 25 pfu/cell Ad*BMP-2*	Porous β-Tricalcium Phosphate (TCP) granule scaffold with a diameter of 1.5–5 mm	2 X 10^7 cells/ml Rabbit BMSCs	50 pfu/cell Ad*BMP-2*	25 pfu/cell Ad*Nell*-1 + 25 pfu/cell Ad*BMP-2* group had the largest bone area and most mature bone structure among the groups.	Xia et al., 2011
Alveolar bone	10 X 10 X 5 mm	10- to 12-year old female rhesus monkeys	pDNA-NELL-1, 20 ml	3D-printed bioactive glass block/chitosan nanoparticles (BD/CSn) composites	5 x10^6 cells/ml monkey BMSCs, 200 ml	-	The new bone was extremely close to normal bone in mass, density, hardness, and structure. The bony cortex was smooth and closely connected to the surrounding normal bone.	Zhang et al., 2018

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Spine	Posterolateral intertransverse process spinal fusion at L4–L5	-	10- to 12-week old athymic SD rats	1 × 10^9 pfu Ad*Nell-1*	Freeze-dried human demineralized bone matrix (DBX)	-	-	Rates of spinal fusion is 60% by manual palpation and 70% by microCT and histology (In comparison with previously published paper: when Ad*BMP-2* and Ad*BMP-7* were co-administered, the fusion rate is 73% by manual palpation; the overall mechanical fusion rates for Ad*BMP-2* and Ad*BMP-7* used separately were only 8% and 16%, respectively).	Lu et al., 2007
	L4/L5 spinal fusion	-	10- to 12-week old male athymic rats	10 μg NELL-1 protein lyophilized onto apatite-coated alginate/chitosan particles	220 mg hyaluronan hydrogel + 100 mg DBX powder + 10 mg biomimetic apatite-coated alginate/chitosan microparticles	-	-	3 of the 5 rat spines implanted with NELL-1 were considered fused without any intersegmental motion. 0 of the 5 rat spines implanted with NELL-1-free controls fused. Radiographic images showed new bone bridging the L4 and L5 transverse processes in 4 of 5 rats implanted with NELL-1.	Lee et al., 2009
	L4/L5 spinal fusion	-	12- to 14-week old athymic rats	5μg; 2.5μg NELL-1 protein	β-TCP + sheep DBX	-	-	Rates of spinal fusion were 75% by manual palpation and 88% by radiology for 2.5 μg group; Rates of spinal fusion were 88% by manual palpation and 100% by radiology for 5 μg group.	Li et al., 2010

L3/L4 and L5/L6 spinal fusion	-	Sheep	0.3 mg/ml; 0.6 mg/ml NELL-1 protein	0.4 ml DBX or 0.4 ml heating inactived DBX (inDBX)	-	-	100% fusion was achieved by 3 months in the DBX + 0.6mg/ml NELL-1 group and by 4 months in the inDBX + 0.6mg/ml NELL-1 group. These fusion rates are comparable to published reports on BMP-2 or autograft bone efficacy in sheep.	Siu et al., 2011
L4/L5 Spinal fusion	-	Athymic rats with induced osteoporosis (4 weeks post Ovariectomy [OVX])	33.3 μg/ml or 66.6 μg/ml NELL-1 protein lyophilized onto β-TCP	50 mg β-TCP + 300 μl DBX	0.25 X 10^6 cells/ml or 0.75 X 10^6 cell/ml hPSCs	-	Regular doses of hPSCs or NELL-1 achieved the fusion rates of only 20%-37.5% by manual palpation. These regular doses had previously been shown to be effective in non-osteoporotic rat spinal fusion. Remarkably, the high dose of hPSCs+NELL-1 significantly improved the fusion rates among osteoporotic rats up to approximately 83.3%.	Lee et al., 2015
L3/L4 or L5/L6 spinal fusion	-	5- to 7-year old male Rhesus macaques	1.0 or 1.7 mg/ml NELL-1 protein	25 mg apatite-coated β-TCP + 0.4 cc DBX	-	-	High doses of NELL-1 induces nonhuman primate lumbar spinal fusion.	James et al., 2017
L2 and L4 vertebral body defect	2.0 cm diameter defect in the central portion of the vertebral body	Sheep with induced osteoporosis through OVX, diet control, and steroid administration	0.9 or 2.25 mg NELL-1 protein	1.5 ml hyaluronic acid (HA) + 150 mg β-TCP	-	-	NELL-1 treatment significantly increased lumbar spine bone formation. Histological analysis revealed a significant increase in bone area and osteoblast number and decrease in osteoclast number around the implant site.	James et al., 2016

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Long Bone	Femur	Femoral distraction at a speed of 0.25 mm every 12 h for 14 days	Adult male SD rats	1 × 10^9 pfu Ad-GFP-*NELL-1* given at the mid-distraction period	-	-	-	The bone union rate was significantly higher with Ad-GFP-*NELL-1* than with Ad-GFP (9/9 vs. 4/9 rats) or saline alone (5/9 rats) at day 56. Less callus but more mature cortical bones formed, and better biomechanical properties with Ad-GFP-*NELL-1*.	Xue et al., 2011

Femur	6-mm critical size mid-diaphyseal femoral segmental defect	12- to 14-week old athymic rats	1.5 mg/ml; 0.6 mg/ml NELL-1 protein	75 μl DBX derived from Rhesus monkey	-	-	Both doses of NELL-1 treated groups had significantly greater bone formation compared to the NELL-1-free group, with bone volume increasing with increasing NELL-1 concentration.	Li et al., 2011
Femoral head	Surgical induced ischemic osteonecrosis	SD rats	1×10^9 pfu Ad*Nell-1*	-	-	0.1 mg/kg Zoledronate	The femoral head was at good shape in the combination group, while mildly flattened femoral head was seen in the placebo group. MicroCT assessment showed significantly higher total and bone mineral volume in the combination group than in the placebo group.	Fan et al., 2013
Tibia	Tibia distraction lengthened for 7 days at a rate of 2 mm/day after 3-day lag	Skeletally mature male New Zealand White rabbits	50 μg NELL-1; 25 μg NELL-1 + 25 μg BMP-2	-	-	50 μg BMP-2	The peak load of the lengthened tibia increased by 71% in the combined treatment group, 54% in the BMP-2 group, and 25% in the NELL-1 group compared to the control group, respectively.	Zhu et al., 2011
Radii	0.15 mm Orthodisc generated open osteotomy in the middle third of the radial shaft	10-week-old female CD-1 mice	Weekly injection of NELL-PEG, 1.25 mg/kg	-	-	-	Systemic injection of NELL-PEG resulted in improved bone mineral density of the fracture site and accelerated callus union, substantially enhanced callus volume, enhanced callus mineralization, enhanced biomechanical properties, augmented bone regeneration.	Tanjaya et al., 2018

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Whole Body	OVX induced Osteoporosis	-	10-month-old female SD rats	600 μg/ml NELL-1 lyophilized onto TCP, intramedullary injection immediately after OVX	50 μl of 0-50-mm TCP	-	-	OVX+ NELL-1/TCP femurs showed resistance to OVX-induced bone resorption showing BV and BMD levels similar to that of SHAM femurs at 8 weeks post-OVX. Histology showed increased endosteal-woven bone, as well as decreased adipocytes in the bone marrow of NELL-1-treated femurs compared to control. NELL-1-treated femurs also showed increased immunostaining for bone differentiation markers osteopontin and osteocalcin.	Kwak et al., 2013
	Gonadectomy induced osteoporosis	-	12-week-old B6 mice	1.25 mg/kg NELL-1 protein, intravenous (IV) injection 5 weeks post OVX, the injection was given every 48 hrs with 4 weeks in total	-	-	-	Systemic NELL-1 treatment induced significant bone formation in both non-OVX and OVX mice.	James et al., 2015
	OVX, controlled diet and steroid induced osteoporosis, L1, L3, L5	-	Sheep	0.6, 1.5 or 3.0 mg/ml NELL-1 protein, intrabody vertebral injection 4 months post OVX	600 μl HA, 50 mg β-TCP	-	-	Live CT scans performed monthly after NELL-1 injection showed a significant increase in BMD and bone volume in NELL-1-treated vertebrae.	James et al., 2015
	-	-	3-month-old female C57BL/6 mice	1.25 mg/kg NELL-PEG IV injection performed every 4 or 7 days	-	-	-	Systemic NELL-PEG therapy administered every 4 or 7 days significantly increases not only femoral and lumbar BMD and percent bone volume, but also new bone formation throughout the overall skeleton after 4 weeks of treatment. Immunohistochemistry revealed increased osteocalcin expression, while TRAP staining showed reduced osteoclast numbers in NELL-PEG groups.	Kwak et al., 2015

-	-	3-month-old female C57BL/6 mice	2.5 mg/kg NELL-PEG intraperitoneal (IP) or subcutaneous (SC) injection performed every 7 days	-	-	-	Weekly NELL-PEG injection via IP administration successfully enhanced the overall bone quality.	Tanjaya et al., 2016
Nell-1 haplo-insufficiency resulted low bone mass	-	Mice	1.25 mg/kg NELL-1 protein IV injection performed every 48 hrs with 4 weeks in total	-	-	-	*Nell-1* deficiency results in bone fragility with diminution of the *Sca-1*$^+$ MPC population; Systemic NELL-1 demonstrates anabolic effects with *Sca-1*$^+$ MPC expansion.	James et al., 2017

	Defect site	Defect size	Species	Nell-1 Dose	Scaffold	Seeding Cells	Other Growth Factors/ Medications	Conclusion	Reference
Ectopic bone formation	Thigh muscle	-	5- to 6-week old BALB/c nude mice	50 pfu/cell Ad*Nell-1*	-	5 million adenoviral encoding gene transferred primary adult goat BMSCs	50 pfu/cell Ad*BMP-2*	*In vitro*: There was no significant difference between the Ad*Nell-1*- and Ad*BMP-2*-transduced BMSCs on their osteogenic differentiation ability; *In vivo*: BMP-2 induced significantly larger bone mass with a mature bone margin and central cavity filled with primarily fatty marrow tissue. Nell-1 samples had significantly less bone mass but were histologically similar to newly formed trabecular bone mixed with chondroid bone-like areas.	Aghaloo et al., 2007
	Thigh muscle	-	Mature male nude mice	1×10^9 pfu Ad*Nell-1*	-	-	1×10^9 pfu Ad*BMP-2*, or 5×10^8 pfu Ad*Nell-1* + 5×10^8 pfu Ad*BMP-2*	Injection of Ad*Nell-1* alone stimulated no bone formation within muscle; however, injection of Ad*Nell-1* + Ad*BMP-2* stimulated a synergistic increase in bone formation compared with Ad*BMP-2* alone.	Cowan et al., 2007
	SC sites on the back	-	6-week-old male athymic nude mice	50–80 pfu/cell Ad*Nell-1*	β-TCP	2×10^7 cells/ml rat BMSCs	-	The percentage of new bone area in Nell-1 group was (18.1±5.0)%, significantly higher than those of untransduced group (11.3±3.2)% and LacZ group (12.3±3.1)%.	Hu et al., 2009

Biceps femoris muscles	-	6-week-old SCID mice	15, 100, or 300 mg NELL-1 protein lyophilized onto TCP	50 mg TCP particles 200-300 μm in diameter + human cancellous bone chip	2.5 X 10^5 human pericytes	-	NELL-1 promotes: 1) osteo-chondrogenic differentiation and endochondral bone formation; 2) engraftment and tissue integration; 3) angiogenic effects of purified pericyte.	Zhang et al., 2011
Biceps femoris muscles	-	8-week-old male SCID mice	300 μg/μl NELL-1 protein lyophilized onto TCP	15 mg TCP + 100 μl Ovine DBX	2.5 X 10^5 human perivascular stem cells (hPSCs)	3.75 μg BMP-2	Results demonstrated the osteogenic potential of hPSCs and the additive effect of hPSC + NELL-1 on bone formation and vasculogenesis. Comparable osteogenesis was observed with NELL-1 as compared to the more commonly used BMP-2.	Askarinam et al., 2013