Exploration of the Effect of NO on Bone Metabolism and Development of a Microneedle System for Transdermal Sustained Delivery of NO Donor to Treat Estrogen Deficiency-Induced Osteoporosis

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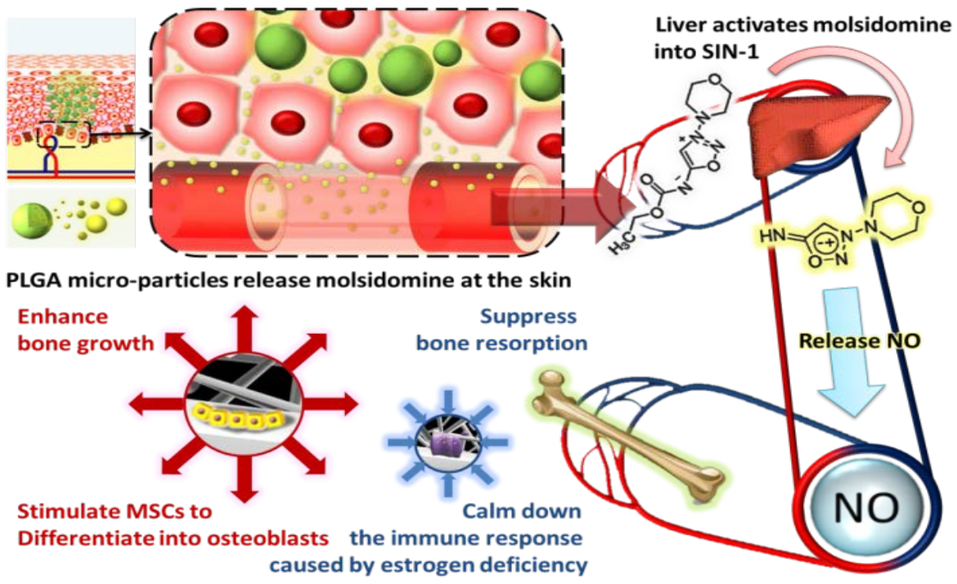
Abstract

In postmenopausal women, estrogen deficiency causes rebound of thymus, over-activation of T cell and osteoclasts, thus inducing osteoporosis. Appropriate concentrations of nitric oxide (NO) have been shown to promote the growth and differentiation of osteoblasts and suppress bone resorption through regulating thymocytes and T cells for the treatment of osteoporosis. In this study, we use cardiovascular drug, molsidomine, as a NO donor, and find its optimal oral dosage for osteoporosis. Oral administration of low-dose (2 μg/day) molsidomine significantly reduced TNF-α levels and bone marrow adiposity in the tibia compared to the untreated OVX group.

To improve convenience of administration and reduce dosing frequency, poly(lactic-co-glycolic acid) (PLGA) MPs were developed for sustained delivery of low-dose molsidomine. The OVX mice were randomly assigned to the following groups: OVX-control, OVX-empty (15mg drug-free MPs), OVX-low dose (5 mg MPs), OVX-medium dose (15 mg MPs), or OVX-high dose (30 mg MPs). Compared to the OVX-control group, all drug-loaded MP groups increased bone volume (BV/TV) and bone mineral density (BMD). Notably , the OVX-medium dose group showed the strongest effects on BV/TV, BMD, and TNF-α levels.



Effective dose range of molsidomine

Healthy control group

Sham
Without ovariectomy and administration

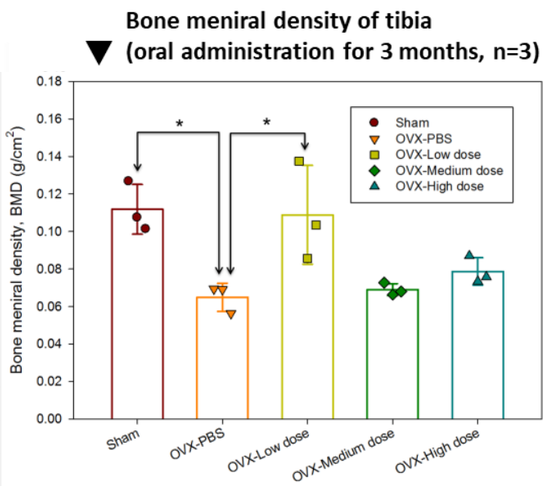
Ovariectomized group (OVX)

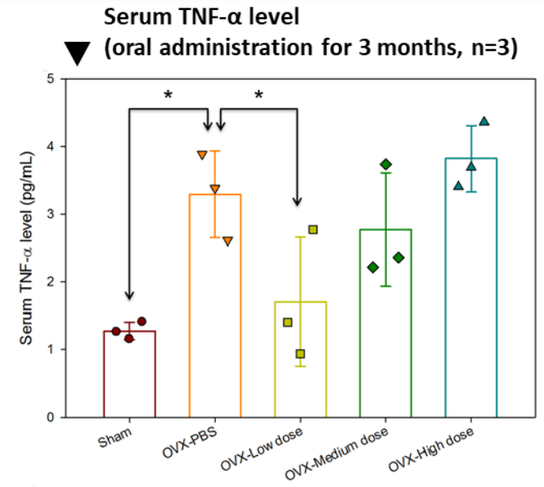
OVX-PBS
Ovariectomized and oral administration of ***PBS*** per day

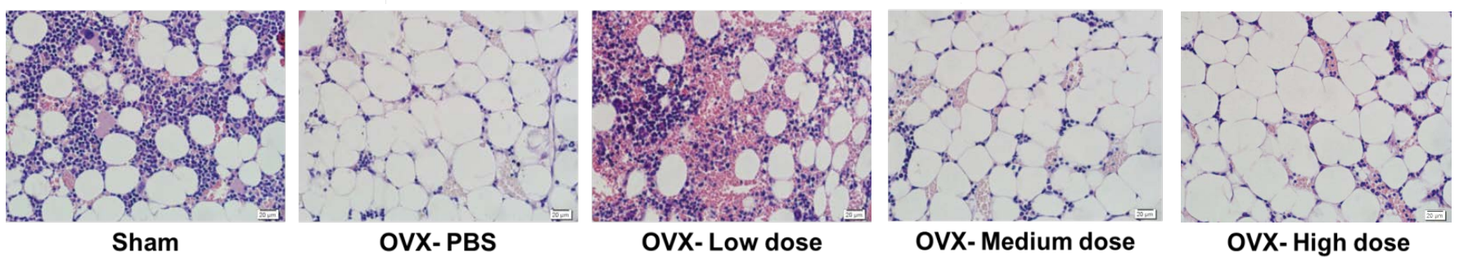
OVX-Low dose
Ovariectomized and oral administration of ***2 μg/mouse*** per day

OVX-Medium dose
Ovariectomized and oral administration of ***60 μg/mouse*** per day

OVX-High dose
Ovariectomized and oral administration of ***200 μg/mouse*** per day



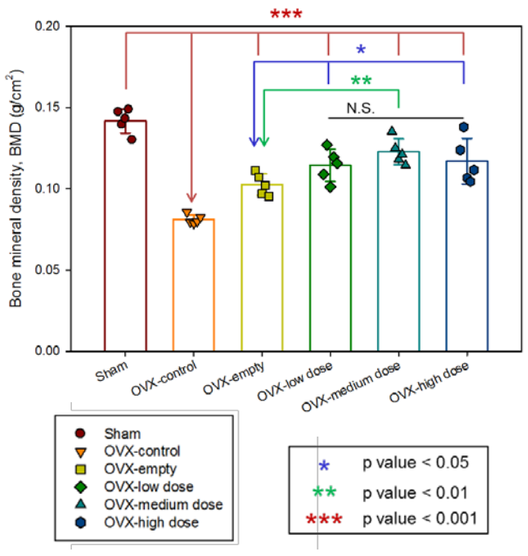


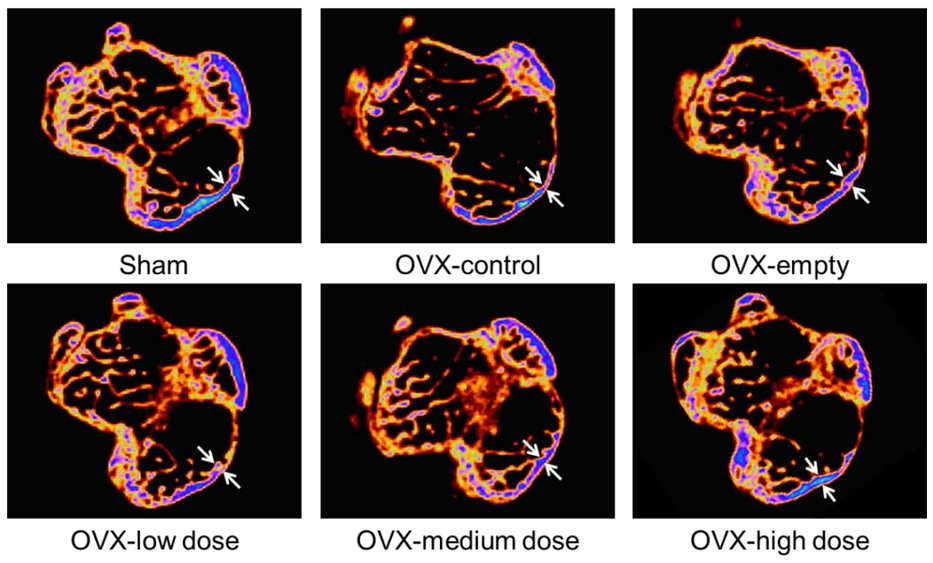


▲ **H&E stain of mice tibia bone for oral administration of molsidomine (n=3)**

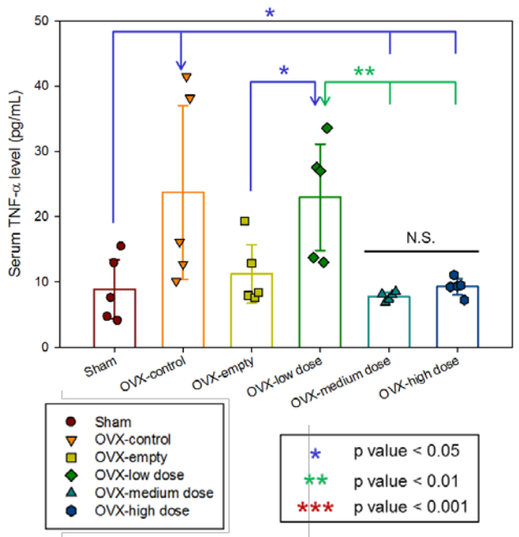
Therapeutically effective of molsidomine-loaed MPs

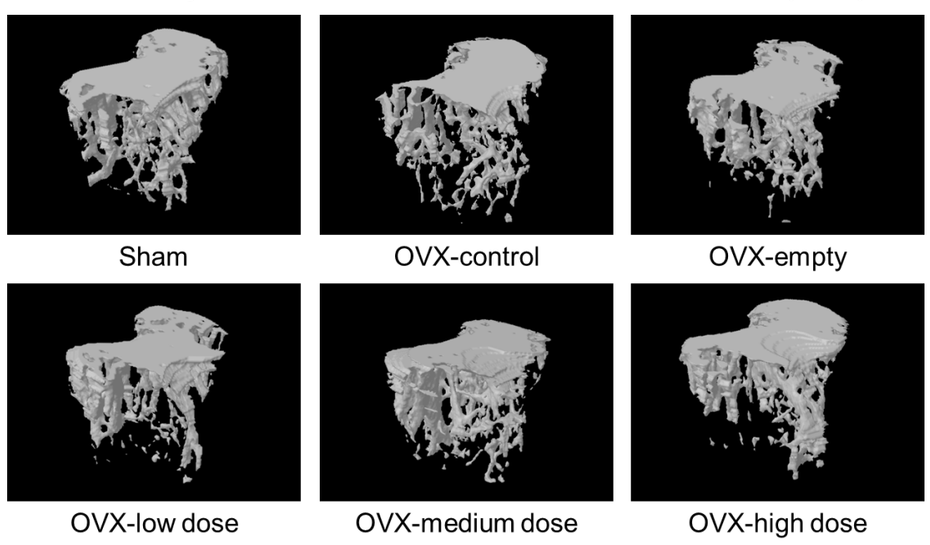
▼ **Molsidomine-loaded PLGA particles for 2 months (BMD of tibia, n=5)**



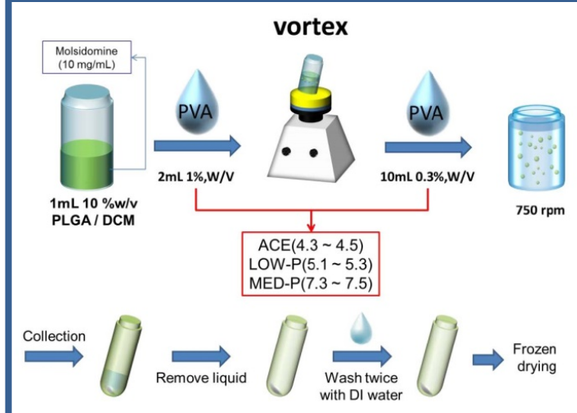


▼ **Serum TNF-α level for subcutaneous injection of molsidomine-loaded MPs (n=5)**



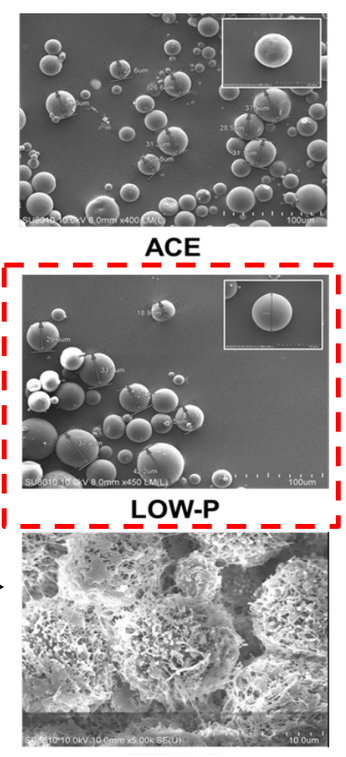


PLGA microparticles

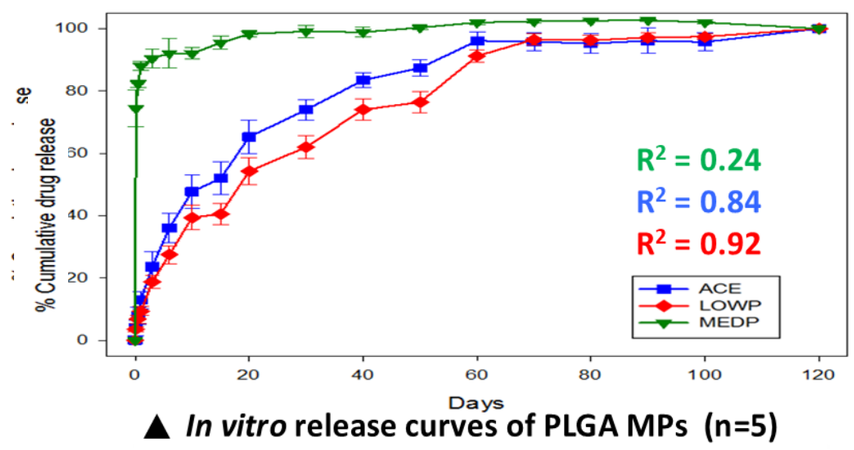


▲ **Schematic of PLGA microparticles by using single emulsion**

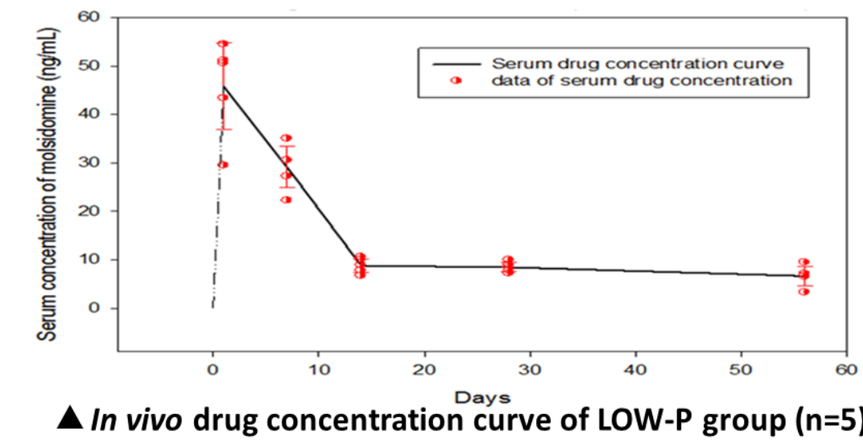
SEM images for three groups of PLGA MPs fabricated at different pH values ▶



% EE of LOW - P = 2.29
% LC of LOW - P = 0.23



▲ ***In vitro*** **release curves of PLGA MPs (n=5)**



▲ ***In vivo*** **drug concentration curve of LOW-P group (n=5)**

Conclusion

- Nitric oxide (NO) can suppress bone loss and increase of bone adiposity through restraint of TNF-α secretion.
- Combination of molsidomine and PLGA MPs which showed approximate zero-order release (LOW-P) can release slowly up to 120 days.
- 15 or 30 mg molsidomine-loaded PLGA MPs for the treatment of osteoporosis is the most effective in every therapeutic assessments, including micro-CT analysis, bone adiposity and serum TNF-α level.