# Assessment of bone healing agents for promoting bone regeneration in spaceflight

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## INTRODUCTION

Segmental bone defects secondary to high-energy trauma may not spontaneously heal and orthopedic surgeons call on the use of bone graft substitutes. Bone morphogenetic protein-2 (BMP-2) and thrombopoietin (TPO) are an FDA approved and a novel therapeutic alternative, respectively, to revitalize these bone grafts thus stimulating osteogenesis. With the growing global interest in space exploration and colonization, these drugs may be used in the absence of loading conditions, which may interfere with their mechanisms and actions.

## GOAL

**To evaluate the effects of BMP-2 and TPO on regenerating bone in a femoral bone defect model in mice housed on the ground or in space.**

## MATERIALS & METHODS

- Sixty, 9-week-old, male, C57BL/6 mice underwent a 2-mm femoral defect surgery
- Ground mice were housed at Kennedy Space Station (5-day asynchronous controls to match conditions) and Spaceflight mice (Flight) were housed aboard the International Space Station (ISS) for 4-week.
- Each group was further subdivided into three groups according to the treatments: Ground-Saline; Ground-TPO; Ground-BMP-2; Flight-Saline; Flight-TPO; and Flight-BMP-2 (n=10/group).
- **Figure 1** illustrates the major events leading up to and after launch.
- Bone regeneration in femurs was assessed by micro-computed tomography (μCT).
- All statistical analyses were performed with RStudio by using two-way and three-way analysis of variance, followed by Tukey's post hoc test. p values less than 0.05 were considered statistically significant.

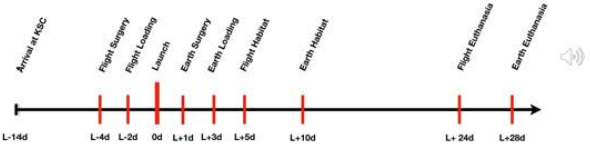


**Figure 1**: Timeline detailing the overall experimental design including launch preparation/mouse acclimation, launch, and mouse euthanasia.

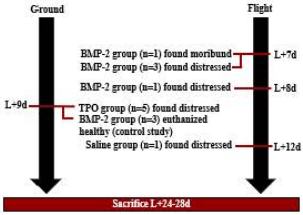


**Figure 2**: Chronology of incidents occurred during the spaceflight that led to unscheduled euthanasia.

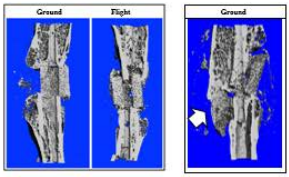


**Figure 3**: Microcomputed (μCT) images of femurs containing SBD approximately 4 weeks after surgery, demonstrating no bone bridging in either the ground or flight groups. Hardware failures (white arrow) were only observed in ground mice

## CONCLUSIONS

**Non-union remains a challenge among patients with limited weight bearing because most options to induce osteogenesis are based on pathways that perform best with loading. Here, we show that TPO induces bone regeneration even under unloading conditions.**

## ACKNOWLEDGEMENTS

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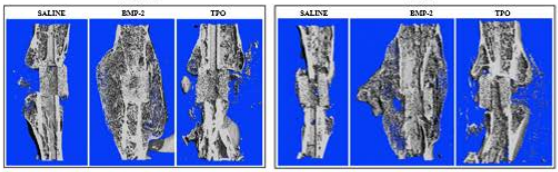


**Figure 4**: μCT imaging of fracture femurs from **ground group** displayed no signs of bone regeneration were observed in the non-treated mice (SALINE), an abundant presence of callus formation with bridging was seen in the BMP-treated mice. Although, TPO did not induce complete bone bridging, the treatment induced new bone formation at a highly mineralized level. While in **flight mice** no signs of bone regeneration were observed in the non-treated mice (SALINE), an abundant presence of callus formation with bridging was seen in the BMP-2 and TPO-treated mice. Although BMP-2 led to a larger callus formation, TPO results in callus with better microarchitecture, higher mineralization, and without the apparent side-effects observed with BMP-2 treatment.

## RESULTS

- Of the 30 mice housed on the ISS (**Figure 2**), 1 died (BMP-2) and 5 were euthanized early based on NASA veterinarian recommendation: 4 (BMP-2) and 1 (saline).
- Of the 30 mice housed on the Earth (**Figure 2**), 5 (TPO) were euthanized early based on NASA veterinarian recommendation due to observed aggressive behavior.
- Zero hardware failures were observed within the spaceflight group.
- In the ground-based group, one out of five scaffolds (20%) exhibited rupture during the study and was excluded from the results (**Figure 3**).
- No new callus formation in the Ground-Saline or Flight-Saline groups (**Figure 3**), confirming the critical size of our segmental bone defect, which does not spontaneously heal.
- We detected callus formation which resulted in bone bridging, for both Ground-BMP-2 and Flight-BMP-2 mice.
- On ground, BMP-2 increased callus volume by three-fold (p=0.08) and SMI by 17% (p=0.01) when compared to the Ground-Saline group (**Figure 4**).
- In flight, BMP-2 increased callus volume by nearly six-fold (p<0.0001), BV/TV by more than two-fold (p=0.001), and SMI by 50% (p=0.02) when compared to the Flight-Saline group (**Figure 4**).
- Comparing the effects on ground or space, BMP-2 resulted in greater callus volume when used in flight (p=0.003), but with lower density (p=0.08), fewer trabeculae (p=0.03), and higher trabeculae separation (p=0.04).
- With TPO treatment, we detected bone bridging in our Flight group, but not on Ground (**Figure 4**). Here, in flight, TPO increased TV and BV/TV by 35%, Conn.D by 173%, callus density by 61%, and Tb.N by 33%, when compared to Flight-Saline mice.
- Furthermore, TPO treatment in flight significantly decreased trabecular separation when compared to Flight-BMP-2-treated mice (p<0.05). Comparing the effects on ground or space, TPO resulted in a more dense callus (p=0.05) and higher trabeculae connectivity (p=0.001), when applied in flight.

## DISCUSSION

- No hardware failures in the spaceflight group confirms the efficacy of our rodent model as an effective SBD model to study bone regeneration in space.
- BMP-2 has remarkable bone regenerative properties.
- While TPO appears to heal bone more slowly than BMP-2, the formed callus appears to be of better quality and microarchitecture. Notably, TPO's mechanism of action does not depend on gravity.
- Furthermore, it is important to highlight that the BMP-2 bone healing data are from the 5 surviving mice, where 50% of the spaceflown mice treated with BMP-2 died or were euthanized early as NASA veterinarians deemed them to show sufficient signs of distress.
- By contrast, all BMP-2 treated mice on Earth survived to the end-points (of note all reagents used were from the same batch and there were a total of 30 mice treated with BMP-2 on the ground: 10 baseline control mice, 10 vivarium control mice, and the 10 identical ground control mice discussed here).
- While the exact cause of death/distress of spaceflight mice was not clear from post-mortem pathological assessments (but was not due to aggression), these findings highlight the importance of further exploring this phenomenon as medical kits are currently being designed for treating humans for many medical problems, including fractures, that will likely occur while living in space long-term such as that anticipated with colonization of the moon or mars..