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Dear Dr. Bajaj,

Thank you for the additional review of our manuscript. We have addressed your constructive comments and have made revisions according to your suggestions. Please find below each comment addressed individually, as well as our attached updated manuscript with tracked changes. We hope that you find our responses satisfactory, and we look forward to hearing from you.

Thank you,

Christopher J. Doro, MD

Department of Orthopedics and Rehabilitation

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**Editorial comments:**

1. **The editor has formatted the manuscript to match the journal's style. Please retain the same.**

We have retained the formatting style.

1. **Please address all specific comments in the manuscript.**

Please see Manuscript Comments below for our point-by-point responses.

1. **Once done please ensure that the highlighted text is no more than 2.75 pages including headings and spacings.**

We have ensured that the highlighted text is less than 2.75 pages. It is slightly over 2 pages.

1. **Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”**

All of the figures used in this manuscript are original and have not been used in previous publications.

**Manuscript comments:**

**What kind of complexed mRNA? What is the significance with respect to the present study? (Line 244)**

The Gaussia luciferase mRNA is complexed with a lipidic transfecting agent (Lipofectamine MessengerMAX), which serves to stabilize the mRNA molecules and enhance transfection efficiency. We have added the following clarification to Step 7.2.

“Allow for the mRNA*-lipid* complexes to form by incubating for at least 5 min at room temperature. *The lipidic transfecting agent* will condense *the mRNA molecules, stabilizing them and enhancing transfection efficiency*.”

Injection of these reporter mRNA complexes causes successfully transfected cells to express luciferase protein, which can then be visualized with bioluminescence imaging. Please see Comment 7 below for a more detailed explanation of the significance of these results.

**How? (Line 276, regarding positioning for radiographs)**

We have clarified Step 8.1 regarding the positioning of the animal during radiographic imaging. We have also removed “coronal” from Step 8.1. We have added the following substep below to describe AP imaging.

“8.1.1. While the rat is in sternal recumbency, advance the surgical hindlimb forward, flexing at the hip and stifle joint. Flex the stifle joint to approximately 90 degrees. Tape the paw plantar side down, close to body wall. Position the tibia forward from the femur to eliminate possibility of superimposing the bones. To provide slight abduction of the hip, place a translucent sponge (approximately 15 mm thick) in the groin region. Then obtain an anterior-posterior (cranial-caudal) image of the femur.”

**Please include post anesthesia recovery steps as well. (Line 280)**

We have added a step to address post-anesthesia recovery in the surgical section and imaging section to ensure proper monitoring.

“6.16. Remove the rat from the nose cone, remaining on the heating pad, and monitor continuously until the rat is able to consistently maintain an upright posture. At this point, place in a clean cage to recover.”

“8.3. Remove the rat from the nose cone and monitor continuously until the rat is able to consistently maintain an upright posture. Then, place back into cage.”

**Need some result for this part. (Line 322)**

We apologize for being unclear. The callous shown at the 4-week timepoint in Figure 3 is our result demonstrating the significant bone healing. We have combined the sentences below to avoid confusion.

“Defects containing rhBMP-2 soaked sponge demonstrated significant bone healing as early as 4 weeks after surgery, as shown by the radiopaque callous bridging across the defect in **Figure 3**.”

**Please mark this in the figure as well. (Line 324, regarding mineral deposition)**

The new bone growth and periosteal callous are labeled in Figure 4 as NB and PC, respectively. We have added the following reference to the figure:

“By 12 weeks, significant new mineral deposition *(****Figure 4****, NB: new bone, PC: periosteal callous)* has formed throughout the defect.”

**We cannot have data not shown. Please either remove this sentence or include data for the same. (Line 333)**

We have removed the sentence.

**Please bring out clarity on why this result is important. (Lines 339-340, regarding bioluminescence imaging)**

For the purposes of this manuscript, bioluminescence imaging of tissues expressing the luciferase enzyme demonstrates just one potential experimental application where our surgical procedure may be advantageous. Due to the easy removal and replacement of the external fixation plate, longitudinal *in vivo* imaging of the site is possible. By demonstrating successful transfection of cells *in vivo*, we also show that the cells at the defect site are amenable to gene delivery. This is an important precedent for future studies that may use therapeutic genes to elicit a healing response in our defect model. Bioluminescence and fluorescence are also commonly used by many research teams as biomarkers, so our results of successful localized bioluminescence are promising for future studies using *in vivo* imaging to monitor biological changes.

We have added the following sentence to the end of the Results section to show clarity.

“This is promising for future studies relying on bioluminescence or fluorescence to measure biological changes such as gene or protein expression during the healing process.”