POST-YIELD DAMAGE IN BONE MATRIX TRIGGERS INTRACELLULAR CALCIUM SIGNALING IN OSTEOBLASTS
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Introduction
Bone sustains microdamage during physiological activity which is repaired by basic multicellular units. The mechanisms by which bone cells target damaged regions are still unclear. It was reported that damage zone of notched bone samples undergoing post-yield deformations release calcium ions [Sun et al., 2012]. Importantly, when notched samples were seeded with bone cells, it was observed that the extracellular calcium efflux ([Ca$_{o}^{2+}$]) from damaged bone matrix triggered intracellular calcium signalling ([Ca$_{i}^{2+}$]) in osteoblasts on the damaged area. [Sun, et al., 2012]. Prior studies have not investigated the associations between microdamage and local activation of [Ca$_{i}^{2+}$] signalling closely. This study aimed to fill that gap by employing a dual staining method that allowed visualization of microdamage and [Ca$_{i}^{2+}$] fluorescence on the same sample.

Methods
Three cortical bone wafers (0.2 mm thick) with blunt notches were machined from bovine bone using methods described earlier [Sun et al., 2010]. MC3T3-E1 cells were seeded on bone wafers to near confluence [Ca$_{i}^{2+}$] and incubated with 5μM of Fluo4-AM (Invitrogen) to label intracellular calcium. The mechanical loading was performed on a microtensile stage positioned on a UV epifluorescent microscope and calcium imaging system (Olympus, Incyt™). Samples were loaded under constant displacement rate until the emergence of damage zone as manifested by a dark region at notch tip. The [Ca$_{i}^{2+}$] was recorded before and after loading to identify cells responding to damage in ten randomly selected cells per sample (Fig 1A). The damage zone was labelled by 1mM of calcine blue (Sigma) in the loaded state. Images were analyzed by ImageJ (NIH).

Results
Cells in the damage zone demonstrated greater [Ca$_{i}^{2+}$] fluorescence than the unloaded baseline fluorescence as tabulated:A small fraction of cells were invisible prior to loading and fluoresced anew upon damage. The distribution of cells with increased [Ca$_{i}^{2+}$]

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ in fluorescence (%)</td>
<td>26±13</td>
<td>22±13</td>
<td>33.6±31</td>
</tr>
<tr>
<td># of fluorescing cells</td>
<td>21</td>
<td>53</td>
<td>42</td>
</tr>
<tr>
<td># Cells in/around damage</td>
<td>17/4</td>
<td>44/9</td>
<td>39/3</td>
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</table>

largely coincided with the calcine blue stained damage zone (100 out of 116 cells in all three samples, Fig. 1 & Table).

Discussion
In this study, it was found that intracellular calcium related fluorescence was increased in cells located in damaged regions. Earlier work demonstrated the release of calcium ions from damaged bone and it appears that extracellular calcium release triggers [Ca$_{i}^{2+}$] pathway in osteoblasts. Future work will focus whether the downstream pathways of the observed intracellular calcium signalling involves repair response.

Acknowledgement
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References