

## Microgel-Enhanced Delivery of Adenosine to Accelerate Fracture Healing

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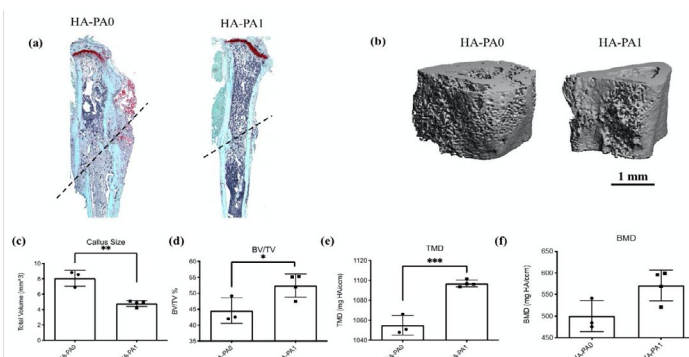
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**Purpose:** Extracellular adenosine (ADO) has been shown to play a key role in bone health by supporting osteoblastogenesis and inhibiting osteoclastogenesis. However, direct administration of extracellular ADO to promote tissue repair is challenging because of the ubiquitous nature of ADO receptors in other tissues and short half-life of ADO in circulation. To deliver ADO to the target tissue, minimally invasive therapies such as in situ-forming injectable scaffolds are highly desirable. Herein, we propose an injectable hydrogel-based scaffold to deliver ADO at the bone tissue and promote fracture healing.

**Methods:** The scaffold was prepared in the form of a composite hydrogel and developed by using ADO containing microgels and cross-linkable hyaluronic acid (HA) polymers. The microgels were developed upon copolymerization of 3-acrylamidophenylboronic acid (3-APBA) and 2-aminoethylmethacrylamide (2-AEMA) conjugated hyaluronic acid (HA-AEMA) in an emulsion suspension. Mixing of the ADO loaded microgels with clickable HA polymers containing dibenzocyclooctyne (DBCO) and azide functional groups (HA-DBCO and HA-Azide) facilitated the entrapment of the microgels, formation of the composite scaffold, and provided injectability.

**Results:** Scaffold showed gradual release of encapsulated ADO over a 2-week period. Negligible cell death was observed upon encapsulation of mouse mesenchymal stem cells (mMSCs) and osteoblastic cell line MC3T3-E1 in the scaffolds after 72 hours. In vivo experiments demonstrated that scaffold with ADO carrying microgel significantly enhances fracture repair in a mouse tibial fracture model with reduced callus size, higher bone volume, and tissue mineral density compared to the non-treated cohorts.

**Conclusion:** Extracellular ADO containing microgel-based scaffolds holds promise as an injectable bone-repairing platform to improve fracture healing.



**Figure 1.** ADO loaded microgel based scaffolds promote callus remodeling. (a) Representative safranin-O staining images of fractured tibiae treated with either HA-PA0 or ADO loaded HA-PA1 scaffold at 21 days. Dotted lines indicate fracture site and subsequent tissue repair. (b) Radiographs, corresponding 3D reconstructions, of the callus regions of fractured tibiae. Tissues were harvested at 21 days following injury. (c-f) Callus size, bone volume ratios (BV/TV) of calluses, tissue mineral density (TMD) and bone mineral density (BMD) at 21 days. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

See the meeting app for complete listing of authors' disclosure information. Schedule and presenters subject to change.