Controlled Delivery of Activin A via Hydrogels Promotes Bone Regeneration

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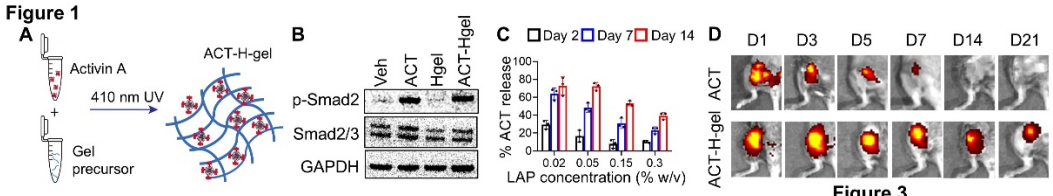
INTRODUCTION: Bone normally heals through a highly coordinated regenerative program, yet 5–10% of fractures progress to delayed healing and a substantial fraction to nonunion. Periosteal mesenchymal progenitors are the principal effectors of repair; however, clinically effective biologics that safely and selectively stimulate these cells remain limited. BMP2 and 7 are FDA-approved drug for spine fuision and open tibia fractures to stimulate osteogenesis. But their applications are constrained by adverse events and delivery issues. Activin A is another member of the TGFβ superfamily of growth factors. We recently found that it enhances proliferation and differentiation of periosteal mesenchymal progenitors in culture and improves fracture healing in aged mice. To translate this finding into clinic, we designed a hydrogel-mediated, controlled delivery system of Activin A and examined its therapeutic effect, as well as potential side effects, in a mouse drill-hole model.

METHODS: ***Hydrogel synthesis and characterization-*** Activin A–loaded methacrylated hyaluronic acid (HA) hydrogels (ACT-Hgel) were generated by mixing Activin A (recombinant human/rat/mouse) with 4% methacrylated hyaluronic acid (MeHA, 50K Da, 50% degree of substitution), and 0.05% w/v photoinitiator phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) in 20 µl PBS and photocrosslinking in situ with 410 nm light (10 mW cm^{-2}) for 1 min. Encapsulation efficiency was measured from the fluorescence intensity of supernatants after washing Cy7-ACT-Hgel with PBS. Formulations were tuned by varying LAP concentration (0.02/0.05/0.15/0.3% w/v). Cumulative release was quantified by fluorescence intensity of supernatants incubating with Cy7-ACT-Hgel at 37 °C for up to 3 weeks. BMP2 hydrogel (BMP2-Hgel) was prepared in a similar manner. ***Cell bioactivity-*** ACT-Hgel with or without 2 µg Activin A were cultured in DMEM (10%FBS) to allow for the release of Activin A. The media were collected 3 days later and added to ATDC5 cells for 1 hr, followed by immunoblotting of phospho (p)-Smad2 and total Smad2. ***Drill-hole model***- All animal work was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. Ten-week-old male *C57BL/6* mice received a 0.8-mm diameter unicortical drill-hole defect in the diaphysis part of right femur. ***In vivo retention-*** Cy7-labeled Activin A with or without hydrogel was applied to drill-hole defect and photocrosslinked. Longitudinal IVIS imaging was performed to quantify local fluorescence decay. ***Therapeutic testing-*** Mice were implanted with hydrogel, Activin A, ACT-Hgel, BMP2, or BMP2-Hgel at the drill-hole site immediately after surgery (n=6-8/group). The amount of growth factor was 1 µg/mouse. Femurs were collected 7 and 21 days later for microCT scanning. The cortical defect area and the intramedullary area were contoured separately to calculate trabecular bone volume fraction (BV/TV). Additional bones collected on day 7 were processed for cryosections followed by Osterix staining. ***Statistics***- Data are expressed as means ± SD and analyzed by t-tests or one-way ANOVA using Prism.

RESULTS: We adopted an injectable hydrogel using an HA derivative, MeHA, to deliver Activin A (Fig. 1A). The efficiency of encapsulation was 91.8%. Immunoblot showed that this hydrogel phosphorylates Smad2, an Activin A downstream target, in mesenchymal cells as potently as free Activin A (Fig. 1B). By adjusting the concentration of photoinitiator LAP, we could efficiently control the release of Activin A from hydrogel during UV photo-crosslinking (Fig. 1C). Longitudinal IVIS imaging revealed that Cy7-labeled ACT-Hgel exhibits sustained fluorescence at the bone defect site from day 1 to day 21, whereas Cy7-labeled Activin A clears rapidly within the first week (Fig. 1D), indicating that hydrogel markedly extends local retention of Activin A. To study in vivo therapeutic effect, we implanted hydrogel, free Activin A, and ACT-Hgel at the mouse drill-hole site and performed microCT 7 and 21 days later to analyze bone regeneration (Fig. 2). BMP2 and BMP-Hgel were also included as controls. On day 7, both ACT-Hgel and BMP2-Hgel induced drastic osteogenesis in the bone marrow next to the defect site. Free growth factors had similar effects, but not as potent as those with hydrogel. On day 21, intramedullary trabecular bone disappeared, and cortical bone defect was bridged in all groups. Analyzing cortical bone area revealed that compared to hydrogel alone, ACT-Hgel and BMP2-Hgel increase BV/TV by 1.6 and 1.7-folds, respectively, which are significantly higher than those induced by free Activin A (1.2-fold) and BMP2 (1.1-fold) only. Notably, BMP2-Hgel, but not ACT-Hgel, caused massive heterotopic bone formation in adjacent muscle tissue. Mechanistically, we observed a 3.2-fold increase in osteoblasts around the drill-hole site at day 7 in ACT-Hgel-treated bones using Osterix staining (Fig. 3).

DISCUSSION: Our study engineered an Activin A hydrogel system to accelerate bone regeneration. This therapeutic strategy meets key criteria for an osteoregenerative biologic: it preserved canonical signaling (robust p-Smad2 activation), enabled tunable sustained release with prolonged local retention, and enhanced bone repair. Importantly, it appears safer than the current clinical standard, BMP2, as it does not induce ectopic ossification. Moving forward, we will further optimize this system to achieve greater efficacy with lower doses and reduced side effects and evaluate its performance in large animal bone defect models to facilitate clinical translation.

SIGNIFICANCE: Activin A is critical for bone repair and can be exploited therapeutically via hydrogel delivery.



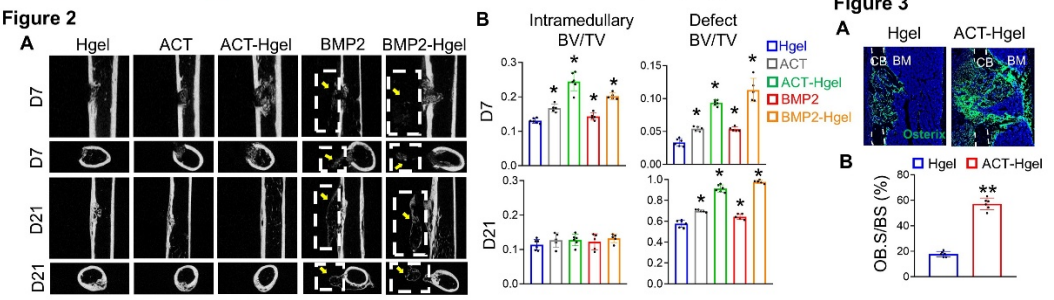


Figure 1. Synthesis and characterize ACT-Hgel. (A) Schematic diagram of ACT-Hgel synthesis. (B) The activity of Activin A in hydrogel was measured by Western blot of p-Smad2 in ATDC5 cells treated by conditional media from vehicle, ACT, hydrogel (Hgel), or ACT-Hgel. (C) The controlled release of Cy7-ACT from Cy7-ACT-Hgel, prepared with varying LAP concentrations, was measured at days 2, 7, and 14 of incubation in PBS. (D) Representative IVIS images of mouse hindlimbs treated with Cy7-ACT or Cy7-ACT-Hgel at various time points post drill hole injury. n=3 mice/group. **Figure 2. ACT-Hgel accelerates bone healing with no obvious side effects in neighboring muscle.** (A) Sagittal (top) and transverse (bottom) cross-sections of microCT images of femoral midshaft at day 7 and 21 after drill hole injury. Arrows within rectangles point to ectopic bone formation in muscle. (B) Bone mass at intrabmedullary and cortical bone defect sites was quantified. n=5-6 mice/group. **Figure 3. ACT-Hgel promotes osteogenesis.** (A) Osterix staining images at the bone defect site. CB: cortical bone; BM: bone marrow. (B) Quantification of osteoblast surface per bone surface (OB. S/BS). n=6 mice/group. *P < 0.05, **P < 0.01, ***P < 0.001 vs Hgel.