# Why Not Use a Parathyroid Hormone-Loaded Hydrogel Rather than Systemic Parathyroid Hormone to Rescue Impaired Fracture Healing in Diabetes?: A New Proof-of-Concept Study

Dennis L Caruana, Will Mao Jiang, Francis Young-In Lee[1]
[1]Yale Univ School of Med/Dept Ortho Surg

INTRODUCTION:

Intermittent parathyroid hormone (PTH) treatment is a well-known bone anabolic regimen, but directed local delivery of PTH has not been evaluated for enhanced fracture care. Type 2 diabetes mellitus (T2DM) accounts for 90-95% of diabetes mellitus cases.

METHODS:

T2DM increases complication rates following orthopaedic disorders. T2DM human patients and animal models often show deficient bone, reduced formation of the fracture callus, and insufficient fracture repair. T2DM has also been associated with decreased function of stem cells. Fracture-activated stem cells (FASCs) are a special class of mesenchymal stem cells of diverse tissue origins that appear transiently during the early stages of fracture healing and possess PTH receptors. The purpose of this study is to establish a method of rescuing impaired fracture healing with locally delivered hPTH1-34 releasing hydrogel and investigate the effects of locally delivered hPTH(1-34) on FASCs *in vivo* in mouse models of T2DM.

RESULTS: All mouse models of T2DM showed increased body fat, fasting blood glucose, and body weight compared to C57BL/6J controls matched for age and sex. T2DM mice showed a reduced size fracture callus, deficient fracture gap bridging (incomplete ossification of chondroid callus), and reduced endochondral callus bone mass, which were rescued with local delivery of hPTH(1-34) (Figure 3). In the diet induced T2DM model, early callus formation was enhanced at day 7 by treatment with locally delivered hPTH(1-34) but not with systemic administration of hPTH(1-34) (Figure 4).

DISCUSSION AND CONCLUSION:

These preliminary data show enhanced fracture healing in mouse models of T2DM using hydrogels at the fracture site to locally deliver hPTH(1-34) thereby supporting a potential cost-effective, therapeutic role of for hPTH(1-34)-loaded hydrogels in the early stages of fracture healing. We will formally test our hypothesis by using more samples at many different timepoints for meaningful statistical validation, biomechanical testing, mCT, and pre/post-hoc power analysis. In conclusion, local PTH-loaded hydrogel treatment enhances fracture healing.

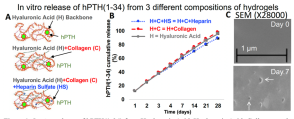


Figure 1. *In vitro* release of hPTH(1-34) from Hyaluronic Acid, Hyaluronic Acid+Collagen, and Hyaluronic Acid+Collagen+Heparin Sulfide. A. The components and compositions of hydrogels. Collagen was added to stimulate matrix. Heparin was used to retain growth factors. B. In vitro release kinetics of 1.2 µg (=40 µg/kg *in vivo*) of hPTH(1-34) in µl of heparail (1%) hydrogels prior to crosslinking. The cumulative release of hPTH(1-34) (N=2;±SEM). C. Generation of micropores during degradation, allowing release from deep regions.

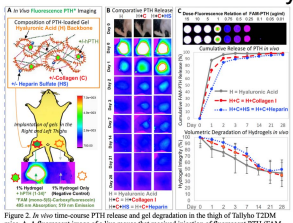


Figure 2. *In vivo* time-course PTH release and gel degradation in the thigh of Tallyho T2DM mice. A. A fluorescent image of a live mouse that received injection of fluorescent PTH (FAM-PTH(1-34)-gel in the right thigh and gel only in the left thigh following 3 different types of 100 µl 1% hydrogels with FAM-PTH(1-34 500 µg/kg (N=3). B. Time-course imaging of 3 different types of implanted PTH-loaded hydrogels. C. Addition of heparin sulfate (H+C+HS) provides more prolonged release, delayed saturation of PTH release, and slower volumetric degradation *in vivo* (N=3). Therefore, we deliberately chose the H+C+HS gel.

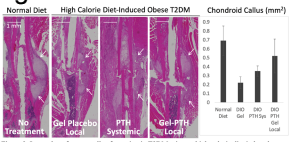


Figure 3. Less robust fracture callus formation in T2DM mice on high-calorie diet induced mouse model of T2DM in comparison to age-/sex-matched mice on normal diet assessed on post-fracture day 7. There is a strong trend of enhanced soft callus (chondroid) formation by locally delivered hPTH(1-34) compared to placebo or systemically administered daily injection of hPTH(1-34) at day 7 (n=3; H&E, x1).

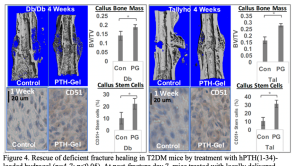


Figure 4. Rescue of deficient fracture healing in T2DM mice by treatment with hPTH(1-34)-loaded hydrogel (n=4-7; p<0.05). At post-fracture day 7, mice treated with locally delivered hPTH(1-34) (PTH-gel) showed increased CD51+ fracture-associated stem cells compared to placebo (gel vehicle)-treated mice. At day 28, PTH-gel treated mice showed increased callus mineralization compared to placebo-treated mice.