Chemical Homing for Localized Delivery of Extracellular Matrix Cues to Bone Fractures to Accelerate Repair

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INTRODUCTION: Delayed fracture healing is a major health issue involved with aging. Therefore, strategies to improve the pace of repair and prevent non-union are needed in order to improve patient outcomes and lower healthcare costs. Tissue engineering has long understood the importance of the extracellular matrix (ECM) in promoting wound repair, often relying on decellularized ECMs to act as scaffolds for repair or isolating anabolic fragments from the ECM to promote wound repair. Many of the cells involved in tissue repair rely on the right cues to be provided from the ECM to initiate a repair response. Current investigative strategies using ECMs for bone fracture repair have relied on the local application of ECM peptide-coated implants or -impregnated cements, or demineralized bone to stimulate the accelerated repair. Some of the cements and coatings have relied on using fragments of collagen[1], fibronectin[2], laminin[3], osteonectin[4], bone sialoprotein[5], and osteopontin[6]. These cements and implants have proven effective, but they are incredibly invasive to implant and are limited to a single application. It is very difficult for a drug to maintain a therapeutic concentration from a single dose. Our hypothesis was that these ECM fragments could be effective as a systemically administrable drug if attached to our acidic oligopeptide bone fracture-targeted drug delivery system, which can safely and effectively be used to deliver therapeutics to the site of a bone fracture. This would open the possibility for the development of these compounds as pharmaceuticals that could be delivered noninvasively and have sustained dosing throughout the fracture repair, thus increasing the efficacy of the treatment without surgical implantation. By utilizing acidic oligopeptide bone fracture targeting developed by our lab, we can localize extracellular matrix cues specifically to the site of bone damage from a subcutaneous injection at a distal site. This method allows for repeat dosing and a noninvasive route of administration, which further allows us to constantly amplify the cues from the ECM to promote repair in just one region from a systemic administration, thus making the surgical application and coating of implants unnecessary. This bone fracture-targeted platform also allows for greater specificity of the drug's accumulation leading to lower required doses and lower side effects.

METHODS: Acidic oligopeptide-targeted conjugates and free untargeted versions of collagen fragments (P15, CTC) osteopontin fragments (ODP, CBM, CBD) and integrin ligands (ITGA5) were synthesized using standard Fmoc solid-phase peptide synthesis. Osteogenic gene's expression patterns were assessed in MCTC3-E1 cells to ensure the conjugates' activity. Once the activity of the conjugates was confirmed, they were tested in a midshaft-stabilized femur fracture model in 12-week-old Swiss Webster mice. Femur fractures were induced using a drop weight fracture device. The mice were dosed subcutaneously each day for three weeks with an n=10 for each of the different drug dose combinations. Fracture healing was assessed using microCT. Morphometric parameters were quantified in the 100 widest slices of the fracture callus. Trabecular thickness (Tb.Th.), trabecular spacing (Tb.Sp.), total volume (TV), and volume of calcified callus (BV) were calculated. Fractured femurs were tested for strength in a four-point bend to failure. Peak load, yield load, stiffness, displacement post yield, work-to-fracture, and deformation data were generated. Statistical analysis was performed using a one-way analysis of variance (ANOVA) and a Tukey post-hoc analysis with significance reported at the P value of 0.05. All animal experiments were performed in accordance with protocols approved by Purdue University's Institutional Animal Care and Use Committee (IACUC)

RESULTS: The in vitro results indicated that the targeted versions of these anabolic ECM fragments retained similar anabolic activity to that of their untargeted forms, indicating that the conjugation wasn't interfering with the receptor binding portion of the fragments. In replicated animal experiments it was shown that these targeted ECM fragments improved midshaft femur fracture healing both mechanically and structurally. The best-performing ECM cue was ITGA5—a fibronectin memetic that is a synthetic integrin alpha 5 ligand that binds to the integrin alpha 5 integrin on the surface of mesenchymal stem cells and promotes their differentiation into osteoblasts via the integrin-mediated cell signals FAK and ERK1/2-MAPKs[2]. We found that, by targeting this compound, we were able to achieve an 89% increase in BV/TV of the fracture callus relative to saline control. We were also able to achieve a significant 216% increase relative to a saline control in work-to-fracture. Additionally, targeted ITGA demonstrated a significant 98% increase in stiffness and a 75% increase in maximum force relative to the saline control. In the targeted drugs, no side effects were observed even at very high doses. A histological evaluation of the liver and kidneys also demonstrated no significant difference between saline and the treated groups. No off-target skeletal effects were observed. The treated contralateral femurs in every ECM drug group showed no significant difference when compared to the saline control contralateral femur. We found that, by stabilizing the disulfide cyclization in ITGA, the activity of the drug could be increased by improving the stability. The targeted ECM fragments were able to improve fracture healing and stimulate a more rapid bone formation at the site of injury.

DISCUSSION: Attaching a bone-targeting ligand allowed the ECM fragments to be administered systemically rather than having to be surgically implanted to improve fracture healing. This delivery platform allows for a new way to utilize the ECM cues to promote wound healing that does not rely on implantation. This improvement in administration method could vastly improve ECM fragment implementation and development as therapeutics. ITGA shows promise as a future therapeutic, as it significantly improved the healing rate of bone fractures in multiple in vivo models. The ITGA molecule was able to develop a hard callus fast enough that the fractured femur could reach the average femoral pre-fracture strength in three weeks compared to eight weeks for the saline control mice, representing a dramatic reduction in healing time, which could reduce the time a patient spends suffering with immobility. This improvement has the potential to reduce the cardiovascular comorbidities associated with patients' immobility. This new drug administration for these anabolic ECM fragments has the potential to provide a new, non-invasive treatment course in which a more sustained therapeutic effect can be elicited with less drug. It has helped create a safe alternative to surgical implantation for these compounds, allowing for a more wide-spread use of these compounds.

SIGNIFICANCE / CLINICAL RELEVANCE: One in four people over the age of 65 who break their hips will die within a year, due to the complications associated with their prolonged immobility caused by slow fracture healing. Strategies such as the one we have presented here will improve the pace of repair and prevent non-union, thus substantially improving patient outcomes, lowering healthcare costs, and decreasing the number of fracture-related morbidities. Here, we demonstrate that systemically-administered, targeted ECM fragments can be used to accelerate fracture healing. This method opens the possibility for the development of these compounds and other ECM fragments as therapeutics for bone fracture repair.

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