Improved Spinal Fusion through Targeted Delivery of Abaloparatide

Jeffery J Nielsen[1,2], Stewart A Low [2], Christopher Chen[2] , Xinlan Li[2], Mary Niedrauer [2], Mini Thomas[2], Ephraim Mbachu[2], Lina Trigg[1], Madeline Tremby[1], Philip S. Low[1]
[1]Purdue University, West Lafayette, IN, [2]Novosteo Inc., West Lafayette, IN *For more information contact: nielsej@purdue.edu*

Significance

Over half a million spinal fusions happen in the United States, with costs over $120k and recovery times over 3 months, there is a need for cheaper, faster, less invasive, and more efficient treatments for people to regain mobility. Here a novel method for administering drugs to spinal fusions is demonstrated and a novel targeted anabolic is demonstrated to be more effective than BMP2 in stimulating spinal fusion.

Introduction

With over half a million spinal fusions performed in the United States every year, and with recovery time increasing with age, there is a need for alternative and affordable treatments. Extended postoperative immobility significantly impacts the risk for comorbidities and quality of life for patients. Current therapeutic approaches rely on the application of a large amount of therapeutic at the time of surgery, which leads to undesirable side effects, such as ectopic mineralization, osteoclast-mediated bone resorption, and inappropriate adipogenesis.(1,2) Our lab has developed a bone targeting system based on acidic oligopeptides that is more biocompatible, and has a more relevant therapeutic half-life than bisphosphonates; and lacks the skeletal side effects that plague bisphosphonates. We have previously demonstrated this system to be effective at selectively delivering therapeutics to bone fractures from a systemic injection at a distal site. We found here that the same targeting system could be used non-invasively to systemically administer therapeutics that chemically home to the spinal fusion. We evaluated the ability to localize therapeutic in a rat posterolateral lumbar spinal fusion model and found that we were able to specifically target drugs to fusion sites in fusions performed with collagen sponges, bone granules and mineralized collagen, suggesting promise in broad application in the existing surgical procedures. Additionally, we found that this system could be used to deliver an anabolic like abaloparatide to a spinal fusion and accelerate its repair relative to treatment with BMP2 in both male and female Sprague Dawley rats. This acceleration of healing could dramatically reduce the burden of disease and reduce the need for revisionary surgeries.(3) This system allows for repeat dosing and thus lower doses are needed to maintain therapeutic levels of the drug. Targeting also improves the safety of the drugs as it prevents off target localization that leads to ectopic mineralization. Current pharmaceutical application of BMP2 struggles from leakage of the compound BMP2, which causes bone to grow into the surrounding soft tissue, windpipe, and spinal cord. All existing strategies require a surgery to apply the drug and only allow for one application. With our platform, we can administer drugs multiple times and continue to stimulate bone growth in the region for a longer time and faster with less-invasive drug administration, thus potentially allowing people to regain their post-surgery mobility more quickly.

Figure 1: GSK3β inhibitor labeled with ^{125}I and imaged at 24h using SPECT/CT. A) Free GSK3β inhibitor is quickly excreted and shows no affinity toward fractured bone. A) When the GSK3β inhibitor is conjugated to an acidic oligopeptide, the majority of the signal is observed in the fracture callus of the femur, with trace concentrations of drug observed in sites of high remodeling.

METHODS: Once acidic oligopeptides spinal fusion targeting was established an efficacy study in both male and female skeletally mature Sprague Dawley rats(n=10/sex) was performed using a bilateral L4-L5 posterolateral lumbar spinal fusion as before with a 5x7.5-mm collagen sponge (RCM6 Ace Surgical supplies). 60 rats were randomly assigned to one of three treatments phosphate buffered saline vehicle control, 10μg BMP2, or 38nmol/kg twice a week of acidic oligopeptide targeted Abaloparatide (Abalo46-DGlu20). The initial dose of all drugs was administered on the sponge and then vehicle control and Abalo46-DGlu20 were administered subcutaneously twice a week for 8 weeks. The spinal fusion mineralization was assessed using a Quantum GX μCT from PerkinElmer. Each rat was scanned weekly during weeks 3-8. The scans were blindly scored by 2 separate individuals. At the end, the lumbar was removed and were blindly manually palpated and submitted to mechanical evaluation via 3-point bend (Instron). All animal experiments were performed in accordance with protocols approved by Purdue University's Institutional Animal Care and Use Committee (IACUC).

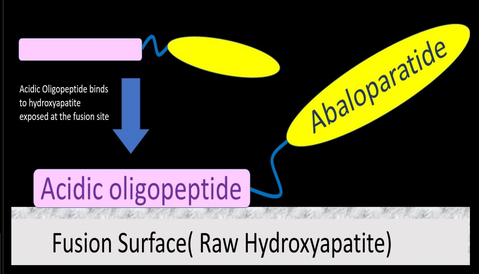


Figure 2: We synthesized fracture-targeted anabolic agents by synthesizing Abaloparatide factors (yellow) in tandem with a spacer (blue) and a hydroxyapatite-binding acid oligopeptide (pink).

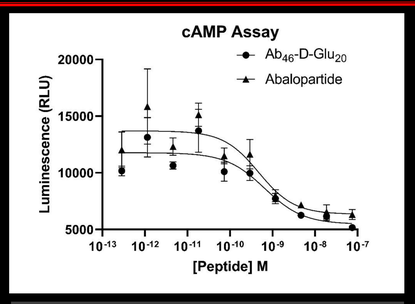


Figure 3: The activity of targeted Abaloparatide relative to free abaloparatide. Activity was assessed in a cAMP-Glo assay on UMR106 cells treated with drug for 1 hour. EC50 0.48nM and 0.66nM, respectively. Addition of targeting moiety affected the engagement of

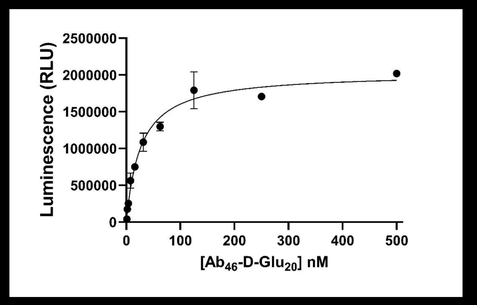


Figure 4: *In Vitro* Assessment of binding affinity of Acidic oligopeptide targeted abaloparatide to Hydroxyapatite. Even with the addition of linker and large abaloparatide payload the acidic oligopeptides had a high affinity interaction with hydroxyapatite. The KD binding constant was calculated to be 27 nM.

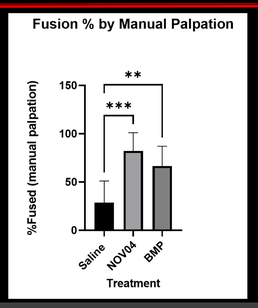


Figure 8 (Above): In vivo fusion healing efficacy of targeted abaloparatide on male Sprague Dawley rats (n=10) after 8 weeks after bilateral posterolateral lumbar fusion of L4-L5. analysis. The lumbar region was excised postmortem and manual palpitated and evaluated for fusion between L4 and L5

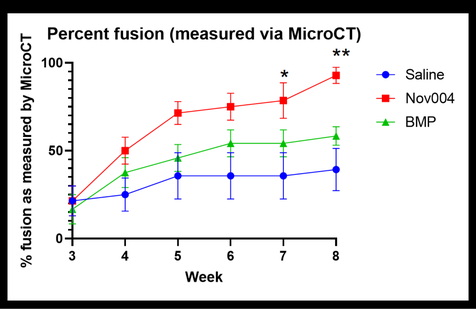
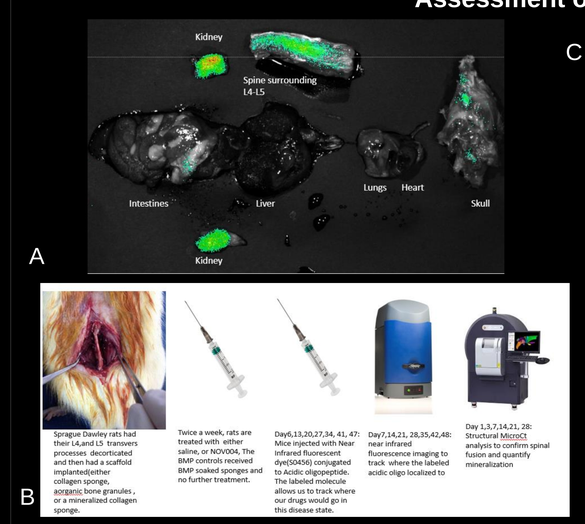


Figure 9: : In vivo fusion healing efficacy of targeted abaloparatide on male Sprague Dawley rats (n=10) over 8 weeks after bilateral posterolateral lumbar fusion of L4-L5. Fusion is assessed by completed osteointegration as seen by micro CT this was assed in a randomized fashion by 2 individuals blinded to the treatment groups

Assessment of Targeting to Different Spinal Fusion Scaffolds



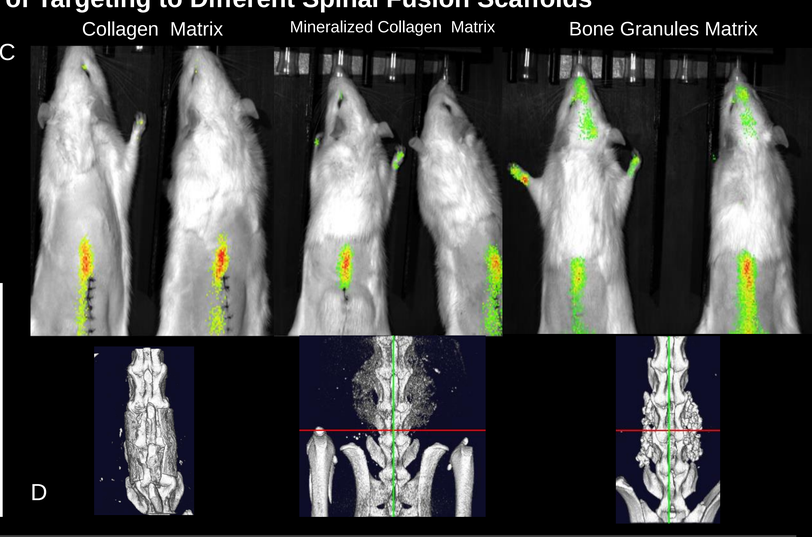


Figure 5: To evaluate the ability to localize therapeutics to spinal fusions, female Sprague Dawley rats(n=3) underwent a L4-L5 posterolateral lumbar spinal fusion using collagen sponge, aorganic bone granules wrapped in collagen or a mineralized collagen scaffold soaked in 10μg BMP2 Using a low-speed burr, the L4 and L5 transverse processes cortical bone and the facia joint between the two vertebrae were removed, and a drug-soaked collagen sponge was placed on each side of the vertebrae. The scaffold was soaked to saturation in the drugs for 10 minutes. They were imaged in a μCT(Perkin Elmer) longitudinally every week for 8 weeks and injected with a near inferred dye(S0456) acidic oligopeptide conjugate and imaged 24 hours later with a spectral imaging system (AMI) for 1 second with excitation at 745 nM and emission collected at 810 nM.
(A) Localization of acidic oligopeptide targeted near inferred dye in rat with collagen scaffold 2 weeks after surgery 24 hours post injection
(B) longitudinal imaging study design
(C) Targeted fluorescent biodistribution of targeted near-IR fluorescent molecule (S056) 24 hours post-injection in bilateral posterolateral lumbar spinal fusion (2 weeks post-surgery) in three different main scaffold types for spinal fusions. Localization primarily occurs in spine. Localization see on paws and head are due to grooming with paws contaminated by dye urinated out.
(D) 3D reconstruction of three different types of spinal fusion scaffolds that were evaluated 4 weeks post surgery

Efficacy Study In Both Sexes

Figure 6 : Sprague Dawley rats(n=10/sex) was performed using the same surgical procedure as before with a 5x7.5-mm collagen sponge (RCM6 Ace Surgical supplies). 60 rats were randomly assigned to one of three treatments phosphate buffered saline vehicle control, 10μg BMP2, or 38nmol/kg twice a week of acidic oligopeptide targeted Abaloparatide (Abalo46-DGlu20). The initial dose of all drugs was administered on the sponge and then vehicle control and Abalo46-DGlu20 were administered subcutaneously twice a week for 8 weeks. Images here are median density images for each group after 5 weeks of treatment. (A) represents female rats and (B) are male rats

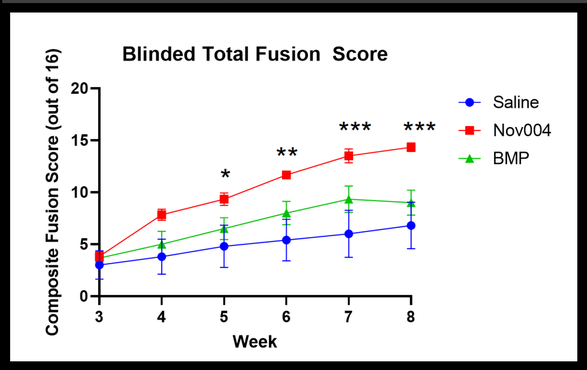


Figure 7 : In vivo fusion healing efficacy of targeted abaloparatide on male Sprague Dawley rats (n=10) over 8 weeks after bilateral posterolateral lumbar fusion of L4-L5. analysis. Total Fusion Score. Scores were assigned (0-4) on osteointegration, density, bridging of both bilateral spinal fusions in micro CT scans of the rats. Scores were assigned by two individuals blinded to the treatment reviewing randomized samples. Total score is sum of all 4 scores combined. Significance determined by One-way ANOVA.

RESULTS

We found that our targeted fluorophores were able to localize selectively in the spinal fusion over the rest of the body in all three types of scaffolds evaluated from a subcutaneous injection at a distal site. We also found that the treatment group receiving Abalo46-DGlu20 had their scaffolds mineralize earlier than either the vehicle control or BMP2 treatment groups in both male and female rats with significant differences being observed as early as 4 weeks post-surgery. Abalo46-DGlu20 had more consistent success in fusion of the vertebrae than the other 2 treatments. BMP2 performed better in male rats than in female rats while no other sex differences were observed in the other treatment groups. There were no significant differences in total volume of the scaffolds but the density of the mineralized scaffold was greatest in the Abalo46-DGlu20 treated group.

Discussion

The ability to localize therapeutics to different types of scaffolds will prove useful in using this system to develop future therapeutics that can be administered at low doses noninvasively over longer periods of time to stimulate more robust healing responses that achieved with the current bolus administration system. This will help to lower off target effects and hopefully reduce the time a patient must spend recovering. The results with Abalo46-DGlu20 showed that this therapeutic we developed for bone fracture healing previously has potential in stimulating bone repair outside of bone fractures. This is a potentially promising application of this therapeutic and future studies will have to be done to further develop it for this purpose. The potential earlier mineralization stimulated by Abalo46-DGlu20 seemed to be responsible for the improvement the successful integration of mineralized scaffolds with the transverse processes and could play a role increasing the success rate of fusions in the future. The noninvasive method of this drugs administration should allow for the ease of its augmentation into current clinical practices.

References

1. 1. Martin BI, Mirza SK, Spina N, Spiker WR, Lawrence B, Brodke DS. Trends in Lumbar Fusion Procedure Rates and Associated Hospital Costs for Degenerative Spinal Diseases in the United States, 2004 to 2015. Spine (Phila. Pa. 1976). 2019;44(5):369–76. A
2. 2. James AW, LaChaud G, Shen J, Asatrian G, Nguyen V, Zhang X, Ting K, Soo C. A Review of the Clinical Side Effects of Bone Morphogenetic Protein-2. Tissue Eng. - Part B Rev. 2016;22(4):284–97.
3. 3. Hunter DJ. Spondylolysis and spondylolisthesis: prevalence and association with low back pain in the adult community-based population. 2013;34(2):199–205.

Acknowledgments

The Indiana Clinical and Translational Sciences Institute
NIH TL1 TR002531, UL1 TR002529, R44DE028713