Development of Imaging Probes for Fracture Healing Response

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01 | Introduction

- More than 16 million fractures are treated in the United States each year
- 10% of fractures are complicated by delayed union or non-union
- Fractures create five primary problems: bleeding, susceptibility to infection, disproportionate interfragmentary strain, bone hypoxia, and an inability to bear weight

The APR is the body's hormonal response system to injury. The APR first resolves lethal problems such as bleeding and susceptibility to infection in the "survival phase," then transitions to the "repair phase" where strain is reduced by cellular and acellular factors allowing new vasculature to extend across the fracture site, reducing bone hypoxia, and leading to vascular union

- The goal of this study was to develop and validate new imaging agents that enable high-resolution near-infrared fluorescence (NIRF) imaging of bone fracture acute phase response

02 | Background

- Acute Phase Response: The body's response to fracture injury

- Hematoma: acts to resolve bleeding and eliminate potential sources of infection
- Fibrin: trap bacteria at the site of tissue, preventing infection
- Neutrophils: acting to destroy pathogens at fracture sites
- Restoration of bone strength
- Macrophages remove necrotic tissues and fibrin matrix for ossification
- Cartilage form to resolve strains
- Vascular growth provides nutrients and progenitor cells needed to form stable bone

- If the APR is insufficient or inappropriately exuberant, complications such as hemorrhage, infection/sepsis, SIRS, and severe cases of death. Complication that prolong the survival phase will delay the initiation of repair
- From a micro scale, the basic unit of bone is osteon. In the Nano scale, the bone is primary composed on collagen fiber and hydroxyapatite crystals.
- They are these calcium minerals the are essential ingredient for normal bones. They are what give bones and teeth their rigidity.
- In APR, hydroxyapatite deposition happens in the repair phase.

03 | Synthesis of CyAl 5.5

- Endgroup

- Linker

- Structure CyAl 5.5

04 | Coupling Strategy

- Carboxylic acid activation with NHS
- Coupling of with Pamidronic Acid

Entry	Conditions	Coupling reagent	Observations	Method
1	Cy dye in DMF/H ₂ O (5/3) + 4 eq TEA added to 1.5 eq pamidronic acid in DMF/H ₂ O (5/3) + 2.5 eq TEA	2.5 eq EDCI	Low conversion <15%	HPLC and LC-MS
2	Cy dye in DMF/H ₂ O (5/3) + 4 eq TEA added to 1.5 eq pamidronic acid in DMF/H ₂ O (5/3) + 2.5 eq TEA	2.5 eq PyBOP	Low conversion <15%	HPLC and LC-MS
3	Cy dye in DMF/H ₂ O (5/3) + 4 eq TEA added to 1.5 eq pamidronic acid in DMF/H ₂ O (5/3) + 2.5 eq TEA	EDCI/DMAP (2.5 eq/.35 eq)	25 to 30% yield	HPLC and LC-MS

05 | Hydroxyapatite Deposition

- Low doses of BPP-CyAm 7 were injected into mice to image and monitor the healing process of bone in relation to hydroxyapatite exposure (similar results were seen with the BPP-CyAl 5.5 agent)

Week 1 Week 2 Week 3 Week 4 Week 5

BPP-CyAm7 with fracture

BPP-CyAm7 without fracture

06 | Coupling with Peptides

- Labeling of binding peptides

07 | Matrix Evolution in Transverse Femur

- By 14 days post fracture, the fluorescently labeled fibrin matrix (red) has begun to be cleared. By 21 days post fracture, the presence of type 1 collagen (green) is pronounced at the site of fracture healing in the form of hard callus. As remodeling of the hard callus occurs through 42 days post fracture, there is a decrease in observable type 1 collagen signal

08 | Conclusion

- In short, we designed successfully synthesized novel fluorescent probes for molecular imaging of fibrin, collagen and hydroxyapatite
- In vivo* studies in mice demonstrate the potential for these imaging agents to be applied clinically

Future plan:

- These probes will be used clinically to assess the *bone healing process*
- We hope to use these dyes in conjunction with other binding agents, allowing us to take a multi-imaging-agent approach to integrate the most significant advancements in fracture biology to create a coherent and unified theory of fracture repair. (i.e. image bone, cartilage, and blood simultaneously)

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