Development of Novel Impedance Sensor to Monitor Fracture Healing

Site: University of California, San Francisco	
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Need and Industrial Relevance:

10-20% of fractures result in delayed or non-union in a normal healing population, but incidence rises to 46% when the fracture occurs in conjunction with vascular damage.[1,2] Radiography remains the standard technique to monitor healing, but because it relies on detection of mineralized tissue, it can only diagnose delayed healing at the late stages of fracture repair (weeks to months following injury). We are currently developing a device that utilizes impedance spectroscopy to monitor progression of fracture healing. Our novel sensor system uses an impedance mapping technology that will allow clinicians to non-invasively monitor fracture healing and detect delays in union at a very early stage. This technology will enable earlier intervention in poor bone healing.

Project Aims:

Specific Aim 1 – Quantitatively assess fracture healing in an *in vivo* murine model.

1a) *Develop sensors for use in an in vivo stabilized fracture model.*
We will develop a model for measuring impedance across a stabilized mouse tibia fracture. Novel, minimally-invasive sensor pins will be incorporated into an external fixator used to stabilize mouse tibia fractures and measure impedance across the fracture site during healing.

1b) *Monitor progression of fracture healing through serial impedance measurements and radiographs over the course of healing.*
Our preliminary study has validated our ability to distinguish various tissue types representative of the multiple stages of fracture healing (**Figure 1**). Cartilage is the primary tissue type present in a healing callus in early fracture healing, progressing to cancellous bone. We propose to acquire serial impedance measurements and radiographs at multiple time points after injury (days 7, 14, 21, 28) reflective of the different stages of healing, allowing for unprecedented tracking of changes at the fracture site over the course of healing.

Specific Aim 2 – Determine the earliest time point when fracture non-union can be detected.

2a) *Monitor the progression of fracture healing in an in vivo non-union fracture model.*
Using an existing mouse tibia fracture non-union model[4,5], we will apply the same measurement protocol as described in Aim 1b. Non-union will be confirmed by fluoroscopy and histology.

2b) *Compare normal and non-union impedance measurements to determine the earliest time point when fracture healing complications can be detected.*

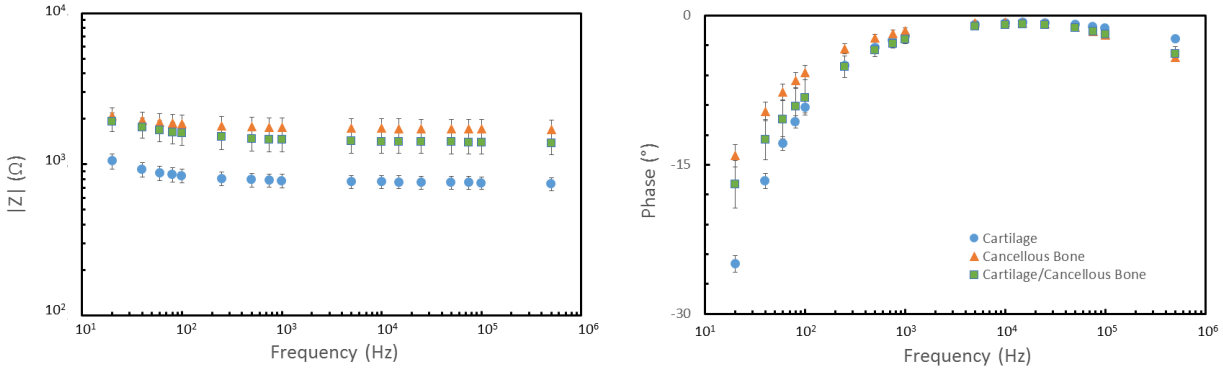


Figure 1 – Impedance magnitude (left) and phase angle (right) measured across a fracture filled with various tissues. Impedance magnitude mainly reflects the conductivity of the tissues; phase angle provides information about how resistive or capacitive the measurement is.

Methods:

Methods 1a) *Develop sensors for use in an in vivo stabilized fracture model.* We will design sensors based on the physical restrictions of the external fixator and in such a way that they do not interfere with the fracture healing process. Sensors will be fabricated as thin printed circuit boards using inert gold as the electrode material, and will take advantage of UV laser cutting to create sensors that resemble insect pins in size, allowing for seamless incorporation into the existing model. External circuitry will be connected at the time of measurement to collect data.

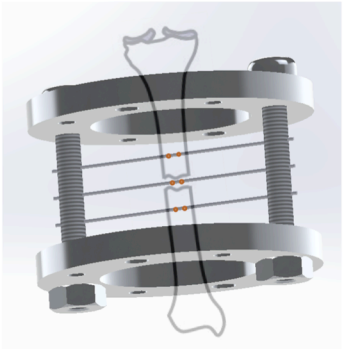
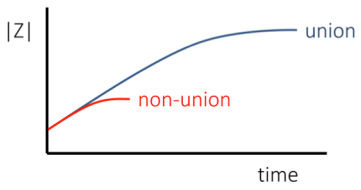


Fig 2 – Diagram of sensors (red) incorporated in external fixator.

Methods 1b) *Monitor progression of fracture healing through serial impedance measurements and radiographs over the course of healing.* These experiments utilize an established murine model of a critical sized segmental defect in an externally stabilized tibia.[3-5] Briefly, a circular external fixator, consisting of two 2cm circular rings held concentrically by two to four threaded rods, will be placed on the right tibia and a 2mm osteotomy created centrally within the fixator. The segmental defect will then be filled with cartilage graft to promote endochondral repair.[5] The sensor pin will be inserted into the grafted tissue to monitor healing as the cartilage transitions to bone. Serial impedance and fluoroscopy measurements will be taken at days 7, 14, 21, and 28 on 20 animals survived for the entire length of the experiment. Additionally, 7 animals per time point will be evaluated by impedance, radiography, and fluoroscopy, and then euthanized for histology and stereology.

Methods 2a) *Monitor the progression of fracture healing in an in vivo non-union fracture model.* The murine model described in Aim 1b will be used in this Aim, however, the 2mm osteotomy will remain unfilled. We have previously demonstrated that this will result in non-union.[5] As in Aim 1b, 20 animals will be monitored throughout 28 days of healing with non-destructive measurements taken at days 7,14, 21, and 28. These measurements will be complemented by radiography, fluoroscopy, histology and stereology measurements taken on animals euthanized at days 7, 14, 21, and 28 (n=7/time point).

<u>Expected Results:</u>



Methods 2b) *Compare normal and non-union impedance measurements to determine the earliest time point when fracture*

healing complications can be detected. Impedance curves from the normal versus impaired healing groups will be plotted and correlated to each other. Since mineralized tissue has a higher impedance signature (Figure 1), we expect to see a plateau from the non-union group demonstrating loss of healing progression. Furthermore, we expect to identify an impedance signature of the fibrous callus that could be used for early detection of non-union.

Milestones:

- Obtain IACUC Approval – Nov 1st, 2015
- Design and validate sensor pins for external fixator – Dec 1st, 2015
- Complete surgeries for Aim 1b – Feb 1st, 2015
- Complete analysis of data for Aim 1b – March 31st, 2015
- Complete surgeries for Aim 2 – May 1st, 2015
- Complete analysis of data for Aim 2 – June 30th, 2015
- Develop system for impedance measurements in a murine spine fusion model – August 30th, 2015

Deliverables:

Quarterly presentation updates:

- December 2015 – conference call
- Spring 2015 – Spring Symposium @ UT
- June 2015 – conference call
- September 2015 – Fall Symposium @ UCSF

Final written report including results - October 31, 2016

Specific work product

- Convert provisional patent to patent application – August 14th, 2016
- First human product prototype – June 1st 2016
- Develop IRB/CHR study protocol – June 1st, 2016

References:

1 Bahney, C. S., et al. The multifaceted role of the vasculature in endochondral fracture repair. *Frontiers in endocrinology* (2015).

2 Dickson, K. F. et al. The importance of the blood supply in the healing of tibial fractures. *Contemp Orthop* **30**, 489-493 (1995).

3 Thompson, Z., et al. A model for intramembranous ossification during fracture healing. *J Orthop Res* (2002).

4 Yu, Y. Y., Bahney, C., et al. Creating rigidly stabilized fractures for assessing intramembranous ossification, distraction osteogenesis, or healing of critical sized defects. *J Vis Exp* (2012).

5 Bahney, C. S. *et al.* Stem cell-derived endochondral cartilage stimulates bone healing by tissue transformation. *J Bone Miner Res* **29** (2014).

General Budget Outline:

Personnel	$	15,000
Supplies	$	8,300
Prototyping	$	5,000
Murine Costs	$	8,000
Total Direct	$	36,300
Indirects (10%)	$	3,630
Total	$	39,930

Start Date:	**End Date**:
October 1, 2015	September 30, 2016