Project Description

1) **Title**: Mechanosensitivity in the bone and cartilage cells of mammals

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LOCATION: NEOMED, C-156

2) **Abstract**: Within mammals, cells of cartilage and bone are mechanosensitive and respond to loads both within their living matrix as well as when loaded in a petri dish (in vitro). However, little is known of differences in the responsiveness of these cells in animals that live in extreme habitats. To increase our understanding of the variation in bone and cartilage cell sensitivity to loads across mammals, this study aims to establish a fundamental understanding of cell mechanosensitivity in terrestrial (mouse), aerial (bat) and marine (whale) mammals. This study uses species specific-primers and standard PCR techniques to compare dynamics of gene expression in cells harvested from bone and loaded in vitro. We hypothesize that the cells of bats will be either just as mechanosensitive or more mechanosensitive compared to the mouse, but those of the whale will be much less responsive to loads compared to mice and bats. Results will establish a new, foundational dataset. We therefore expect our results will add a critical understanding to the physiology of connective tissues across a taxonomically broad survey of mammals. Students interested in participating in this research should demonstrate fluency in PCR and cell culture.

3) **Significance of the Research:** The mammalian skeleton dynamically responds to loads but little is understood of how mechanosensitivity to loads differs between mammals that occupy different habitats. We expect that life in an aerial habitat has altered skeletal function, and indeed the wing bones of bats flex and bend with wing beats. Deformation in their skeleton is elastic, and such changes in shape would easily break the bones of most mammals. In comparison, the flipper bones of whales are stiff like terrestrial mammals, but only experience compressive loads and are therefore free from stresses associated with their exceptional mass or shear loading. Although the field of skeletal biology has an understanding of the mechanosensitivity of cells living in the skeleton of mice, we have yet to develop a critical understanding of the cellular physiology and function in mammals that occupy aerial and aquatic habitats. This study will increase our understanding of the diversity of mammalian bone and cartilage cell mechanosensation and allow for unique insights into the cellular consequences of life in an extreme habitat. By comparing gene expression profiles, our data will elucidate the loads at which bone cells begin to respond to their loading environment. Fresh tissues of bats and whales are exceptionally rare. The PI has a colony of bats and conducts field work in the Arctic and has collected cells of whales that are currently ready for analysis. Our contribution will be significant because the data will assist in developing a nuanced understanding of the cellular evolution underlying mammalian radiations into fluid habitats. It is also likely that our results will vertically advance our understanding of cartilage and bone cell plasticity within the mammalian skeleton associated with locomotion in novel habitats.

4) Goals and Objectives of the Research: By comparing gene expression levels, our data will elucidate whether the bone and cartilage cells of bats and whales are uniquely mechanosensitive compared to mice.

5) Research Methods Learned by the Summer Fellow: Applicants should have experience and fluency in PCR and cell culture. First, the researcher will be trained to participate in every phase of project research, including specimen preparation and analyses. Opportunities for students to gain experience with unusual model organisms are rare, and the skills gained through

involvement with this project should substantially broaden the researcher's skill sets. The student will learn to conduct PCR experience and assist in cell culture experiments, harvest tissues, and analyze data.

6) Research Methods and Data Analysis – The cells of whale, mouse and bat cartilage and bone are available for gene expression assays using standard relative PCR and species-specific primers. For cell culture, fresh and frozen cells that are terminally differentiated will be plated onto collagen coated dishes and incubated at physiological temperatures. Cells will be transferred to chamber slides and regular glass slides, and after an acclimatization period, will be loaded within an incubator for short cycles daily, over a period of days and then used for morphological comparisons as well as for gene expression assays. This protocol is already established in the PIs lab.

7) Expected Outcomes – Three possible outcomes are anticipated for this study. Our null hypothesis is that compared to mice, gene expression profiles will be similar between bats, whales and mice. If the bat cells display differences in gene expression, this is likely associated with the exceptionally flexible bone matrix and greater loads in the cartilage associated with flight performance. If the whale cells display differences in gene expression, this is probably associated with life in a compressive loading environment. Our findings, regardless of outcome, will lay the foundation for future work quantifying mechanosensitivity across the skeleton between these taxa. We expect this work to be the first of several publications.

Student Fellow Training/Mentoring Plan: Funding is requested to support one summer research fellow. PI's Cooper is committed to fostering the researcher's development for the summer. This goal will be achieved through a structured mentoring program, as described below.

Research will be conducted in C-156 using cells harvested from Cooper's colony of bats and samples collected from the field. Protocols are already established, and all necessary laboratory equipment and disposables are already in use as this is an ongoing project.

Besides benefiting from working alongside the PI and a technician, the student will be required to attend and present once at weekly laboratory meetings. The Musculoskeletal Research Focus Area - a joint effort of the Department of Anatomy and Neurobiology and the Department of Integrated Medical Sciences at NEOMED – also sponsors a weekly journal club on the general topic of "Skeletal Biology", where the fellow would have the opportunity to share and discuss ongoing research findings and pertinent scientific publications. Finally, the student will design and present a poster for the end-of-program poster symposium at NEOMED.