Christine & T. Jack Martin Research Travel Grant 2008

Final Report

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Synopsis Study centre visits:

1. Prof Adele Boskey, Musculoskeletal Integrity Program, hard Tissue Research Laboratory, Hospital for Special Surgery, NY, USA

2. Dr Nicola Napoli. Campus Biomedico Roma, Rome, Italy.

Conference: Attendance & Presentation

1- First workshop on Bone Tissue: Hierarchical Simulations for Clinical Application Organized by UCLA's Orthopaedic Hospital/Department of Orthopaedic Surgery, David Geffen School of Medicine Institute of Pure and Applied Mathematics (IPAM). April, 2010, Los Angeles.

2- The 37th European Symposium on Calcified Tissues which was organized in association with the United Kingdom Bone Research Society, Scotland, June 2010.

Report

The overall aim when I was awarded the Christine and TJ Martin Research Travel Grant was to travel learn new skills, and enhance my expertise on the study of bone tissue micro-mechanical and material properties. The travel opportunities provided to me by the grant, have been very useful in helping achieve this purpose.

I first attended the first workshop on bone tissue, at the University of California, Los Angeles (LA). The theme of the workshop was hierarchical simulations for clinical simulations. During the workshop, I had the opportunity to interact with leaders in the study of bone tissue material properties such as Maria-Grazia Ascenzi, and David Burr, and exchange views. The discussions we had greatly helped me to improve my knowledge in this area. Prior to attending the workshop, I had started studying tissue mineralization using quantitative backscattered electron microscopy (qBSEM), and tissue micro-mechanical properties (hardness, and elastic modulus) using nano-indentation. The knowledge gained during the workshop was very useful in understanding how experiments should be designed, interpreted and reported in this area. On my return to Melbourne, using the knowledge acquired, I was able to complete my work on the relationship between tissue mineralization and micro-mechanical properties and better interpret the findings.

We demonstrated that the variability in tissue stiffness is about 5 times greater than the variability in tissue mineral density and that, contrary to this common belief, only a small proportion of the variance in tissue stiffness was explained by its mineralization density. These findings suggest that caution should be exercised when using estimates of tissue stiffness derived from tissue mineral density in finite element analyses. We propose that other factors such as the direction of the mineralized fibers within lamellae of osteons is likely to be important. This work is in press in Bone (1) and I have duly acknowledged the Christine and TJ Martin Research Travel Grant which contribution has been invaluable for the completion of this work.

Following LA, I visited Dr Nicola Napoli in Rome as we had collected iliac crest biopsy samples from patients with atypical subtrochanteric fractures after long-term biphosphonates (BPs) therapy and age-related controls. The objective was to examine the effects of long-term BPs therapy on bone micro-mechanical properties. The aim of visit was to work with Dr Napoli on the protocol to be used to examine these samples as well as nurturing this ongoing collaboration. I also used the opportunity to learn about the ongoing work of Dr Napoli on aromatase inhibitors and bone.

I later went to New York City (NYC) in the laboratory of Prof Adele Boskey at the Hospital for Special Surgery (HSS), Musculoskeletal Integrity Program. The laboratory specializes in the study of mechanisms of mineralization of hard tissues using a wide range of techniques including such as Fourrier Transform Infra-Red microspectroscopy (FT-IR), wide angle x-ray diffraction, and imaging using micro-computed tomography. These techniques allow detailed study of mineral and matrix properties. The laboratory also develops new methods as required for this purpose. The ultimate goal of the studies is to improve our ability to assess bone quality and thereby provide further insight needed for new therapies and tissue engineered products. The research agenda in the laboratory therefore fit perfectly with my research agenda and the overall aim of the Christine and TJ Martin Research Travel Grant (i.e., learn techniques to assess tissue mineralization and matrix composition)

While I was interested in the technology used, I elected to use the largest fraction of my time in learning FT-IR. I elected to learn this technique because it allows probing of the composition and physicochemical status of mineral and matrix of bone in normal and diseased tissues and compliments the technology we have in our laboratory in Melbourne. Furthermore, reports of atypical subtrochanteric fractures after long-term biphosphonates (BPs) were just emerging. When I arrived in NYC, Prof Boskey was studying changes in bone mineral and matrix properties associated with long-term biphosphonates (BPs) therapy. This was a perfect collaboration because, in collaboration with Dr Nicola Napoli, we had collected iliac crest biopsy samples from women with atypical subtrochanteric fractures after long term BPs therapy and their age-matched controls with the purpose of examining the contribution of long-term BPs therapy on bone micro-mechanical properties. Examining the effects of long-term BPs therapy on bone mineral and matrix properties would therefore provide additional information and better insights into this issue.

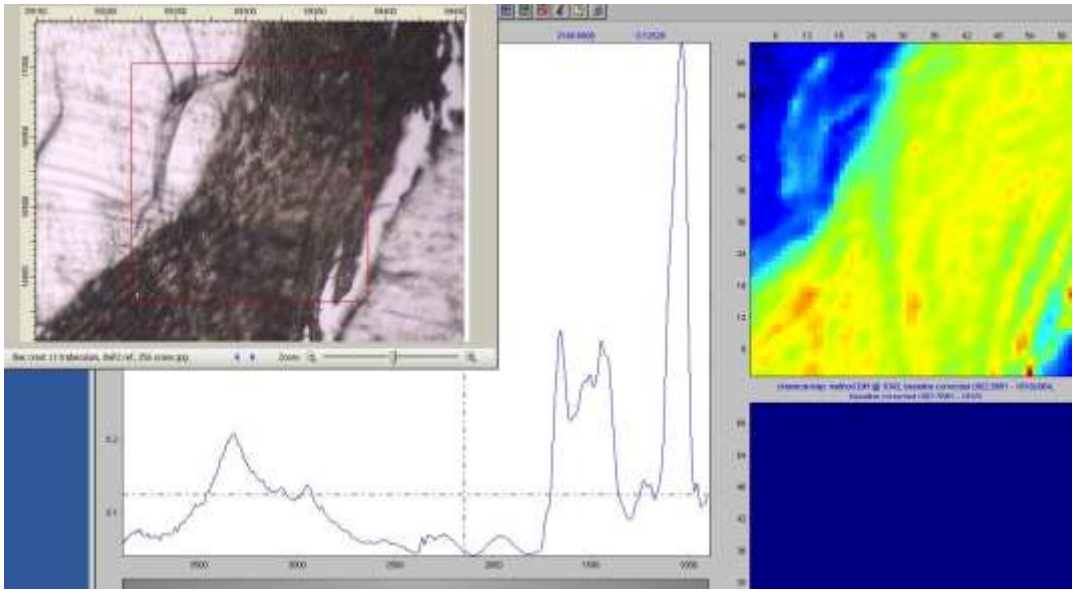
Dr Napoli and I decided that it would be good to combine the two datasets. I

therefore learnt FT-IR with a focus on examining the effect BPs therapy on bone. The laboratory used a transmission mode FT-IR Microscope which requires very thin sections (~ 2 microns). Some my samples were couriered from Melbourne to NYC so I had the opportunity to practice on them.

Apart from learning new skills, I had the opportunity to share with people in the laboratory some the works we have been doing in Melbourne on the micro-structural basis of bone loss and in particular on the contribution of cortical bone loss to total age-related bone loss. The presentation I gave to the laboratory on this topic was very well received and generated a lot of interest.

Besides visiting Prof Boskey, I also used the opportunity of my presence in NYC to visit Prof John Bilzekian and Dr Stephanie Boutroy, Department of Endocrinology, Columbia University, NYC, USA. We have an ongoing collaboration with this team which is aimed at clarifying the effects hyper- and hypoparathyroidism on cortical and trabecular bone micro-architecture. Parathyroid hormone (PTH) excess is believed to produce cortical thinning by endocortical resorption while being anabolic at trabecular sites. However, new evidence suggests that the proposed anabolic effects on trabecular bone may be spurious as high intracortical remodelling adjacent to the marrow causes cavitation in the cortex (trabeularization) leaving cortical remnants of trabecular appearance which may be erroneously measured as trabeculae of growth plate origin. Hence, determination of the effects of endogenous PTH excess requires separation of cortical remnants from trabeculae of growth plate origin. We have recently developed a new method and system encoded in a software which allows us to achieve this separation and therefore clarify the effects of PTH excess on bone. Professor Bilzekian has a large cohort of patients with thyroid dysfunction followed up for a number of years with high-resolution peripheral computed tomography (HRpQCT) images. The overall aim of visit in this laboratory was to progress this collaboration.

On return to Melbourne, it is was essential to use the the skills learnt in NYC, in our laboratory in Melbourne allowing us to get long-term benefits from the visits. To do so, I put a team together that could replicate these techniques. I made contact with Dr Stephen Best, an expert in Spectroscopy. In Melbourne, we were already able to prepare thin sections of ~2 microns as required for transmission mode FT-IR. What was needed was a good source of FT-IR. The best of source of FT-IR in Australia is from the Australian Synchrotron (AS). This offers a much higher resolution than an FT-IR microscope (5 microns vs 20 microns). We applied for beamtime to collect spectra for our samples and were successful. We have now been able collected IR spectra in patients on BPs and controls. An example mineral to matrix ratio in a trabeculum is shown below



I was pleased with the fact that I learnt the technique and I have been able to successfully reproduce it here in Melbourne.

ECTS Meeting, Glasgow, UK

I attended the 37th European Symposium on Calcified Tissues, the premier European bone meeting, in Glasgow, Scotland, which attracted ~ 4000 delegates. I gave a plenary talk there on the contribution of porosity to age-related bone loss. This talk was well received by my peers and was followed by a lot constructive discussions. I enjoyed and learnt a lot from other presentations and posters.

In sum, I am extremely grateful to AMGEN for the sponsorship of this travel award. Prof Jack Martin is a giant in the field and I hold tremendous respect for him. I feel very privileged to have been the recipient of the 2008 Christine and TJ Martin Research Travel Grant. I also thank the ANZBMS committee for the opportunity offered. This grant has been of a great help for my career development.

References

1-Zebaze RM, Jones AC, Pandy MG, Knackstedt MA, Seeman E. Differences in the degree of bone tissue mineralization account for little of the differences in tissue elastic properties. **Bone**. 2011 Mar 6. [Epub ahead of print