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|--------------------|-----------------------------------------------------------------------------------------------------------------------------|
| Name               | H.M. Suranji Wijekoon                                                                                                       |
| Laboratory         | Veterinary Surgery                                                                                                          |
| Year (Grade)       | DC4                                                                                                                         |
| Place of practice  | Department of Pathology and Immunology,<br>Division of Bone and Mineral Diseases, School of Medicine, Washington University |
| Period of practice | May 15 <sup>th</sup> 2017 – June 7 <sup>th</sup> 2017                                                                       |
| Purpose            | Overseas practice on collaborative research                                                                                 |

My main purpose of trip to Washington University was to gain the experience, skill and make research collaboration with renowned institute for bone and mineral disease.

Washington University Musculoskeletal Research Center (MRC) was established through a partnership between the Department of Internal Medicine, Division of Bone and Mineral Diseases, and the Department of Orthopedic Surgery. Its mission is to support and expand research on the musculoskeletal system, foster collaborations within its research base, attract investigators from other disciplines to the field, and train the next generation of musculoskeletal investigators. As a budding researcher, I prefer to expand my knowledge and skill on osteoclast-related research for exploring the novel aspects of this field.

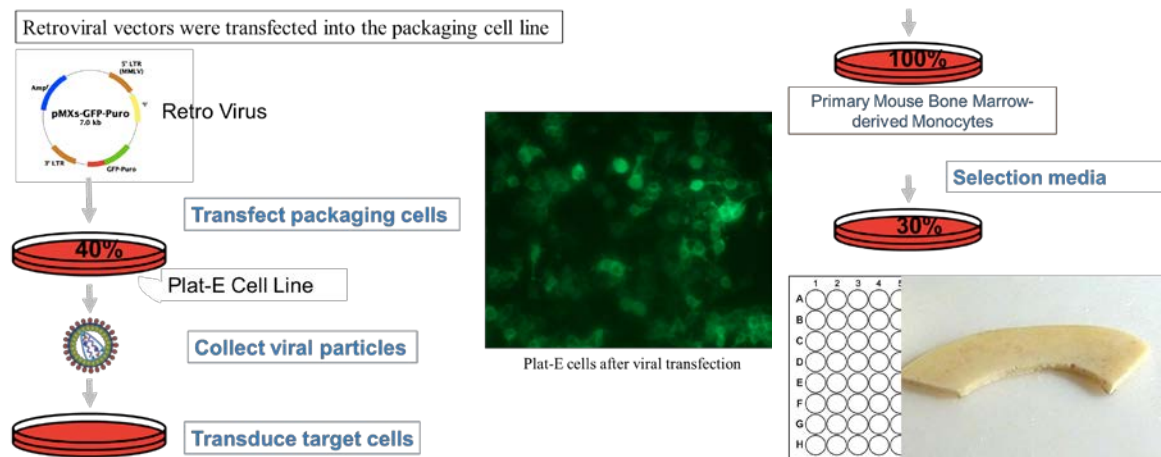


In order to accomplish my objectives, I have selected the laboratory of Prof. Steven L. Teitelbaum who is one of the pioneer researchers in USA related to the field of bone and joint

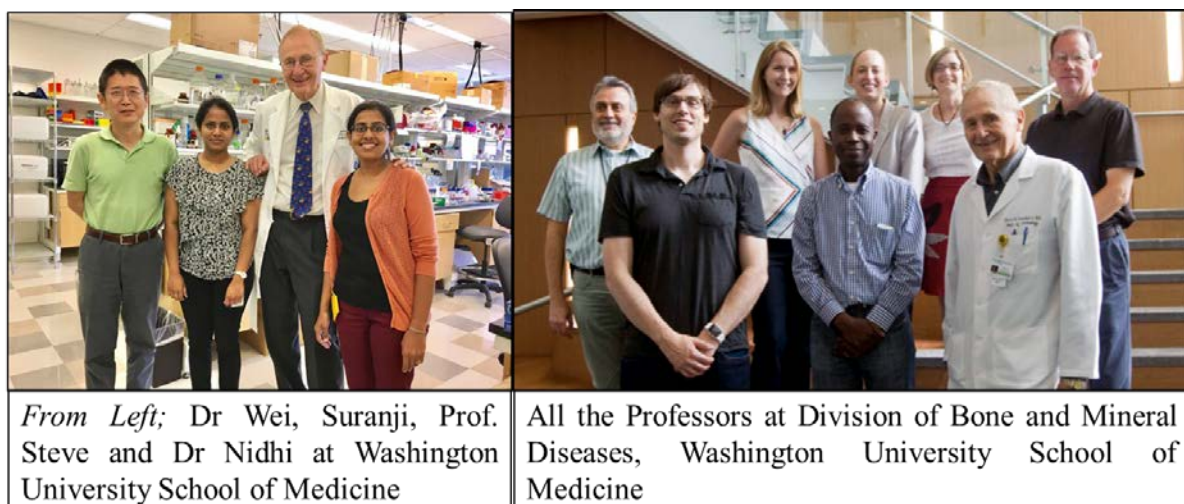
disease. One of the main research projects going at Teitelbaum lab is “understand the relationship of mitochondrial function and osteoclastogenesis”. Osteoclasts are the most mitochondria-rich cells in human and animal. Peroxisome proliferator-activated receptor gamma coactivator 1- $\beta$  (PGC1 $\beta$ ) is a transcriptional coactivator that regulates energy metabolism by stimulating mitochondrial biogenesis and respiration. Previous studies using global knockout mice reported that PGC1 $\beta$  is essential for osteoclast differentiation. To determine the role of PGC1 $\beta$  in osteoclastogenesis, they have generated conditional knockout mice, in which PGC1 $\beta$  is exclusively deleted in myeloid lineage cells (PGC1 $\beta$ LysM). This novel aspect of osteoclast experiments was driven me to choose this place for overseas activity, thereby serve as diplomat to Veterinary Surgery Laboratory, Hokkaido University by creating network among the two laboratories.

At Teitelbaum laboratory, basic ethics and safety handling with orientation were the first lesson for new comers to make them familiar with the settings and procedures. At the same time, I have discussed and modified my working plan for fitting in to next 3 weeks of time. By using their available mouse models, wild type and knock out for mitochondrial transcript factor, I was able to visualize how the cytoskeletal is affect by mitochondrial activity.

To envisage the live cytoskeletal development through time lapse microscope, I have to keep the cell live during the staining of cell wall. It is necessary in many biological and medical studies to introduce fluorescent markers or plasmids into cells to facilitate visualization of filamentous actin, tubulin, or to observe functional alteration. And it is often necessary that researchers insert such materials into cells for various dynamical or drug delivery studies too. For my study, I used retro viral vector to insert into cells of interest. pMX retroviral vectors containing GFP-actin and a blasticidin resistance selectable marker, were transfected into separate platE. The virus-containing supernatant was harvested 48hrs after transfection and then added to mouse marrow-derived macrophage (BMM) previously cultured for 2 days. After 24hr viral incubation, BMMs were selected for 2 days with 1  $\mu$ g/ml blasticidin, before culturing for osteoclastogenesis. Then culture was continued 5 days for osteoclastogenesis. At the end of the experiments, I was able to explore the data regarding how cytoskeletal formation is affected by mitochondrial factors which correlate with inflammatory pathways and differentiation cascade of osteoclast.



My current work at laboratory of veterinary surgery, Hokkaido University is focusing on understand the mechanism of action of pentosan polysulfate sodium (PPS), a novel disease modifying osteoarthritis drug. In order to detect the efficacy of PPS over the inflammatory arthritis, at the beginning I used it for one of the major cell types of bone, called osteoclast. Thereby I studied how PPS act in different inflammatory pathways and other metabolic pathways of osteoclast to regulate their activity at the inflammatory conditions such as rheumatoid arthritis. The skill and techniques I gained from Teitelbaum lab, how mitochondrial function in osteoclasts relates to energy consumption and cytoskeletal formation during bone remodeling might be value added for my future studies on this relevant field. The connection we made with Teitelbaum lab through this trip will use to make collaboration to exchange further technical knowledge, protocols and possible cell lines in future.



Further, I had a chance to visit other labs which are focusing on how connexins and Wnt signaling regulate osteoblast function, role of microRNAs in chondrocyte biology and skeletal biomechanics. During my stay, I have attended all conferences including weekly journal/data

club, weekly laboratory meeting and our Avioli Seminar Series in which senior investigators, both local and visiting, present their research. The opportunity of getting communicate and share the ideas with experts of this field was an immense experience for me and unforgettable moment for my career life.

I would like highlight few points for future candidates who are looking for place for their overseas activity. First, this kind of overseas activity related to collaborative research is one of the most important pieces of the foundation you are laying for a successful career. Since the professional world can be very different from university life, it is important that getting to understand of some of the differences. Second, focus on your communication skills, both written and verbal throughout the training period. When communicating with your co-researchers choose respectful, tactful, and professional language always. Third, show up to work on time, when you are expected. When you leave, be sure to ask your supervisor for an evaluation for your stay and future collaboration.

Finally, I am forever grateful to Prof. Masahiro Okumura, for his enormous help and keeping faith on my competence for the success of this trip. Further, I would like to thank leading program coordinator Prof. Motohiro Horiuchi for approving and supporting me financially. And I want to thank leading staff for their generous help throughout this activity.

(Field Epidemiology • Collaborative Research) Evaluation by supervisor

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|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| Institution • Official title • Name                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Prof. Masahiro Okumura<br><br>(Lab. Veterinary Surgery, Graduate school of Veterinary Medicine, Hokkaido University) |
| Here she reported what she experienced there in the States. She gained her own experience and skills related to the study on the relationship of cellular function and osteoclastogenesis, which would be a major part of her thesis. The research laboratory dedicated to study on osteoclast functions gave her very important opportunity to study one of cutting edges of approach on the subject. Both this novel aspect of experiments and her experience in the different system of laboratory will contribute her to be a globally based reader for the respective field in veterinary science. |                                                                                                                      |

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