This report should be submitted within 2 weeks after you return to Japan.

（Abroad・ Domestic） Internship report form （Student） 2017/12/14

(Year/Month/Day)

Name	Md. Abdul Masum
Laboratory	Anatomy
Year (Grade)	D3
Internship institution	Department of Cellular & Integrative Physiology, School of Medicine, University of Indiana, Indianapolis, USA
Internship period	Internship period: 11/03/2017 - 11/29/2017 (Departure Date from Sapporo: MM/DD/YYYY, Arrival Date in Sapporo: MM/DD/YYYY)
Purpose	For Internship

- The reason why you chose this institute

- It great opportunity for me to involve with various ongoing projects in the Department of Cellular & Integrative Physiology. So, I have acquainted with good research experiences through the ongoing projects in that laboratory.
- I got knowledge about human kidney research as my internship place was in human medical school. So, I could play role to One Health through kidney research on prevalent human kidney diseases in addition to animal disease.
- I have gained many experiences regarding how to manage laboratory and able to create research network.

- Result of the activity （about 800 words、provide photos, tables and figures that clearly show the activities during the period）

I have completed my internship under the supervision of Professor Dr. David P. Basile at the department of Cellular and Integrative Physiology, University of Indiana, USA. Dr. Basile also assigned Dr. Purvi Mehrotra to execute different protocols that I performed in his laboratory.

They arranged a laboratory seminar to show their research activities. I also presented my research activities in Japan at the same seminar. Professor Dr. Basile made outline for my internship activities in that seminar.

Firstly, we isolated endothelial side population (SP) by magnetic cell sorting (MACS) and counted their number using fluorescence activated cell sorting (FACS) method. Endothelial SP is very important for kidney disease as renal endothelial cells rarely participate in regeneration that lead to fibrosis. Dr. Basile with his collaborative group identified stem/progenitor–like endothelial cells in peripheral blood vessels based on their ability to efflux Hoechst 33342 dye. Cells that do this are termed SP cells because of their characteristic appearance in flow cytometry. SP cells appear as a discrete subpopulation at the side of main population cells. They showed that these endothelial-SP cells possess endothelial colony–forming potential *in vitro* and contribute to angiogenesis by generating

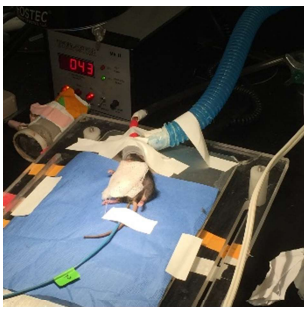
functional mature blood vessels when transplanted into an ischemic limb. The aim of this project was to isolation and characterization of endothelial SP for the purpose of therapeutic use.

Secondly, I performed tyramide signal amplification (TSA) technique to identify renal capillary using CABLIN antibody. TSA was done in combination with our Alexa Fluor dyes to achieve high-resolution signal amplification in cell and tissue applications. Catalyzed Reporter Deposition—is an enzyme-mediated detection method that utilizes the catalytic activity of horseradish peroxidase to generate high-density labeling of a target protein *in situ.* TSA is usually use to increase detection sensitivity up to 100-fold, as compared with conventional avidin–biotinylated enzyme complex procedures.

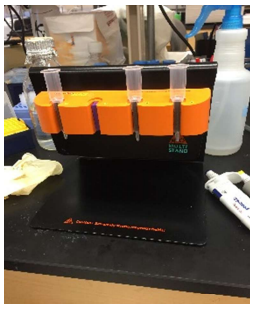
Infiltrating immune cells and their cytokines play important role in the development of kidney disease. That's why, I performed sediment gradient centrifugation (SGC) to isolate mononuclear cells (lymphocyte). Ficoll-Paque separation media was used in SGC. Differential migration of cells during centrifugation results in the formation of layers containing different cell types. The bottom layer contains erythrocytes, was aggregated by Ficoll PM400. The layer immediately above the erythrocyte layer contains mostly granulocytes. At the interface between the plasma and the Ficoll-Paque layer, mononuclear cells were found together with other slowly sedimenting particles (e.g., platelets) with low density.

I also measured serum creatinine value using ELISA to detect the level of kidney injury.

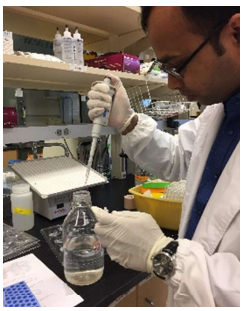
I also attended on several meetings with Dr. Basile and other laboratory members to update the activities. We also discussed about future research collaboration and research opportunity in USA.



Ischemia-reperfusion injury in mice kidney



Isolation of CD31 cells by MACS



Dilution of CD31 cells in FACS buffer

Me with Dr. Basile and his laboratory members

- What do you think the positive impact of the activity will have on your further career path?

- I have gotten good knowledge about the role of endothelium in physiology and pathology of kidney.
- I got many directions or hints for my future research through discussion with Dr. Basile and Dr. Mehrotra.
- Now I have a good research network that will facilitate my future research advancement.
- This internship experience will add worth in my curriculum vitae to get new research position or job.

- Advice for your junior fellows

- It is better to complete internship at earlier stage of graduate course as you could get many helpful hints about research from different expertize.
- Discuss freely with your boss what you want to do now and in future.

Approval of supervisor	Institution・Official title・Name

※1 Send the electronic file to the Leading School section, International Affairs Office
※2 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
※3 The Steering Committee of the Leading Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

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