2021年度　技術交流助成　成果報告（日本留学）

2022 年　3 月　30 日
氏名　Fatma Rashed

留　学　先：東京医科歯科大学
大学院医歯学総合研究科　口腔基礎工学分野
受入先担当者：青木　和広
留 学 期 間：2020年7月1日～　2021年12月31日

1. 留学中に実施した研究テーマ 1. Research themes conducted while studying abroad

Title

"Development of cell-membrane protein-mapping technique for quick screening of novel bone anabolic reagents in treating tumor-induced bone destruction."

Scope and purpose

An atomic force microscope is mostly used as a force sensor or mechanical mapping. However, we will use it as a biological simulation tool. In this mode, we hypothesize that the stimulation of a restricted area, using an AFM probe with a ligand, on the cell membrane receptor could be enough to initiate the osteoblast differentiation. We will compare our results to the conventional in-vitro stimulation using stimulation media with the ligand to search for the most appropriate condition/ ligand morphology that can stimulate osteoblast differentiation most efficiently.

Proposed mechanism:

1. The peptide-modified probe will be cyclically approached and retracted from the cell surface.
2. While monitoring the variation of the force concerning the tip-sample distance.
3. Then, on the binding of the proper peptide to the RANKL on the osteoblast surface, the induction of the osteoblast differentiation cascade is monitored.

留学期間中の研究成果 2. Research results during the study abroad period

First

We found that mature osteoclasts release RANK-expressing extracellular vesicles, which

interact with the RANKL on osteoblasts that functions as a coupling signal acceptor. In other words, RANKL works as a receptor on osteoblast to stimulate bone formation through reverse signaling that can be a potential pharmacological target to recover the bone remodeling balance between bone formation and bone resorption. (**Rashed, F.** et al. First author (2021) The Effects of Recptor Activator of NF-KB Ligand-Binding Peptides on Bone Resorption and Bone Formation. *Frontiers in Cell and Developmental Biology,* 9)

Second

Even though recent experiments have revealed that the created RANK liposome does not have a function as RANK that cannot suppress RANKL-induced osteoclast formation, there is a possibility to develop a bone formation promoting agent using RANK-containing proteo-liposomes (RANK liposomes). We aim to confirm the RANK-liposome work as a ligand to bind to RANK to stimulate reverse signaling. Since the function and structure of proteins are known to be related, it is necessary to acquire a suitable three-dimensional structure for RANK exosome to work physiologically.

Third

We used Hight speed-AFM to reveal the 3-dimensional structure of 1,2-dioleoyl-sn-glycerol-3-phosphocholine (DOPC) liposomes, RANK-Fc, and RANK liposomes. AFM observation revealed that the DOPC liposomes formed a supported lipid bilayer (SLB) of about 3 nm in height on the silicon substrate. The RANK liposomes also formed SLB of about 3 nm in height, and particles of about 112 nm in diameter were observed. Since RANK-Fc was about 55 nm in diameter, these observations suggest that the particles with a diameter of 112 nm were aggregated RANK molecules. Taken together, the reason why RANK liposomes did not work as a RANKL-antagonist could be due to inappropriate topology, which could not bind to RANKL.

2. 今後の研究計画 3. Future research plan

-Force mapping: technique will be used using AFM in real-time *in vivo* to clarify the localization and the quantity of the membrane-bound RANKL on the surface of osteoblasts using a cantilever AFM tip modified by DH01, a novel RANKL-binding peptide.

- Utilizing Mirabody: By using, Random non-standard Peptides Integrated Discovery (RaPID) system with collaboration with Prof. Hiroaki Suga, the University of Tokyo, to find out a peptide drug candidate with high affinity to the target protein and grafting of an Fc fragment into this high-affinity peptide (Mirabody) then testing them *in-vitro* to develop the most efficient RANKL-binding peptide in the activation of reverse signaling and bone formation.

- *Chimera mice:* Generate an extracellular human/ intracellular mice RANKL knock-in

chimera mouse to test the Mirabody (DH01-grafted Fc protein) *in vivo* since DH01 binds only to the human RANKL, not to the mice RANKL.

- *Osteopenic model mice:* Test a new peptide on an osteopenic mice model, which we recently established. The bone mineral density of distal femurs was significantly reduced by the oral bacteria (*Streptococcus mutans*) intravenous inoculation. (Hirohashi, Y., Kamijo, S., Khan, M., Ikeda, M., Oki, M., Matin, K., **Rashed, F.,** & Aoki, K. (2021). Tetracycline, an Appropriate Reagent for Measuring Bone-Formation Activity in the Murine Model of the *Streptococcus mutans*-Induced Bone Loss. *Frontiers in cellular and infection microbiology*, *11*).

3. その他と謝辞（日本での生活・交流の様子など）4. Others and acknowledgments (life and exchanges in Japan, etc.)

I attended several practical sessions with the undergraduate students, such as using image-J Software, calculating different doses of different anesthesia (inhalational and injection) for experimental animals (mice and rats).

Every Thursday morning at 8 am, the research team meeting is held in the meeting room, and one member is asked to present a presentation. I made several presentations, the first one was about my research experience in Egypt, and the following one was a summary of a research paper.

I learned how to do several tissue-culture experiments on the effect of different peptide on osteoclasts activation, I learned to use the micro-CT and the pQCT machine and the fluorescent microscope, I also learned how to make frozen histology and methyl methacrylate tissue sections.

I observed an animal experiment on testing different scaffolds on a calvaria defect of mice in the TMDU animal center and how to make life micro-CT images.

Cultural activities:

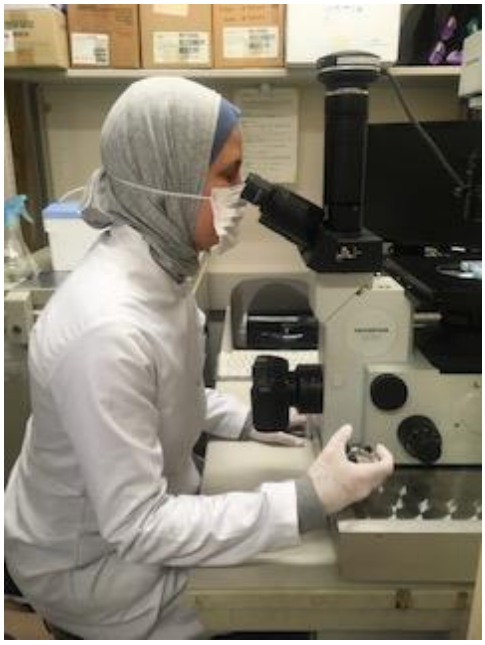
The first thing I did was visit the famous Shibuya crossing and the Hachiko dog, and I ate sushi from a sushi belt bar. Then I saw the Panasonic and sewage museum and the different shopping malls at the Odaiba district. The next weekend, we went to a classical music concert and the anime museum in Suginami City. I went to the Tokyo metropolitan building observatory in Shinjuku and then to the ice skating ring in the Roppongi area. I visited Okutama lake and Tama lake amongst many mountains. I climbed Mount Fuji in September. I also visited Kamakura city and walked around Minato city and visited Tokyo tower in addition to many other touristic famous monuments. I participated two days as a volunteer for Tokyo Olympics, the Tokyo marathon, and several charity activities in Tokyo.

※最後に留学中に技術交流を行っている様子等の写真 2〜3 枚ありましたら、簡単なコメントを添えて、挿入してください。* Finally, if you have a few photos of technical exchanges while studying abroad, please insert them with a brief comment.

List of photos

1. **Group photo of the research team in Professor Aoki's lab.**

2. **Working at the Lab.**



3. **My name on the door.**

分野	役職	氏名
口腔基礎工学分野 Basic Oral Health Engineering (bhoe)	教授	青木 和広
		Kazuhiro Aoki
	准教授	大木 明子
		Meiko Oki
	助教	上條 真吾
		Shingo Kamijo
	院2年	中村 聡倭
		Satowa Nakamura
		廣橋 優奈
		Yuna Hirohashi
	院1年	清水 優里
		Yuri Shimizu
		謝 倉右
	非常勤	Masud Khan
	博PD	大河原 久実
	博自治医	小澤 通子
	博PD	松野 瞳
	ポスドク	Fatma Rashed
	非常勤	天野 均

4. **Climbing mount Fuji**

5. **At the practical session with undergraduate students**

6. **Volunteer at the cycling event in Tokyo 2020.**

7. **Distributing food to homeless people at Ueno Area.**