This report should be submitted within 2 weeks after you return to Japan. Please do not change the formatting

（Abroad・Domestic）Internship report form（Student） 2022/10/11 (Year/Month/Day)

Name	WANG Yanlin
Laboratory	Veterinary Surgery
Year (Grade)	DC 4
Internship institution	Laboratory of Veterinary Surgery, Joint Faculty of Veterinary Medicine, Kagoshima University
Internship period	Internship period: 09/12/2022 - 09/30/2022 (Departure Date from Sapporo: 09/11/2022, Arrival Date in Sapporo: 10/01/2022)
Purpose	To increase the knowledges and skills for treating degenerative joint diseases with mesenchymal stem cell (MSC) therapies, and build a good relationship with the host.

- The reason why you chose this institute

The first one is the host laboratory has a long history of research on degenerative joint disease (DJD) and good achievement in the field of stem cell therapies for joint diseases in animals. My research topic is about the effect of pentosan polysulfate in animals with osteoarthritis. However, I want to find more possibilities for the treatment. I can learn the technique of using mesenchymal stem cells (MSCs) for DJD treatment in animals at the institute since this is one of the current topics in their lab. In addition, they are also studying with other medical treatments besides PPS, which will largely expand my knowledge. The second reason is I have already met Professor Misumi in a symposium before, and our lab has some good relationship with them. I want to keep this good relation not just between the laboratory, but also build a personal connection, which will become a treasure in my future career path.

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

DJD is one of the top healthy issues affects human and animals, especially in the ageing population. For instance, osteoarthritis (OA), a common type of DJD, can cause the breakdown of multiple joint structures and subsequently leads to loss of joint function. Unfortunately, current treatments are mainly palliative and are incapable of preventing the disease progression.

Intra articular injection of MSC to the affected joints provides large amount of exosomes secreted by MSCs. These exosomes have the capacity of promoting cartilage regeneration and modulating the local inflammatory reactions. However, intra articular MSC injection is still facing several challenges. Firstly, the

injection requires a great number of MSCs (around 1.0 x 10^8) to achieve proper effects. Traditional in-vitro expansion by monolayer culture takes time and effort to meet the required cell number. Moreover, MSCs gradually loss their normal phenotype and in monolayer, which will affect their regenerative capacity and exosome production. To overcome these limitations, Professor Misumi and his research team are focusing on developing a feasible procedure for intra articular MSC injection in animals, mainly athletic horses.

The equine medical center of KU, the lab is on 2F

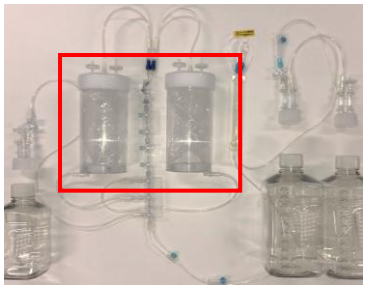
Teaching hospital of KU

The carpal joints are common sites of osteochondral fractures in racing horses, which easily results in traumatic arthritis or OA even after surgical repair. In order to manage this problem, Professor Misumi's teams is collaboration with Japan Racing Association (JRA).

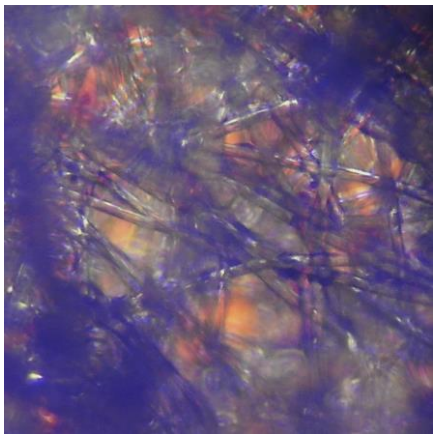
Surgical treatment for horse with palmar osteochondral fractures were performed by surgeon in JRA. During the surgery, synovium tissue of the affect joints or adipose tissue were collected from the horses, then refrigerated transported to Kagoshima University. Equine MSCs (eMSCs) were then released from the tissues by enzymatic digestion and the primary (P0) cells were seeded in monolayer. After around 5-7 days culture, the P0 eMSCs were collected and seeded on 3-D polyester (PET) non-woven fabrics to mimic an in-vivo like condition and cultured for further 12 days to expand the cell number. The 3-D culture was monitored by a highly specialized system designed by FullStem Co. Ltd (Uruma city, Okinawa, Japan). This system provides massive culture for the MSCs (300-500 pieces x 2 bottles every time), and researchers only need to pay a little time to complete the culture. After 12 days, the MSCs culture bottles were sent to JRA in the and collected to inject into the joints. The whole procedure can be finished in 20 days, and this method have shown improvement in clinical symptoms and joint function in a trial. Unfortunately, due to the COVID-19 pandemic, the clinic trial in horse was stopped during my visiting. But they are

also exploring the possibility for applying this procedure in dogs, which I luckily participated in this time.



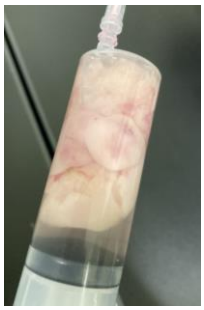
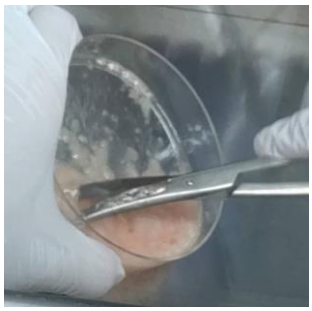



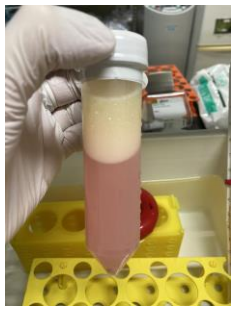
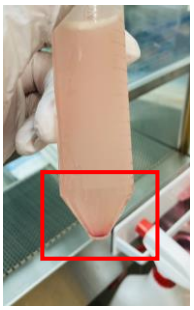
FullStem Achieva-CS automatic culture system **Culture bottles**

Non-woven PET fabric & Micro-structure (purple: MSC with crystal violet)

In the first week of my internship, we collected adipose tissues from a dog received abdominal surgery. The sample was cut into small fragments and digested by 0.1% collagenase I for 90 min, then the aqueous layer was transferred to a new tube. Canine MSCs (cMSCs) were collected by centrifugation and seeded in monolayer.

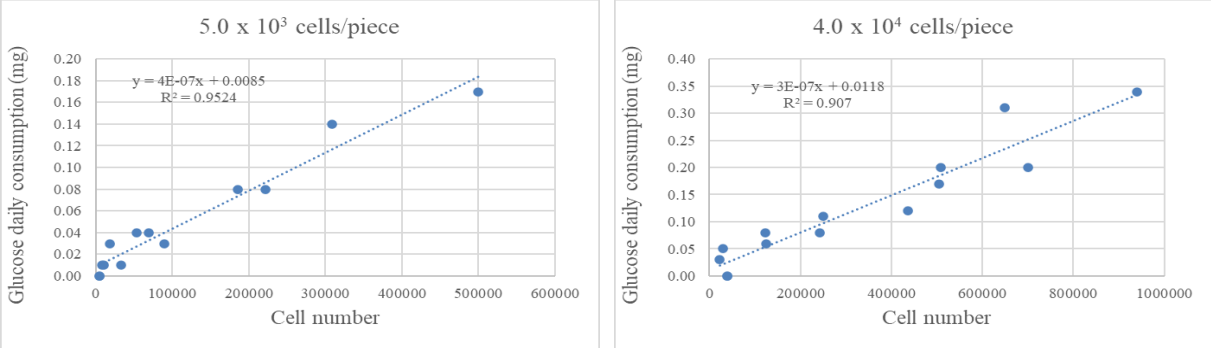



Collecting cMSCs from canine adipose tissue **After digest** **cMSC pellet**

To test the feasibility of cMSCs 3-D culture in PET fabrics and to measure the growth speed of the cells, we performed a series of experiments with the P1 cells in the second and third week. Firstly, we seeded the cMSCs with different densities (5.0×10^3 or 4.0×10^4) in individual PET fabrics to monitor the growth.

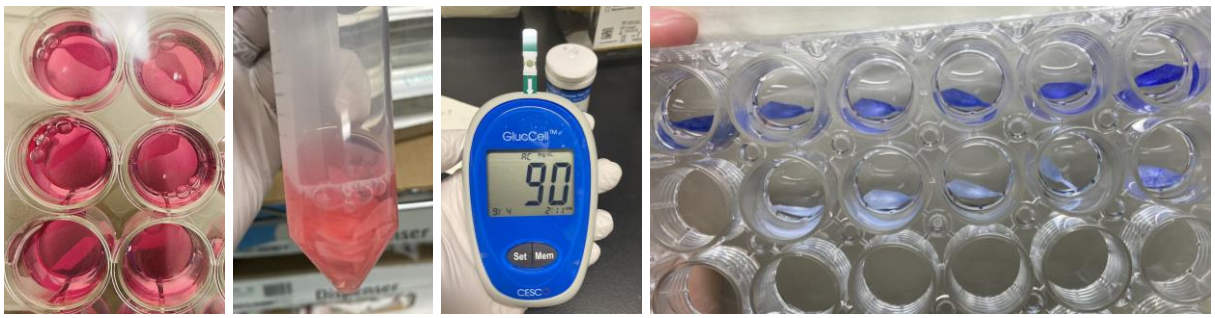
Since we cannot check the cell number during 3-D culture by light microscope, we need an altered measure to monitor cell growth. One convenient way is measuring the glucose consumption. From day 3 to day 8, we collected culture supernatant every day and measure the glucose concentration with a glucose



Results of trial 1: glucose daily consumption positively correlates cell number of cMSCs

meter. Besides the glucose, we also performed crystal violet staining on the fabrics as another indicator. Every day, a fabric was used to recover the cMSCs and the cell number was counted to find the correlation between cell number and glucose consumption. The results indicated that regardless the seeding density, the number of cMSCs showed a positive liner correlation with the daily glucose consumption, and the color of staining also became stronger.

Secondly, we seeded cMSCs in 30 pieces of PET fabrics with standard seeding density (1.0 x 10^4 cells/piece) to check the number of cells that can be obtained after expanded. As the results the glucose consumption started to largely increase from day 7 and the change became smaller from day 14. Finally, we totally collected 2.1 x 10^7 cMSCs on day 15 from 30 pieces PET fabrics. Due to the time limitation, we did not check the MSC properties after collected from PET fabrics. But depending on the data from this short trial, we can presume the cell number could reach the require 1.0 x 10^8 in 2 weeks by using the FullStem system, which suggested this culture system might be suitable for cMSC expand. I am expecting their future progress about the following research.



Trial 1 **Trial 2** **Glucose meter** **Crystal violet staining for cMSCs**

Besides learning the experiment skills, I also built a good relationship with them. They invited me to joint their research discussions in the laboratory. We introduced our current research to each other and exchanged the ideas. This experience from this internship will be very helpful to my future research and career path.

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

My first choice for the future career path is a position in a university as a researcher and a veterinarian in my home country. As the goal of this internship is to learn new skills and to build network with researchers in my major. This internship was a very good chance for me for working in institutes of research and education. Comparing with China, Japan has more developed system of research in veterinary medicine. I hope to have more access to these advanced knowledges when I have a chance, which could accelerate the progression of my research in the future. The development of veterinary medicine requires collaboration between researchers. Since the research topic of my host laboratory is close to mine, this network is very precious in the future to share information and exchange ideas. This internship also expanded my understanding about what a veterinarian can do in the clinical setting, and how to transfer the results in laboratory to clinical usage. Since they do not only perform the basic research in laboratory, but also have experiments in clinical level.

- Advice for your junior fellows

Prepare for unexpected situations. The schedule and contents of our internship could be changed by many factors. For instance, a new wave of COVID-19 pandemic. Even the travel ban is becoming looser and looser, the policy of individual institution would change under the situation, and some activities might be restricted. We need to keep communication with the hosts event after the contents are fixed. When accident really happens, do not be afraid of changes, and immediately re-discuss with the hosts. There will always be more chances. Also be active, do not feel bad to contact frequently with the host. The people in charge could be too busy to response us. And confirming the situation for our own internship should be our responsibility.

Approval of supervisor	Institution・Official title・Name Laboratory of Veterinary Surgery, Professor, Masahiro Okumura

※1 Send the electronic file to the WISE Program 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 for the WISE Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.