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学位論文題目	Study for the development of a fabrication technology of the *Antheraea pernyi* fibroin （サクサン（*Antheraea pernyi*）フィブロインの加工技術開発に関する研究）
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論　文　内　容　の　要　旨

The utilization of wild silk is still in progress and promising in the medical field to be part of regenerative medicine. *Antheraea pernyi*, a long studied wild silk, is known for its high bio-compatibility and good cell adhesion thanks to its RGD sequence in its molecule. However, compared to *Bombyx mori*, the *Antheraea pernyi* is more complicated to manipulate and even the processes in published papers can be difficult to handle. In our study, we looked for a new process to make a regenerated silk fibroin solution with a high success rate in order to overcome the difficulties encountered.

In chapter 1, I summarized the background of my research topics and the recent studies worldwide related with my study as the General Introduction.

In chapter 2, I described the development of this new process for making the regenerative *A. pernyi* fibroin aqueous solution. The preparation goes in three steps: the degumming process for removing sericin proteins, the dissolving in solvent and the dialysis for exchanging the solvent with water. At a first attempt, I followed the reported processes for each step to make the aqueous solution. However, I encountered difficulties to make the solution. Therefore, I reexamined each phase in the preparation process carefully. The degumming process was performed according to the reported process, where cocoons were treated in a 2.5 g/L of Na_2CO_3 solution for 45 minutes at 95~98°C three times. The degumming ratio was 25~30 %, to be enough for removing sericin and the other contaminants. The conditions for dissolving the degummed *A. pernyi* fibroin fibers was examined as the next step. The concentration of LiSCN solution at 10 M reported by previous literatures was insufficient to dissolve the fibers completely. I found the appropriate dissolving conditions to prepare the aqueous solution, which were to treat at 45 ℃ for 1 hr in 15 M LiSCN solution. Dialysis process against pure water only kept turning to gel between 1 to 3 days instead of completing after 4 days as described in literatures. We discovered that adding few amounts of calcium chloride during dialysis helped its stabilization by inhibition of the gelation. The conductivity in the dialysis solution was recorded through the dialysis to track the removing of salt. We found a shorter dialysis time (1 hr for each solution exchange in the case of our system) than the typical procedure was enough to remove salt from the dialysate, and the changed process could prevent gelation through the preparation of the aqueous solution.

In chapter 3, I characterized the new solution by SDS-PAGE and FTIR. The obtained

solution from this improved process was characterized to understand its molecular weight and distribution, and the results were confirmed as similar to the reported papers. Moreover, we found the incorporation of Ca^{2+} ions at 260 ppm in the *A. pernyi* fibroin molecules prepared by the new process. At the present time, we infer the Ca^{2+} incorporation might contribute to the stability of the aqueous solution.

In chapter 4, I described the results of characterizations of *A. pernyi* fibroin coating films fabricated from the aqueous solution prepared by our new process as mentioned in chapter 2. FTIR measurements of chapter 3 presented the secondary structure of our *A. pernyi* fibroin film resembled with the structure of previous reported film. The surface morphology was observed by AFM, and higher roughness on our *A. pernyi* fibroin film was characterized than that on *B. mori* fibroin film. We deduced the difference of molecular aggregation strength between *A. pernyi* and *B. mori* fibroin induced the difference of the roughness. More hydrophilic property and much higher surface potential on our *A. pernyi* fibroin film than *B. mori* were determined by contact angle and zeta potential measurement. Cell adhesion on our *A. pernyi* fibroin film was better than *B. mori* fibroin and TCPS (Tissue culture polystyrene). This result indicates that the RGDS function in the molecules of our *A. pernyi* fibroin can work. Cell mobility on our *A. pernyi* fibroin was half of that on *B. mori* fibroin. The RGDS sequence in *A. pernyi* fibroin molecules may work to inhibit cell moving through the cell adhesion function.

In chapter 5, I concluded my study. In this study, an improved preparation process for *A. pernyi* fibroin aqueous solution with stability and reproducibility was established successfully. The aqueous solution was kept in liquid state without gelation for more than one month in refrigerator. The aqueous solution could turn to gel by raising the temperature. This phenomenon is very important to fabricate *A. pernyi* fibroin to the materials. Thus once the aqueous solution is prepared by the new process, the solution can be stores till fabrication in refrigerator, and desired materials are manufactured by only raising temperature without any harmful regents. Actually, we demonstrated to fabricate the coating film using the *A. pernyi* aqueous solution in this study (in chapter 4). Evaluation of cell culture on the coating film revealed to no toxicity and good cell adhesion and proliferation. *A. pernyi* fibroin in the aqueous solution can be expected as a medical material like tissue engineering scaffold with biocompatibility.