K. Iwasaki, S. Yamaguchi, M. Yasuda, T. Ohta, M. Amano. The novel decellularized bovine medial collateral ligaments for regenerative medicine. Gerontechnology 2008; 7(2):133. Rupture of anterior cruciate ligament (ACL) is one of the most common injuries of the knee with an incidence of 1 in 3000[1]. It is clinically evident that the disrupted ACL is incapable of wound healing. Although the most popular graft for reconstruction is autologous tendon, complications such as knee pain, restriction of motion, and reduced mechanical properties, in addition to sacrifice of autologous tissues are drawbacks. We have developed the novel tissue-decellularization machine to eliminate cells from animal or human donor tissues utilizing microwave and pulsatile circulation technologies[2]. It is anticipated that the cell-free tissues will work as templates for regeneration with autologous cells after transplantation. We investigated to decellularize bovine medial collateral ligaments (MCL) as potential sources for transplantation. **Methods** The decellularization device treats tissues with microwave at the frequency of 2.45 GHz, expecting immersion of detergent into thick tissues by resonance effect of water molecules, because the detergent has an ability to destroy cell membranes. The microwave unit was rotated around the tissue chamber at the constant speed of 6 rpm in order to realize homogeneous treatment. Pulsatile flow and pressure were also applied to the bovine MCL (*Figure 1*) using a pneumatic pulsatile pump in order to enhance immersion of the detergent as well as washout of cellular debris. Deoxycholic acid solution with a concentration of 1wt% was used. A cooling system was equipped to regulate temperature of the working fluid below 37°C. Fresh bovine MCLs were decellularized and uni-axial tensile tests were performed to investigate an influence of the decellularization on mechanical properties. **Results and discussion** Figure 2 shows the hematoxylin-eosin stain for the MCL. In a 24-hour treatment it was successfully demonstrated that cells were able to be removed from MCLs. Natural alignments of collagen fibres were preserved even after the dynamic treatment. The ultimate strength and Young's modulus of the decellularized MCLs were 17.3 MPa, and 170 MPa, those were 70% and 65% of the native bovine MCLs, respectively. However, the ultimate strength of human ACL is approximately 5-6 MPa, the decellularized bovine MCL was considered to have enough strength for transplantation. **Conclusion** The data above demonstrated the potential of the decellularized bovine MCLs as grafts for transplantation. Future animal trials will elucidate the ability for regeneration.

References

1. Frank CB, Jackson DW. Journal of Bone and Joint Surgeons of America 1997;79:1556-1576
2. Iwasaki K, Ozaki S, Kawai T, Yamaguchi S, Eto M, Ohba Y, Umezu M. Proceedings of the 12th International Conference on Biomedical Engineering; 2005; pp in CD-ROM

Keywords: tissue engineering, anterior cruciate ligament, ultimate strength

Address: Waseda Institute for Advanced Study, Japan; E: iwasaki@waseda.jp

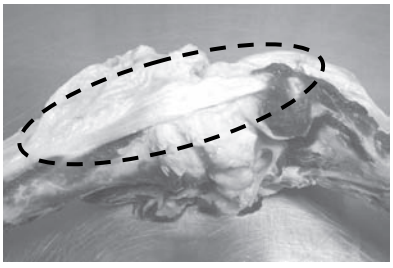
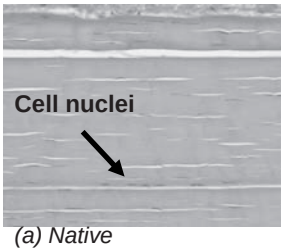
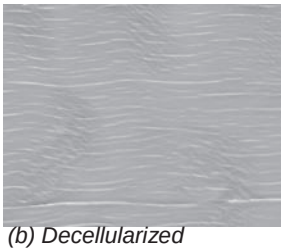


Figure 1 A bovine medial collateral ligament for decellularization



(a) Native



(b) Decellularized

Figure 2 Hematoxylin-eosin stain for the ligament. Black dots represent cell nuclei