Preparation of a decellularized tumor using high hydrostatic pressure (HHP) technology

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Purpose: Decellularized tissue is one of candidate materials for regenerative medicine because of good biocompatibility. Many species of decellularized tissue have been developed as an alternative tissue. In many cases, a decellularized tissue is prepared from an objective tissue and replaced at the target tissue. The replaced acellular tissue shows good tissue regeneration. We also have developed many decellularized tissues, such as aorta, small diameter vessel, cornea and bone marrow, using high hydrostatic pressure (HHP) treatment. This decellularization method can decellularize tissue remaining the extracellular matrix (ECM) structure compared to that of other decellularization method, such as chemical decellularization methods using detergents and enzymes. Recently, we focus on the influence of the ECM structure of decellularized tissue on cellular behavior on/in it. We hypothesize that the maintained ECM structure of the HHP decellularized tissue allow the essential nature and behavior of cell during regenerating of tissue. Previously, we reported that the reconstruction of bone marrow, which occurred at a porcine bone marrow decellularized by HHP technology, which was implanted into rat subcutaneously [1]. This indicates that the typical structural microenvironment was remained in the HHP decellularized bone marrow and induced tissue regeneration ectopically. A tumor has typical tissue structures, which are constructed by cancer cells during its development. We hypothesize that, after decellularization of tumor, the structure and nature of ECM of cancer microenvironment is maintained for the acellular tumor matrix and then its typical ECM microenvironment could stimulate cells. In the present study, the decellularized melanoma was prepared by various decellularization methods.

Methods: B16 melanoma cells were subcutaneously injected to C57BL/6 mouse. After 1-4 weeks, the tumor was harvested and treated by the HHP or SDS methods to remove the cells in the tissue according to previous research [1-3]. They were evaluated by H-E staining, SDS-PAGE and DNA quantification.

Results: Melanoma having various sizes were obtained and treated by different decellularization methods. From H-E staining of the SDS treated melanoma, it was observed that inner cells of melanoma were remained although the cells of near surface were removed (Fig1 (B)). When the melanoma was soaked in SDS solution for long term, the shape was not kept. Controlling of the penetration of SDS detergent is needed to keep balance decellularization and remaining of structure. On the other hand, for the HHP treated melanoma, the cells were removed completely (Fig 1(C)). The black spots were decreased compered to that of the non-treated melanoma. The fibrous structure was remained. Also, for SDS-PAGE analysis of the HHP decellularized melanoma, the number of bands of proteins was decreased compared to the non-treated melanoma.

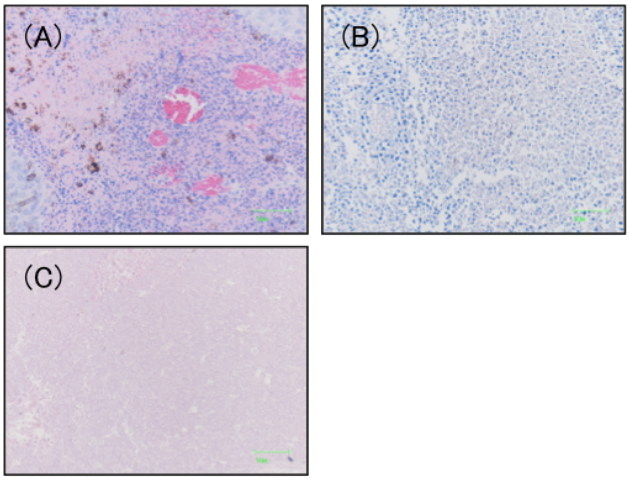

Figure 1. Fig 1. H-E staining of the decellularized tumors. (A) non-treated, (B), SDS-treated, (C) HHP-treated

Conclusions: In the present study, we have successfully developed the decellularization method for a tumor using HHP technology. In future, the reconstruction of tumor by recellularization using cancer stem cells was tried to investigate the effect of tumor ECM microenvironment on cancer development.

References:

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