板状緻密骨に由来する加工移植材の創成とラット生体内での特性評価

I. Introduction

In recent years, the development of various biomaterials is the background of the rapid development of hard tissue regeneration therapy. Bone defects in the oral region such as the alveolar bone and jawbone often require hard tissue regeneration from the viewpoint of oral system function recovery, and local bone regeneration is a major issue in the dental field. For bone augmentation, biological techniques such as bone grafting, engineering techniques using acellular biomaterials such as calcium phosphate ceramics, and their fusion methods have been used. In clinical practice, it is common for compact bone plate to be used for transplantation to be immediately transplanted with freshly perforated autogenous bone. Therefore, we focused on ultrasonic demineralization as a rapid demineralization of perforated bone and further processing inside the bone. In this experiment, adult rat parietal bone-derived compact bone was subjected to physicochemical processing, that is, treatment with mechanical perforation and ultrasonic demineralization by 2% nitric acid. The purpose of this experiment is to observe the ultrastructure of four types of processed bones (FBP group, DFBP group, UFBP group, UDFBP group) with a scanning electron microscope and to observe bone and cartilage induction phenomena in the subcutaneous tissue of the back of the rat after 4 weeks. In the future, the application of processing to human dentin, which is an acellular biomaterial, is considered, and in this experiment, processing of compact bone plate after cryopreservation is attempted.

II. Materials and methods

1. Experiment ：Bone induction using rat processed bone in rat subcutaneous back skin

Wistar rats (female, 12 weeks old, weight: about 250 g) were sacrificed, and the parietal bone from which the periosteum had been removed was removed. The parietal bone was cut into a plate (5×5×2 mm^3), stirred and washed with distilled water for 30 minutes, and stored frozen at -80℃. The first of frozen bone with perforations (FBP) was made by adding 5 perforations by a diamond bar (φ0.6 mm) under water injection on the inner and outer plates of the parietal bone. The second of demineralized frozen bone with perforations (DFBP) was prepared by demineralization (2% nitric acid, 40kHz, 220W, 20℃, 90 minutes) was added to the FBP , and the mixture was stirred and washed with distilled water (500 rpm, 30 minutes) and stored frozen at -80℃. The third of Ultrasonic frozen bone with perforations (UFBP) was prepared by ultrasonication (distilled water, 40kHz, 220W, 20℃, 90 minutes) was added to the FBP , and stored frozen at -80℃. In addition, the fourth of ultrasonic demineralized bone with perforations (UDBP) was prepared by ultrasonication (2% nitric acid, 40kHz, 220W, 20℃, 90 minutes) was added to the FBP , and the mixture was stirred and washed with distilled water (500 rpm, 30 minutes) and stored frozen at -80℃. The CT values of each before and after processing were compared by MDCT, and the surface was observed with a scanning electron

microscope (SEM). Before implantation, ImageJ was used to measure the percentage of crack area per unit surface area. Samples were implanted subcutaneously in the back of Wistar rats (male, 4 weeks old). After 4 weeks, they were sacrificed with pentobarbital Na, and the samples were removed as a lump together with the surrounding soft tissue. Then, hematoxylin-eosin (HE) stained was performed to observe the histological images with an optical microscope.

III. Results and discussion

Observation of the surface before implantation by SEM revealed a fibrous structure that appeared to be acid-insoluble collagen in DFBP and UDFBP. Focusing on the P and Ca elements present on the surface, DFBP and UDFBP were reduced to about 1/10 or less of FBP and UFBP. Since DFBP was the smallest before implantation and 4 weeks after implantation, mineral components such as HAp were removed by decalcification, and the sustained release of BMPs attached to acid-insoluble collagen increased due to the demineralization Probably. From the SEM observation of this experiment, the FBP surface was smooth and the UFBP surface was smooth and cracked. On the other hand, collagen fibers were exposed on the surface of DFBP and UDFBP. In addition, the crack area ratio of UDFBP was about 14.3 times that of FBP. HE staining before implantation showed many depressions on the outer plate of the UFBP that seemed to be cracks, and rupture of the bone matrix at the bone marrow cavity equivalent to the bone remodeling line. In UDFBP, the rim of the medullary cavity was irregular, and the rim was irregular and the bone matrix was torn at the bone remodeling line. Four weeks after implantation, HE staining revealed a crack on the surface of the outer plate with UFBP and a palisade-like fragmentation of the bone matrix equivalent to the bone marrow cavity. In UDFBP, cracks and bone marrow cavity-equivalent parts were mosaic on the outer plate surface. Since osteoinduction was observed only with UDFBP, the surface and internal structure of the compact bone were altered by mineral elution and crack initiation, suggesting that an internal environment for promoting differentiation of osteoblasts and chondrocytes was provided. UDFBP showed a mosaic-like structure from the medullary cavity to the lateral plate, suggesting a composite of extraosseous surface cracks and interlamellar bone matrix cracks. Furthermore, since the processed bone surface cracks were found to expand in the order of FBP, DFBP, UFBP, and UDFBP, the composite cracks were treated with ultrasonic treatment in the same way as the existing cracks expanded by demineralization (Hanba et al., 2017). Although the interstitial bone matrix cracks were less than 300-400 μm (Tsuruga et al., 1997), which were optimal for osteoinduction, the decalcification treatment was considered to cause crack expansion, uneven crack gaps, and uneven distribution of blood vessels. In other words, the demineralized fluid penetrates into the bone matrix due to ultrasonic penetration and combined cracking, and the BMP concentration inside the bone matrix increases due to the osmotic action

and the combined cracks. It was suggested that osteoinduction occurred, and chondrocyte-like cells were found in the area where the crack gap was narrow and blood vessels were poor.

IV. Conclusion

The processing of perforated plate-shaped compact bone (ultrasonic nitric acid demineralization) results in a structural change that allows cells and blood vessels to penetrate by the formation and expansion of cracks in the bone matrix. This is thought to have contributed to the osteoinduction of the space.