Preparation of decellularized mandible bone with periodontal membrane for periodontal reconstruction

Naoko Nakamura[1], Ai Ito, Kwangwoo Nam[1], Tsuyoshi Kimura[1], Toshiya Fujisato[2], Takashi Tsuji[3], Akio Kishida[1].
[1] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University
[2] Faculty of Engineering, Osaka Institute of Technology
[3] Center for Development Biology, RIKEN

Purpose:

Dental implant has become a major treatment for lack of teeth. In the case of the decreasing the mandible bone for supporting the dental implant, the enlargement of bone is performed using various treatments. The problems of dental implant are the lack of the periodontal membrane that can be got the feeling such as chewiness and firmness of foods and can prevent the infection. Here, we investigated the reconstruction of the unit of periodontal tissue, including the mandible bone, periodontal membrane, and artificial tooth. The unit of periodontal tissue is expected to implant into the lack of mandible bone. In this study, we prepared the decellularized mandible bone remained the periodontal membrane and evaluated the reconstruction of the unit of periodontal tissue using artificial tooth.

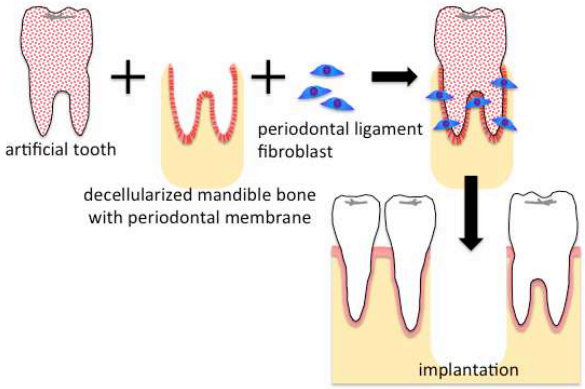

Fig. 1 Design of a novel periodontal treatment.

Methods:

The units of mandible bone with molar were harvested from mouse. They were treated by high hydrostatic pressure and washed by saline in order to remove the cells from tissue[1)]. The molar were pulled out from the decellularized units of mandible bone with molar after treatment of SDS solution or not. The decellularization was evaluated by H-E staining and DNA quantitation. For *in vitro* and i*n vivo* study, periodontal ligament fibroblasts were harvested from rat periodontal membrane around incisor. The cells (2×10^6 cells/sample) were seeded on the decellularized periodontal membrane that was remained on the decellularized mandible bone. After seeding of rat periodontal ligament fibroblasts to periodontal membrane, they were implanted under the renal capsule of rats (Wister rat, female, 0.15kg). As a model of artificial molar, molars that were obtained from decellularized units of mandible bone with molar were used. They were demineralized by EDTA-2Na solution. The demineralized molars were recalcified by alternate immersion of $CaCl_2$ in Tris-HCl and Na_2HPO_4 solution[2)]. The recalcified molars were inserted to recellularized periodontal membrane on decellularized mandible bone *in vitro*. They were implanted under the renal capsule of rats. The evaluation was performed by H-E staining.

Results:

The units of mandible bone with molar were decellularized by high hydrostatic pressure treatment. The periodontal membranes were observed on the molar that was pulled out from the units. In the case of treatment of SDS solution before the pulling out the molars, the periodontal membranes were maintained on the mandible bone after molar extraction. Therefore, two type of decellularized mandible bones with or without periodontal membrane were prepared.

In order to evaluate the recellularization of decellularized periodontal membranes by periodontal ligament fibroblasts, the cells were seeded on the decellularized mandible bone with or without periodontal membrane. The periodontal ligament cells were observed not only on but also inside the decellularized periodontal membrane, although the orientation of the cells were not similar to original tissue. The decellularized mandible bones with or without periodontal membrane were implanted under the renal capsule of rats after seeding the periodontal ligament cells. Three weeks after implantation, they were not observed the inflammation and absorption under renal capsule. Many cells were observed for both of decellularized mandible bones with and without periodontal membrane. For with periodontal membrane sample, the orientations of the cells were similar to original, indicating the cells oriented along the collagen fibers of periodontal membrane.

As a model of artificial molar, we here used the molars that were pulled out from decellularized mandible bones in order to fit the decellularized mandible bones, it would be needed to prepare artificial tooth using CAD/CAM. The molars were demineralized and recalcified by alternate immersion treatment. The observation of scanning electron microscope showed that the surfaces of artificial molar were covered with calcium phosphate. After implantation of the artificial molar with decellularized mandible bone, the artificial molars were adhered to periodontal membrane of decellularized mandible bone, indicating that the decellularized periodontal membranes were recellularized and reconstructed.

Conclusions:

Decellularized mandible bone with periodontal membrane could reconstruct the unit of periodontal tissue using artificial tooth. It suggested that a novel treatment for periodontitis and mandible bone defect.

References:

1) Nakamura N. et al. Advanced Biomedical Engineering 2013;2:95-100.
2) Taguchi T. et al. Biomaterials 2002;22:53-58.