Abstract

Research on the development of 3D bioprinting scaffolds for bone replacement in the craniomaxillofacial region

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Graduate School of Dentistry,
Health Sciences University of Hokkaido

Shotaro YAMAZAKI

【Introduction】

In the craniomaxillofacial region, bone grafting is used to treat gaps caused by congenital osteogenesis imperfecta, cleft lip and palate, and orthognathic surgery. Autologous bone has the best biocompatibility, osteoconductivity, and osteoinductivity, but the amount that can be harvested is limited. Therefore, artificial bones made of hydroxyapatite (HA) are sometimes used. HA also has biocompatibility and osteoconductivity, but it has the disadvantage that it remains without bone replacement (remodeling) for a long period of time. Residual HA hinders tooth movement and causes serious side effects such as tooth root resorption. Therefore, there is a need to develop bone grafting materials that remodel appropriately.

3D printers have been used in the medical field in recent years because they can create complex three-dimensional structures that are difficult to create using conventional processing techniques, and they can also easily design objects according to individual orders. There is. Furthermore, bioprinters that print by mixing biomolecules and cells with ink have been developed and are attracting attention. In this study, we mixed osteoblasts and BMP-2, a bone morphogenetic protein, into a bioink containing alginate-based nanocellulose fibers, and used an extrusion-type 3D bioprinter to create a bioink with a diameter of 8.0 mm and a thickness of 1.0 mm. We prepared a scaffold and investigated its potential for use as a bone filling material.

【Materials & Methods】

1. Creating a scaffold

Using CAD software, we designed a disc-shaped scaffold with a diameter of 8.0 mm and a thickness of 1.0 mm, with a cross-shaped structure in the center. Scaffolds were printed using a 3D bioprinter using an alginate-based bioink.

2. Characterization of scaffold samples

The FT-IR spectrum of the bioink was measured using the kBr method. The dynamic viscoelasticity of the bioink was measured using a cone-type rheometer. The cross section of the scaffold was observed using SEM.

3.Immersion test

The scaffolds were immersed in the medium for 14 days, and dimensional changes on the medium were measured.

4.Cell survival test

Cells were mixed into the scaffold, and the cytotoxicity caused by printing was quantitatively evaluated by photometry using LDHAssay.

5. Cell localization within the scaffold

The localization of cells within the scaffold was observed using a confocal laser microscope using double staining with calcein AM and PI.

6. Calcification properties

The calcification concentration of each cell concentration and BMP-2 concentration was stained with Alizarin Red S, and the degree of calcification was quantitatively evaluated using photometry.

7.Animal experiment

An 8.0 mm bone defect was created in the calvaria of a 10-week-old male Wistar rat, and a scaffold was implanted.

8. Micro CT analysis

Imaging was performed using an inspeXio SMX-225CT (Shimadzu) with a tube current of 160 μA, a tube voltage of 70 kV, and a resolution of 7 μm. Bone density (BV/TV, %), trabecular bone (Tb.Th, μm), and BMD (mg/cm3) in the skull were measured using analysis software (TRI/3D-BON, Ratoc).

9. Nanoindentation test

Samples containing new bone and existing bone were collected from the collected rat calvaria, and the mechanical properties (hardness and elastic modulus) of the new bone and existing bone were measured using a nanoindentation test.

【Results & Disccusion】

1. Analysis of scaffold samples

From the FT-IR spectrum, the bioink had the characteristics of SA and NCF. Rheological tests showed remarkable frequency-dependent shear thinning behavior, indicating that it had ideal dynamic viscoelasticity.

Characterization of the scaffold revealed that the SA-based bioink has biocompatibility and viscoelastic properties suitable for bioprinting.

2. Scaffold surface observation and immersion test

In the SEN image, a porous network structure was observed in the cross section of the scaffold. It is inferred that the porous network structure is advantageous for cell differentiation, proliferation, and colonization of new blood vessels. In the immersion test, there was no significant change in dimensions after 14 days of culture. This suggests that it remains at the wound site during the early stages of bone healing and maintains its shape and function.

3. Cell viability

No cytotoxicity due to printing was observed with LDHAssay. Confocal laser microscopy revealed that cells were localized uniformly in both two and three dimensions.

4. Calcification properties

Quantitative evaluation using Alizarin Red S staining photometry showed that the most calcification was observed at a cell concentration of 2.4 x 104 cells/ml and a BMP-2 concentration of 100 μg/ml. It was revealed that the mineralization ability of the scaffolds depended on the cell concentration, and the addition of BMP-2 significantly increased the mineralization.

5. Bone remodeling

Micro-CT images showed that no bone regeneration could be observed in the untreated group and the group in which cells and growth factors were not mixed, but significant bone regeneration was observed in the group in which cells and BMP-2 were mixed. There was no significant difference in BMD between new bone and existing bone, and Tb.Th had significantly more new bone and BV/TV had significantly more existing bone. No significant difference was observed in the hardness and elastic modulus of new bone and existing bone. It was suggested that new bone has mechanical

properties equivalent to existing bone.

【Conclusion】

A 3D-printed scaffold made from an alginate-based bioink with nanocellulose fibers mixed with osteoblasts and BMP-2 exhibits excellent osteoinductivity, osteoconductivity, and osteogenicity, and also rapidly regenerates. It has been shown that it has the potential to be used as a modeling bone substitute material.