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Development of a biointegrated mandibular reconstruction device consisting of bone compatible titanium fiber mesh scaffold

Makoto Hirota [a, *], Takaki Shima [b], Itaru Sato [a], Tomomichi Ozawa [a], Toshinori Iwai [a], Akihiro Ametani [b], Mitsunobu Sato [c], Yasuharu Noishiki [d], Takahiro Ogawa [e], Tohru Hayakawa [f], Iwai Tohnai [a]

[a] Department of Oral and Maxillofacial Surgery, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama, 236-0004, Japan
[b] Medical Device Department, HI-LEX Corporation, Inc, 1-12-28 Sakae-cho, Takaraduka, 665-0845, Japan
[c] Department of Applied Physics, School of Advanced Engineering, Kogakuin University, 1-24-2 Nishi-Shinjuku, Tokyo, 163-8677, Japan
[d] Division of Artificial Organ Science, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama, 236-0004, Japan
[e] Laboratory for Bone and Implant Sciences, The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, Division of Advanced Prosthodontics, Biomaterials and Hospital Dentistry, UCLA School of Dentistry, 10833 Le Conte Avenue, Box 951668, Los Angeles, CA, 90095-1668, USA
[f] Department of Dental Engineering, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama, 230-8501, Japan

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ABSTRACT

Coating biomaterials with a thin hydroxyapatite (HA) was proven effective in enhancing bone compatibility. Segmental bone defects are considered as the most difficult defect to repair in bone regeneration therapy. We developed submicron-thin HA-coated titanium fiber mesh scaffolds to reconstruct immediately loaded segmental mandibular defects and evaluated their bone compatibility *in vitro* and *in vivo*. Human osteoblasts attachment, proliferation, and osteocalcin expression in non- and HA-coated scaffolds were evaluated. A 10-mm long segmental bone defect in a rabbit mandibular bone was reconstructed with non- or HA-coated scaffolds, which were removed at 9 and 21 weeks, to evaluate the mechanical strength of the bone-scaffold connection and the bone formation around the scaffold. Expression of osteocalcin was greater in HA-coated scaffolds. *In vivo* bone formation in HA-coated scaffolds was greater than that in non-coated scaffolds at 21 weeks. Newly formed bone in HA-coated scaffolds mostly restored bone continuity. Scanning electron microscopy identified strong integration of the bone and HA-coated scaffolds. The mechanical strength of the bone-scaffold connection was 3-fold greater in HA-coated scaffolds than that in non-coated scaffolds. These results suggest that a thin HA-coated titanium fiber mesh scaffold is a bone-compatible mandibular reconstruction device in immediately loaded segmental defects.

1. Introduction

Segmental bone defect is considered a most difficult defect to repair in bone reconstructive and/or regenerative therapy and no existing therapy has been proven to be fully satisfactory [1,2]. Repair or regeneration of large bone defect is still a challenge for surgeons and researchers [2]. Treatment for segmental defect of the mandible in particular is difficult because mandibular defect causes not only functional but esthetic problems. Loss of mandibular continuity following tumor ablative surgery places patients under severe strain and impairs their quality of life. The mandible is the only movable load-bearing bone of the face. The mandibular bone constantly moves and is loaded during speech, mastication, eating, and swallowing immediately/early after surgery. Bone tissue generally has a good healing capacity; however, biomechanical instability due to a large defect and constant loading, which exactly corresponds with a situation after segmental mandibular bone resection, limits intrinsic regeneration potential [1]. The defect, i.e., critical-sized bone defect, cannot be expected to spontaneously heal.

Many methods of mandibular reconstruction disregard the various forces acting on the bone, leading to failure of the

* Corresponding author.
E-mail address: mhirota@med.yokohama-cu.ac.jp (M. Hirota).

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reconstruction [3]. The current options for mandibular reconstruction include free bone grafts, particulate cancellous bone and marrow grafts, vascularized free bone flaps, and tissue-engineered bone [4]. Patients are often put on a soft diet for an extended period and face a prolonged wait before the completion of dental rehabilitation, because early or immediate occlusion is difficult in these reconstruction methods. Mandibular reconstruction using vascularized free bone flaps is the most reliable treatment because it facilitates early bone healing. However, the procedure involves a long operation time and significant morbidity. Furthermore, height and width of vascularized bone flaps are insufficient for optimal dental implant placement. Tissue-engineered bone using a combination of absorbable materials, bone marrow stromal cells, and osteogenic factors has been recently developed, however it is not yet fit for clinical application [4–6]. The method has the potential to reduce the morbidity related to autogenous bone harvesting, but concerns remain regarding the absorbance of materials without bone replacement, long duration of osteogenic cell maturation, and oncogenic potential of growth factors.

Titanium (Ti) fiber mesh scaffold is a porous material comprising a web form of Ti fibers with a diameter of 20–50 μm and that can be formed into any shape. Ti fiber mesh scaffold shows sufficient biological compatibility and strength; therefore, Ti fiber mesh-based bone regenerative materials have been investigated [7–9]. Reportedly [10], a type I collagen coating on Ti fiber mesh accelerated the differentiation of rat bone marrow cells into osteoblasts. Moreover, Ti fiber mesh coated with TiO2 coating accelerates the differentiation of rat bone marrow stromal cells into osteoblasts [11] and facilitates uniform bone formation in rat bone defects [12].

The biocompatibility and osteoconductivity/osteoinductivity of hydroxyapatite (HA) coating are widely utilized in orthopedics and dental implant surgery [13,14]. These coatings, which include calcium phosphate, provide a bone-like mineral matrix that simulates the bone formation: this environment is necessary for osteoblast attachment and may drive osteogenic differentiation [15,16]. Various techniques, including magnetron sputtering, plasma spray methods, and physical vapor deposition, can be used to place a thin coat of HA on Ti materials [17–20]. However, achieving a uniform thin layer of HA on a complicated structure such as a tissue-engineering scaffold is challenging using these methods. Recently, the molecular precursor method, a new technology that applies a thin coating of HA, was described [21]. In this method, scaffolds made from Ti fiber mesh are immersed into a molecular solution ant then heated, creating a thin HA coating both on the surface and inside scaffold [21]. Accelerated trabecular bone formation occurs within Ti fiber mesh scaffolds created using this method and implanted into trabecular bone defects in rabbits [21,22]. Previously, we reported that the HA coating of Ti fiber mesh scaffolds enhanced the functional activity of osteoblasts [23], and that bone formation within Ti fiber mesh scaffolds implanted into rat cranial bone defect was rapidly enhanced by a thin HA coating applied using the molecular precursor method [24,25].

Enhanced osteoconductivity of Ti fiber mesh scaffold could be advantageous in not only rapidly connecting to the bone tissue but stabilizing the reconstruction. Rigid stability of reconstruction would provide favorable environment for bone healing even in critical-sized bone defect of immediately loaded site. Accordingly, we hypothesized that Ti fiber mesh scaffolds could be used as reliable reconstructive materials for immediately loaded segmental mandibular bone defects by a thin HA-coating on Ti fiber. In this study, we evaluated the utility of a thin HA-coated Ti fiber mesh scaffolds as reconstruction devices for immediately loaded segmental mandibular bone defects in rabbits. We evaluated bone formation inside and outside the Ti fiber mesh scaffolds and the mechanical strength of the bone-scaffold connection to compare the efficacy of non- and HA-coated Ti fiber mesh scaffolds for the reconstruction of immediately loaded segmental mandibular bone defect.

2. Materials and methods

2.1. Ti fiber web scaffold sample characterization

Ti fiber mesh scaffolds (Hi-Lex Corporation, Takaraduka, Japan) with a porosity of 87% were prepared from Ti fibers 20 μm in diameter. Cubic three-dimensional (3D) scaffolds (10 × 10 mm, 5 mm thick) containing pores with a mean size of 95 μm (range 80–110 μm) were manufactured (Fig. 1A). It was possible to place a dental implant into the scaffold with slight deformation but rigid primary stability using 40 Ncm torque (Fig. 1B, C). Before use, the Ti fiber mesh scaffolds were sterilized in an autoclave at 121 °C for 15 min. The mechanical strength of the scaffold was evaluated via the three-point bending test. A testing machine (Instron 5544 Electro-mechanical Testing System, Instron, Norwood, MA, USA), equipped with a 2000-N load cell and a bending lot, was used to load the middle line of the scaffold vertically downward at a crosshead speed of 1 mm/min.

2.2. Thin hydroxyapatite coating of Ti fiber web scaffolds

The HA coating of Ti fiber mesh scaffolds was performed according to established methods [21,22]. Briefly, a precursor solution was prepared by mixing Calcium-ethylendiaminetetraacetic acid/amine complex and dibutylammonium diphosphate salt in ethanol at a Calcium (Ca):Phosphorous (P) ratio of 1.67. The Ti fiber mesh scaffolds were soaked in this solution for 20 min with ultrasonic treatment. Subsequently, the scaffolds were preheated in a muffle kiln at 60 °C for 20 min followed by heating at 600 °C for 2 h under atmospheric conditions. The immersion of Ti fiber mesh scaffolds into the precursor solution and the heating process were repeated thrice, because this reportedly coats the scaffold interior effectively. The fiber structure of the scaffold before and after HA coating was visualized by field-emission scanning electron microscopy (FE-SEM, JSM-6340F, JEOL, Tokyo, Japan) at an accelerating voltage of 5 kV. The specimens were coated with platinum before FE-SEM imaging. The elementary distribution of Ca, P, and Ti before and after the coating was evaluated by energy dispersive x-ray spectroscopy (EDX) (XL30, Philips, Eindhoven, Netherlands).

2.3. Human osteoblast cell culture

Freeze-preserved normal human osteoblasts (CC-2538, Takara Bio, Ohtsu, Japan) were disseminated into a 75-cm^2 flask at a concentration of 5000 cells/cm^2. An osteoblast culture medium kit (CC-3208; Takara Bio Inc), primarily consisting of ascorbic acid, fetal bovine serum, and antibiotics, was used to evaluate cell proliferation. The culture medium was changed the day after dissemination and every 3 days thereafter. Cells were grown until they were 80% confluent, detached by trypsinization and suspended in a culture medium. After centrifugation of the cell suspension for 5 min, the supernatant was removed, and the cells were suspended in osteoblast differentiation-inducing medium at a concentration of 5×10^4 cells/ml. This medium was prepared by adding OGM™ Differentiation SingleQuots® (CC-4194; Takara Bio Inc.), consisting of hydrocortisone hemisuccinate and beta-glycerone acid, to an osteoblast culture medium (CC-3208; Takara Bio Inc.).

A Ti fiber mesh scaffold was placed in a 24-well plate, and the cell suspension was disseminated on to it at a concentration of 1 ml/well (5×10^4 cells/well), followed by culture at 37 °C in a 5%