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Expanded View

Clinical review of bone regenerative medicine and maxillomandibular reconstruction

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ABSTRACT

We have studied bone regenerative medicine to employ autologous bone marrow stromal cells and platelet-rich plasma as tissue-engineered osteogenic materials. Although our studies have been successful to a certain degree, advancing to clinical applications, the strategy for practical use of this method has to be changed, because the environment surrounding bone regenerative medicine has evolved dramatically. Therefore, we have changed our focus from cells to cell-conditioned media, and we found that the latter contains growth factors and matrices released from the cells and promotes regeneration of tissues by recruiting endogenous stem cells and precursor cells, and thereby started clinical research based on these findings. However, the amount of regeneration achieved is minimal (mm), and the centimeter-scale reconstruction of segmental bone defects is still a challenge. We have focused our attention on distraction osteogenesis, which is an excellent model for in vivo tissue regeneration of segmental defects, and we have investigated and clarified its mechanisms at a cellular level, based on tissue regeneration processes. The strategy has been investigated to promote vascularization, as it should precede tissue regeneration. A potential approach for enabling tissue regeneration that mimics distraction osteogenesis without using a distraction device is discussed.

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1. Introduction

Various materials such as calcium phosphate-based artificial materials, allogenic bone-derived materials, and heterogenic bones are used to repair bone defects in the oral and maxillofacial regions. However, autologous bones are considered the best option in terms of efficacy and safety. Unfortunately, bone grafting can cause stress at the donor site, limiting its application. Hence, bone regenerative medicine has been studied extensively to overcome these limitations. We have been working in this field and have attained a certain level of success. However, defects that occur at the time of mandibular segmental resection may have characteristics that are considerably different from those of small bone defects, in terms of size, morphology, vascularization, the presence or absence of investing soft tissues, and other factors. Hence, we have not been able to accomplish mandibular reconstruction by simply

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applying bone regenerative medicine; thus, free vascularized autologous grafting is required. In this article, we will consider regenerative medicine-based methods to treat such large bone defects, and also report the investigated design of a bone regenerative medicine approach and a plan for maxillomandibular reconstruction.

2. Approaches to bone regenerative medicine

We have performed translational research in bone regenerative medicine using tissue-engineered osteogenic material (TEOM), a complex of autologous bone marrow stromal cells and platelet-rich plasma, prepared from bone marrow aspirate and the peripheral blood [1]. This effort was intended to differentiate osteogenic cells from the bone marrow stromal cells, growth factors contained in the platelets, and the fibrinous network (a type of gelatinized plasma) to function at the bone defect site in the body as the three important elements for tissue regeneration (i.e., cells, signals, and scaffolds, respectively). The standard method for the preparation and use of the TEOM is as follows. First, the eligibility of patients should be considered based on predetermined criteria, for example, whether the patients have anemia or infections. Then, peripheral blood samples are collected for the preparation of autoserum to be used for cell culture. Either 200 or 400 mL of blood should be collected once a month for 3 months, and the sera separated from these samples should be cryopreserved. Then, inserting a dedicated centesis needle into the iliac crest under local anesthesia collects a few milliliters of the bone marrow aspirate. Wall-adherent cells are separated from the collected aspirate and cultured for about 1 month. Once the cell count has reached a certain level, they are allowed to differentiate into osteogenic cells for approximately 1 week. Peripheral blood is then collected to prepare platelet-rich plasma, which forms a complex with the cultured cells. Gelatinization of this complex with thrombin and calcium chloride results in the formation of the TEOM. The TEOM is prepared at the time of use and is injected into or filled in the bone defect site. With regard to the amount of regenerated bone obtained from the TEOM, when the procedure was applied to the space made at the time of maxillary sinus floor lifting in 16 sinuses in 12 patients, the height at 2 years after the procedure was 8.8 mm ± 1.6 mm [2]. The procedure was effective in patients with vertical ridge resorption [3] or an alveolar cleft of >10 mm [4], and it was also possible to guide unerupted teeth to a proper position. As described earlier, sufficient bone regeneration could be obtained to support dental implants using the TEOM at the site of maxillary sinus floor lifting. However, some studies reported that the bones regenerated if blood clots were maintained [5]; hence, the application of the procedure needs to be reexamined.

3. Changes in the environment surrounding bone regenerative medicine

As the number of clinical research studies based on regenerative medicine increases, the regulation of regenerative medicinal applications should be considered. In 2006, the Ministry of Health, Labour and Welfare, Japan, established the “Guidelines on clinical research using human stem cells” (hereafter referred to as “guidelines”) [6]. Before this, our institution consisted of a cell-processing facility that had sufficient equipment and a quality control system to ensure safety, and it was accredited by the International Organization for Standardization (ISO 13485) [7]. Because the guidelines are applicable only to research to be started after 2006, and not to that had been started before 2006, we decided to reconsider the content of our prior research based on the new guidelines.

Human allogenic mesenchymal stem cells were first marketed in 2010 for bone augmentation in the oral and maxillofacial regions in the United States of America (US) in 2010 [8]. In Japan, a bovine-derived bone substitute was approved for periodontal use in 2011, almost 20 years after the approval in the US and Europe [9]. These products were sold for tens of thousands of yen; especially, the TEOM products cost >500,000 yen for the material alone. Furthermore, the cost of construction of the initial facility for the aforementioned cell processing was 200 million yen, and the annual maintenance and operation costs exceeded 60 million yen. Hence, the cost of TEOM was estimated to be exorbitant. New cell preparation devices, such as isolators or automatic culture machines, that do not require the extensive cell-processing facilities and can be installed for a cost of several tens of millions of yen, have been developed; thus, the expenses for establishing and operating facilities have been reduced. However, in any case, considering the cost-effectiveness of the materials, there is little hope for practical use of the TEOM.

As the number of studies on bone regeneration from the TEOM increases, the efficacy of this method has gradually become clear. Bone regeneration using the TEOM depends greatly on the morphology of defects. When the number of bony walls surrounding the defect decreases, the amount of regenerated bone also decreases proportionately; in addition, the TEOM is not effective for segmental defects. No specific relationship has been observed between the number of cells applied to the bone defect site and the amount of regenerated bone. Furthermore, cells that are autologous but are cultured ex vivo have substantially lower survival rates after introduction into the body [10,11], and these cells often regenerate tissues indirectly rather than directly, that is, endogenous stem cells and precursor cells recruited by paracrine effects regenerate the tissue at a higher rate.

4. Changes in the strategy for realizing bone regenerative medicine

As legal regulations on regenerative medicine become more stringent, burdens on facilities and personnel, and costs for cell culture have increased. Moreover, the mechanisms of tissue regeneration have become clearer. We thus have had to change our strategy for bone regenerative medicine (Fig. 1).

A German group [12], conducting studies similar to ours using osteogenic cells derived from the periosteum, demonstrated the limitations of bone regeneration [13]. In order to avoid the problems associated with cell culture, researchers began to apply bone regenerative medicine without the use of cell culture [14]. Their new method was intended to obtain mononuclear cells by centrifuging the bone marrow aspirate and to apply the mononuclear cells together with bovine-derived bone substitute to the bone defect site. This procedure can be completed in the operating room without a specialized cell-processing facility or culture.

We have investigated a different type of bone regenerative medicine wherein cell culture is performed but the cells are not introduced into the body. Here, we have focused on the application of cell-conditioned medium rather than conditioned cells. The cell-conditioned medium contains growth factors and substrates that are released from the cells. If endogenous stem cells and precursor cells could be recruited by the application of the conditioned medium to the bone defect site, bone regeneration may be obtained. Because this procedure does not include the introduction of processed cells into the body, it is outside the scope of the guidelines. In addition, if active components in the conditioned medium are identified, and their formulation becomes possible in