Comparative study of new bone formation capability of zirconia bone graft material in rabbit calvarial

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PURPOSE. The purpose of this study was to compare the new bone formation capability of zirconia with those of other synthetic bone grafts. **MATERIALS AND METHODS.** Twelve rabbits were used and four 6-mm diameter transcortical defects were formed on each calvaria. Each defect was filled with Osteon II (Os), Tigran PTG (Ti), and zirconia (Zi) bone grafts. For the control group, the defects were left unfilled. The rabbits were sacrificed at 2, 4, and 8 weeks. Specimens were analyzed through micro computed tomography (CT) and histomorphometric analysis. **RESULTS.** The Ti and Zi groups showed significant differences in the amount of newly formed bone between 2 and 4 weeks and between 2 and 8 weeks ($P<.05$). The measurements of total bone using micro CT showed significant differences between the Os and Ti groups and between the Os and Zi groups at 2 and 8 weeks ($P<.05$). Comparing by week in each group, the Ti group showed a significant difference between 4 and 8 weeks. Histomorphometric analysis also showed significant differences in new bone formation between the control group and the experimental groups at 2, 4, and 8 weeks ($P<.05$). In the comparison of newly formed bone, significant differences were observed between 2 and 4 weeks and between 2 and 8 weeks ($P<.05$) in all groups. **CONCLUSION.** Zirconia bone graft material showed satisfactory results in new bone formation and zirconia could be used as a new synthetic bone graft material. ***[J Adv Prosthodont 2018;10:167-76]***

KEYWORDS: Zirconia; Titanium; Bone graft material; Newly formed bone

INTRODUCTION

Many kinds of bone graft materials are being used for the reconstruction of defects in the oral and maxillofacial regions or for bone graft treatment accompanied by dental implant. Among these materials, autogenous bone has been considered a gold standard graft material with successful results.[1] However, autogenous bone has limitations, including morbidity of the donor site, insufficient amount of bone, and absorption during the healing period.[2] Although allogeneic and xenogeneic bones have been used as substitutes for autogenous bone, theses have the disadvantages of a possible immune response and the transmission of infectious disease.[3,4]

Recently, there has been remarkable progress in manufacturing techniques of bone substitute materials, and synthetic bone has been widely used; it has biological compatibility and osteoconduction that provides an excellent scaffold for the formation of new bones.[5] With the development of synthetic bone graft production technology, synthetic bone has become the main material for replacement of autogenous bone in dental operations.

Hydrate calcium phosphate synthetic bone, often referred to as hydroxyapatite, has biological compatibility and a structure that is similar to actual human cancellous bone.[6] However, hydroxyapatite is a biodegradable graft material in the human body and cannot maintain its original volume of graft.[7] Absorption of the grafted material leads to an unaesthetic result, especially in the anterior regions of mouth.

Titanium is commonly used as a dental implant as it has biocompatibility and is non-absorbable, and thus titanium bone graft material can maintain the volume of graft and

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alveolar bone.[8,9] However, titanium graft material cannot be used in the esthetic zone due to its unique metallic shade.[10]

Among the materials that can be used as bone grafts, zirconia is being studied as a material to replace existing alloplastic bone grafts and has shown excellent biological compatibility. Yttria-stabilized tetragonal zirconia polycrystals (Y-TZP) has excellent mechanical strength and biological compatibility.[11-20] Y-TZP is a non-absorbable material,[21] and with its color similar to those of natural teeth and alveolar bone, it can be used as bone substitute material in the anterior region of mouth and thin gingiva where esthetics are required.[22]

The production of a block bone graft that accurately fits an extensive defect is possible by capturing precise three-dimensional (3D) images of the bone defects using diagnostic imaging systems, such as 3D computed tomography (CT) and measuring the length, volume, and shape of the defect. The 3D imaging technology that is already being used for dental implants has excellent accuracy.[23,24] In comparison to other graft materials, zirconia is expected to play a better role as a block graft material because it is easier to manufacture and has a porous structure with an appropriate strength, and errors can be adjusted during the operation.

The ideal size and structure of synthetic bone graft materials have been reported in numerous studies. In most studies, a cribriform structure, which is similar to human bone, is recommended, and bone grafts with a granular shape have been designed for easy manipulation and control during bone graft surgery. Each granule has interconnected pores that maintain blood circulation and allow interaction among cells, thereby facilitating bone regeneration and bone growth. Although results are controversial, new bone formation is usually active at a pore size of 150 to 500 μm,[25,26] with the appropriate particle size of graft material for new bone formation being from 0.5 to 1.0 mm.[27]

The purpose of this study was to compare the new bone formation capability of zirconia with those of synthetic bone grafts. In this study, the 6 mm calvarial defects of New Zealand white rabbits were filled with synthetic, titanium, zirconia bone graft materials. After 2, 4 and 8 weeks, the newly formed bones of the groups were compared through micro CT and histomorphometric analysis.

MATERIALS AND METHODS

A cribriform-shaped polyurethane was used to manufacture a zirconia porous scaffold (Filtrocell, Eurofoam, Troisdorf, Germany). The polyurethane was dipped in 2% NaOH solution for 30 minutes before being washed with distilled water. It was then stored for 24 hours at room temperature.

Zirconia powder 100 g (TZ-3Y-E, Tosoh Co., Yamaguchi, Japan) calcined at 600°C for 2 hours to remove impurities. Triethyl phosphate 6 g (TEP) was added to ethanol, which was then placed in a ball mill for 24 hours to produce a slurry. In this slurry, Polyvinyl butyral 6 g (PVB) was added, mixed, and then stored for 24 hours. The polyurethane that had been previously processed was dipped into the slurry three times. To maintain the interconnected pore structure, the remaining slurry was removed by centrifuging at low speed and dried at 60°C for 10 minutes. This process was repeated twice, after which the polyurethane was kept at room temperature for 24 hours.

For sintering of this material, the temperature was increased to 600°C at the rate of 1°C per minute and then maintained for 2 hours to remove the polyurethane. The temperature was then increased again to 1550°C at the rate of 3°C per minute. After cooling, the zirconia graft material was placed in ethanol for 10 minutes and washed with an ultrasonic cleaner before being dried. The dried zirconia was crushed and made into granules, which were sorted by size using 0.5 and 1.0 mm with sieves. The granules were sterilized with ethylene oxide gas and stored in microtubes.

The titanium bone graft material (Tigran PTG, Tigran Technologies AB, Malmö, Sweden) consisted of porous titanium granules made of commercially pure titanium. The Ti granules were between 0.7 mm and 1.0 mm in size, irregularly shaped and non-resorbable. The granules have interconnected pores that provide a framework for new bone growth.

The synthetic bone graft material (Osteon II, Genoss Co., Suwon, Korea) was composed of hydroxyapatite 30% and beta-tricalcium phosphate 70%. It has an interconnected pore structure similar to that of human cancellous bone. The granules were between 0.5 mm and 1.0 mm in size.

This experiment received approval from the Institutional Animal Care and Use Committee (IACUC) of Cronex (CN20140528-1) and was conducted in accordance with the guidelines of the IACUC.

Twelve healthy New Zealand white male rabbits (weight 2.5 - 3.0 kg) were randomized into three groups. Each rabbit was anesthetized with 15 mg/kg of zoletil (VIRBAC S.A., Carros, France) and 5 mg/kg of xylazine (Bayer Korea Co., Ansan, Korea), and then 5 mg/kg of biotril (Komipharm Co., Shihung, Korea) was injected as a preoperative antibiotic. The hair of the rabbits was removed using an electric shaver, and the operation site was sterilized with a betadine solution before covering with a surgical drape. A midsagittal incision was made from the frontal region to the occipital region in the ventral position. The flap to the periosteum was retracted to the side. Four bone defects with a diameter of 6 mm were formed by using a trephine bur (Hager & Meisinger GmbH, Hansemannstrasse, Germany) with careful drilling. One defect was unfilled for the control group, and three defects were grafted with Os, Ti, and Zi as bone graft materials. The defects were filled with the proper volume of graft materials. The periosteum was sutured with 4-0 Vicryl suture threads (Johnson & Johnson Medical GmbH, Norderstedt, Germany). Finally, the skin was sutured with 4-0 Blue Nylon (Ailee Co., Busan, Korea). All rabbits recovered after the operation with no complications and were administered with biotril for 3 days after surgery. Each operation was performed under sterile conditions. At 2, 4, and 8 weeks, rabbits were sacrificed by administering 25 mg/kg of Succipharm (Komipharm Co., Shinhung, Korea).