Introduction
Banked allogeneic bone grafts have been used widely in clinic for the repair of bone defects. Massive allografts are thought to function through an osteoconductive pathways, demineralized bone was reported to be osteoinductive. Morcelized allograft which has been used to fill bony defects in revision arthroplasty to fulfill a mechanical role, as well as to provide a scaffold for new bone formation has yet to be elucidated the potentiality of osteoinductive function.

Infections and disease transmissions due to the usage of allograft tissues have motivated careful procurement and sterilization processing in bone graft banking. Secondary sterilization is required to limit disease transmission and contamination. Gamma irradiation is a widely accepted secondary sterilization procedure. A dose of 20 kGray or higher has been reported to alter the mechanical properties of bone. The effect of gamma irradiation on in vivo biological properties of morcelized allograft bone has not been well reported.

Our previously study found a negative dose-dependent effect of gamma irradiation on bone formation and remodeling of morcelized human bone grafts in nude rat defect model [1]. A statistically significant decrease in new bone formation was found in the defect packed with the morcelized grafts treated with 25 kGray gamma irradiation when compared with the 0 and 15 kGray groups. Evidence of osteoinductivity was found only in the defects packed with zero or low dose treated grafts.

This study focused on the mechanism investigation by testing the expression and distribution of bone morphogenetic protein (BMP) 7 which is a growth factor produced mainly by osteoblast, core binding factor α1 (CBFA1) which is a transcription factor governing osteoblast differentiation, and proliferating cell nuclear antigen (PCNA) which is a cell proliferating marker.

Materials and Methods
Morcellized human bone grafts were obtained as described previously, which were treated with gamma irradiation at the dose of 0, 15 or 25 kGray.

A bilateral tibial window (5×8mm) was created in the anteromedial aspect of the tibia of 21 CBH/rnu rats (male, 13-week old) following ethical approval. The defect was packed with the randomly numbered bone grafts, or left empty as negative control. All animals were killed at 3 weeks post-operation. The tibias were harvested, fixed, decalcified, embedded and sectioned medially-laterally into 5µm sections in serial. Haematoxylin & eosin (H&E) staining and Immunohistochemical staining (IHC) were performed for histological analysis and the evaluation of the protein expression of the detected factors.

The primary antibodies were monoclonal antiPCNA (PC10), goat polyclonal anti-BMP7 (N19) and goat polyclonal anti-CBFA1 (C-19) (Santa Cruz). DAB+ substrate-chromogen system was used which forms a brown product at the target antigens.

The distribution and the intensity of the staining were assessed by two independent investigators and recorded. The images of the staining were taken under normal light microscope at 20x magnification and 4 images per section were used for quantitative analysis using BIOQUANT NOVA PRIME image analysis system v6.50.10 (BIOQUANT Image Analysis Corp., TN, USA). The region of interest (ROI) is in the center of defect away from the host cortex (Figure 1). A one-way ANOVA was used for the comparisons of IHC intensity among the different gamma irradiation groups. The coefficient of variation is 10.1% and 12.5% for inter- and intra-person.

Results
The H&E staining showed the empty defects were filled with primarily disorganized loose connective tissues at 3 weeks. Some new bone in-growth arising from the intact cortex, bridged by the implanted grafts was noted in all defects packed with the morcellized grafts treated with different dosages of gamma irradiation. New bone formation was also noted around the implanted bone graft far from the host cortex in the zero or 15 kGray groups.

CBFA1 and BMP7 stained mainly the osteoblasts and some mesenchymal like cells lining around the intact cortex, the new bones adjacent to the host cortex bridged by the implanted grafts and in the loose connective tissue far from the host bone (Figure 2). A negative correlation of immunostaining of CBFA1 and BMP7 and gamma irradiation was presented by quantitative analysis. The expression of proliferation marker PCNA showed no correlation with the irradiation (Figure 3).

Discussion
Massive allograft bone is believed to function as an osteoconductive scaffold and lack of osteoinductivity. The mineralized statues, limited surface areas, lack of vascularization and the removal of the cells are the same time may expose growth factors such as BMPs to the surfaces.

In this study, a negative dose dependant bone formation capabilities of the allograft bone chips treated with 0, 15 or 25 kGy gamma irradiation in rat defects was founded. The same correlation was found with the expression of BMP7 and CBFA1. In the other word, the low bone formation rate in the high dose gamma irradiation group (25 kGy) may due to the less expression of BMP7 and/or CBFA1. The gamma irradiation reduces in vivo osteoconductivity and osteoinductivity of morcellized bone without affecting the proliferation of the host cells.

Reference:

**Queensland Bone Bank, Holy Spirit Hospital, Brisbane, QLD, Australia

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