Biological characterization of human blood-derived material (CGF) and its cellular response under back skins of nude mice

Biological characterization of human blood-derived material (CGF) and its cellular response under back skins of nude mice

2019

Graduate School of Dentistry,
Health Sciences University of Hokkaido

ONJI Keiko

Introduction

Graft materials from a patient's own body components have been developed for tissue regeneration. Human blood-derived fibrin with platelet concentrates has been studied to improve bone healing by facilitating recruitment, proliferation, and maturation of the cells. Platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF) constituted three varieties of the autologous platelet concentrates (Dohan DM et al., 2006; Dohan Ehrenfest DM et al., 2010). The CGF is the most advanced generation of platelet concentrates prepared automatically by a special centrifuge device (Anitua E et al., 2008). It is characterized as stiffer, denser, and richer growth factors with fibrin matrix. The CGF releases various growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin-like growth factors (IGFs), transforming growth factor-β1 (TGF-β1), which stimulate cell proliferation, angiogenesis, and matrix remodeling. Although BMP-2 (Asahina I, 2014) was also detected in CGF, but the concentration was significantly lower compared with PDGF, TGF-β1, and VEGF. Until now, the sequential behavior of human CGF alone has not been clear *in vivo*, and a graft of human CGF with recombinant human BMP-2 (rhBMP-2) has not been attempted in ectopic sites.

The aims of this study were to observe the microstructures of human blood-derived fresh CGF as glue and membrane, and to evaluate the behavior of human CGF membrane as a delivery scaffold of rhBMP-2 in subcutaneous tissues of nude-mice.

Materials and Methods

Sixty milliliters of blood was collected in six sterile vacuum glass tubes from healthy peripheral venous blood and immediately centrifuged in a special centrifuge device to get three blood fractions as platelet poor plasma (PPP), concentrated growth factor (CGF), and red blood cell (RBC) layer. The middle layer as CGF glue was collected by a sterile tweezers, and placed in the sterile petri dish. Half of the yellow glue (CGF) was pressed using a

stainless-steel compression device to transform into CGF membrane (5x5x2 mm^3). Forty microliters of rhBMP-2 solution (0.025 g/l) was added into the CGF membrane (5x5x2 mm^3) to prepare CGF/rhBMP-2. CGF glue, CGF membrane, the buffy coat layer, and the RBC layer were evaluated for morphological and histological characteristics by scanning electronic microscopy (SEM) and hematoxylin & eosin (HE) staining.

Twenty-four nude mice (5-week-old, male) were used for the assessment of *in vivo* ectopic bone formation. Mice were received the CGF glue or the CGF membrane as the controls and the CGF/rhBMP-2 membrane as the experimental group in the subcutaneous tissues, and harvested at 7, 10, and 14 days after the graft. Harvested samples were evaluated for histological examination and histomorphometric measurement was conducted to compare the size of the CGF.

Results

The CGF glue exhibited mature 3D-fibrin network assembled from several fibrillary elements with various thickness. Multiple platelets were found trapped on the thick polymerized fibers observed by SEM. Histologically, the CGF glue revealed homogenous light pink fibrin matrix and the CGF membrane showed non-uniform irregular pinkish shape due to compression. The buffy coat layer, the boundary between the CGF and the RBC layer, showed attach to the bottom of the CGF layer, and mixture of leukocytes and erythrocytes.

The CGF glue appeared clearly as highly dense fibrin fibers. The glue architecture became unstructured bundles with multiple clear spaces from 7 to 10 days. The bundle areas were acellular, and undifferentiated mesenchymal cell-like cells were observed in the clear spaces. The CGF membrane revealed a bundle-bending mass with round-type cells including various thick and irregular bundles. The cellular invasion into the CGF membrane was higher compared with the CGF glue at 7 and 10 days. The immunohistochemical staining for osteopontin showed the fragmented CGF was negative, and for the staining of Elastica van

Gieson, the CGF remnants were stained with red. At 14 days, the grafted CGF glue and membrane samples totally disappeared in the subcutaneous tissues. Bone and cartilage induction didn't occur at 7, 10, and 14 days. The CGF/rhBMP-2 membrane showed dispersed and disorganized bundles revealed as wavy structure. Undifferentiated mesenchymal cell-like cells were observed in the spaces between bundles at 7 and 10 days. At 14 days, several induced bony islands with round- and trabecular-shapes and active cuboidal osteoblasts were lined with capillary. The CGF membrane was replaced by bone, cartilage, and connective tissues at 14 days. The immunostaining for osteopontin showed the immunoreactivity was detected in the induced bone matrices, and the induced bone were strongly stained with red in Elastica van Gieson.

Conclusion

CGF materials are 100% autologous blood products, and they can be prepared immediately for surgery. Both the CGF glue and the CGF membrane alone were almost absorbed at 14 days by plasmin degradation. The present study indicates that the CGF membrane supported the bone-inducing capability of rhBMP-2 as absorbable sticky fibrin matrix. These results demonstrated that a fresh, human CGF membrane might act as a short-term biological scaffold for the delivery of rhBMP-2.

References

Anitua E, Aguirre JJ, Algorta J, Ayerdi E, Cabezas AI, Orive G & Andia I. Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. J Biomed Mater Res B Appl Biomater 84:415-421, 2008.

Asahina I. Bone morphogenetic proteins: their history and characteristics. J Hard Tissue Biol 23: 283-286, 2014.

Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J & Gogly B. Platelet-rich

fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 101: e37-44, 2006a.

Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J & Charrier J B. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. J Periodontol 81(4):546-555, 2010.