

Supporting Information

Bioresorbable bone graft composed of RGD-enriched recombinant human collagen polypeptide induced neovascularization and regeneration of mature bone tissue

Takahiro Hiratsuka^{*,1}, Izumi Ogura¹, Ai Okamura¹, Hideo Fushimi¹, Kazuhiro Yamaguchi¹, Ichiro Nishimura²

¹Bio Science & Engineering Laboratory, Research & Development Management Headquarters, FUJIFILM Corporation, Kanagawa 258-8577, Japan

²Weintraub Center for Reconstructive Biotechnology, UCLA School of Dentistry, Box951668 CHS B3-087, Los Angeles, CA 90095, USA

Corresponding author's email address: takahiro.hiratsuka@fujifilm.com

Figure S1. Overview of the synthesis and purification process of RCPPhC1 from working cell bank (WCB) of *Pichia pastoris* strain X-33 carrying pPICZ-RCPPhC1 plasmid.

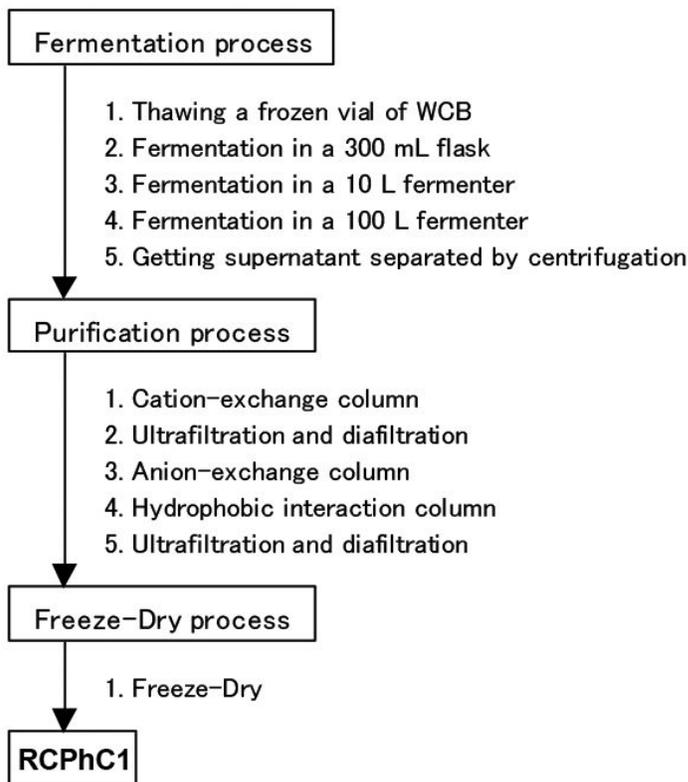
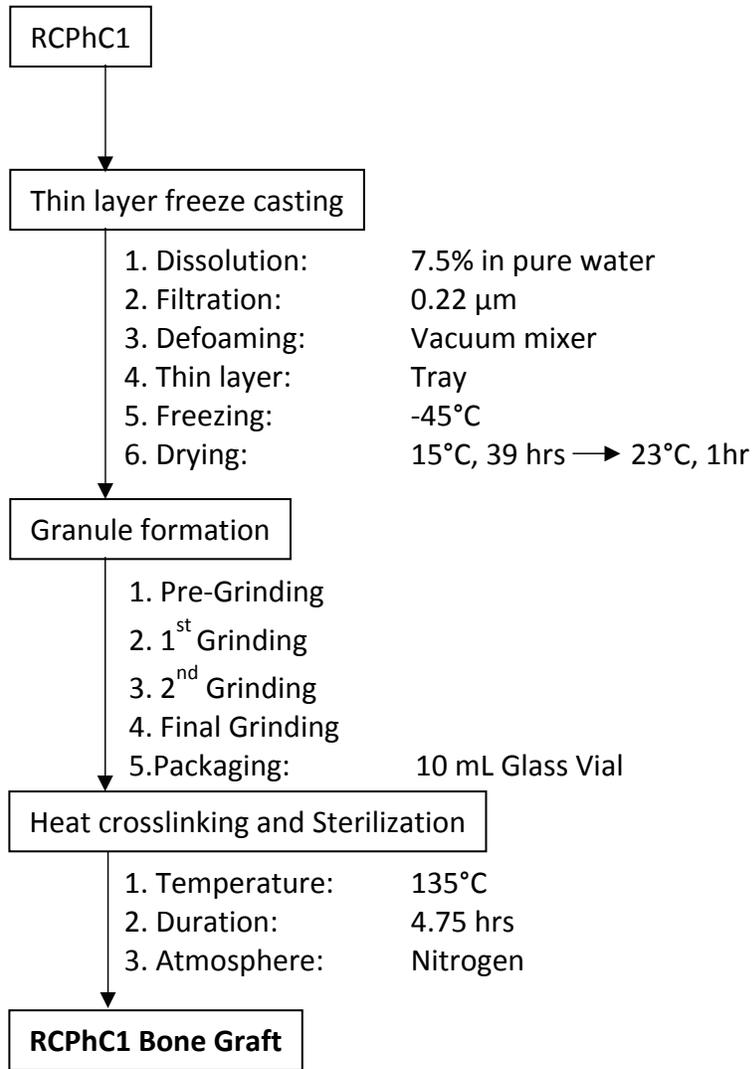


Figure S2 A. Overview of the manufacturing process of RCPHC1 Bone Graft. **B.** RCPHC1 Bone Graft granules.

A



B

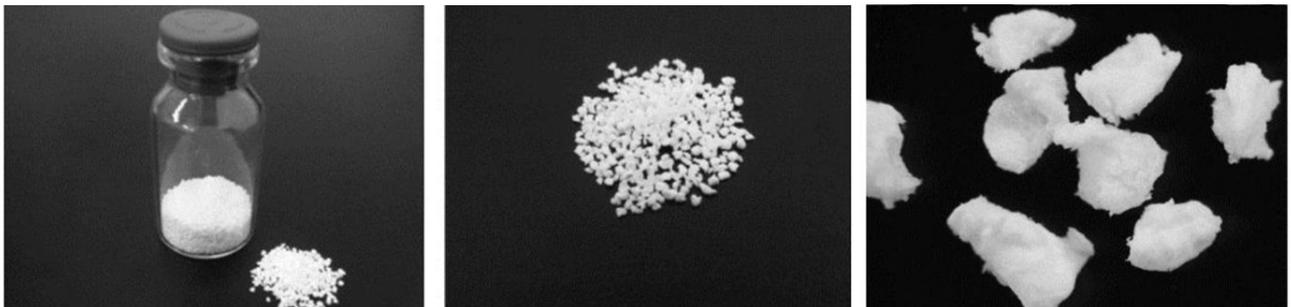


Figure S3. Difference in the bone formation ability due to each heat cross-linking time in rat calvarial defect model (4 weeks after implantation). This RCPHC1 Bone Graft was used with the condition of the red dashed line frame. Statistical analysis was performed using one-way ANOVA with Tukey multiple tests to compare treatment groups. (From Fushimi et al., Communications Materials, 2020. <https://doi.org/10.1038/s43246-020-00089-9>)

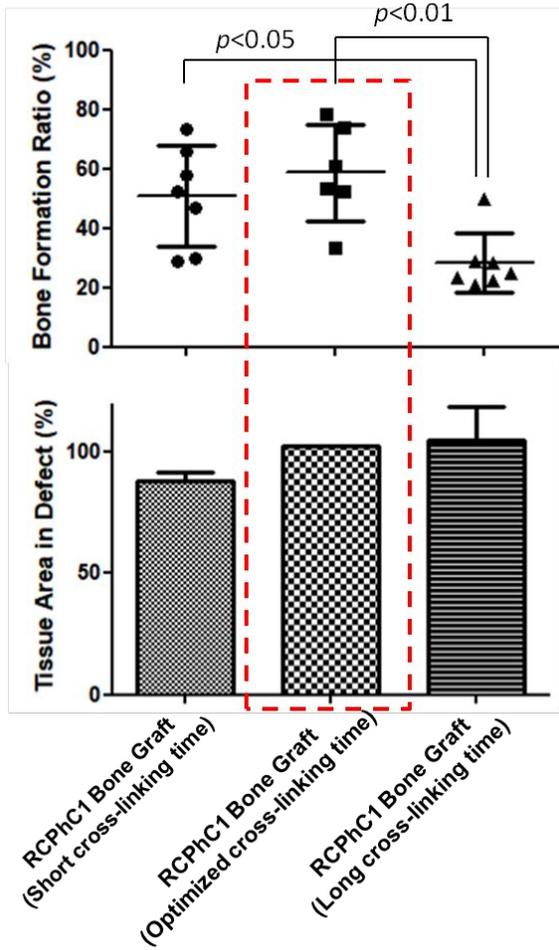


Figure S4. Cell adhesion of RCPHC1 Bone Graft. NIH-3T3 that stained calcein AM were incubated for 24hrs on RCPHC1 Bone Graft. The fluorescence labeled cells were observed.

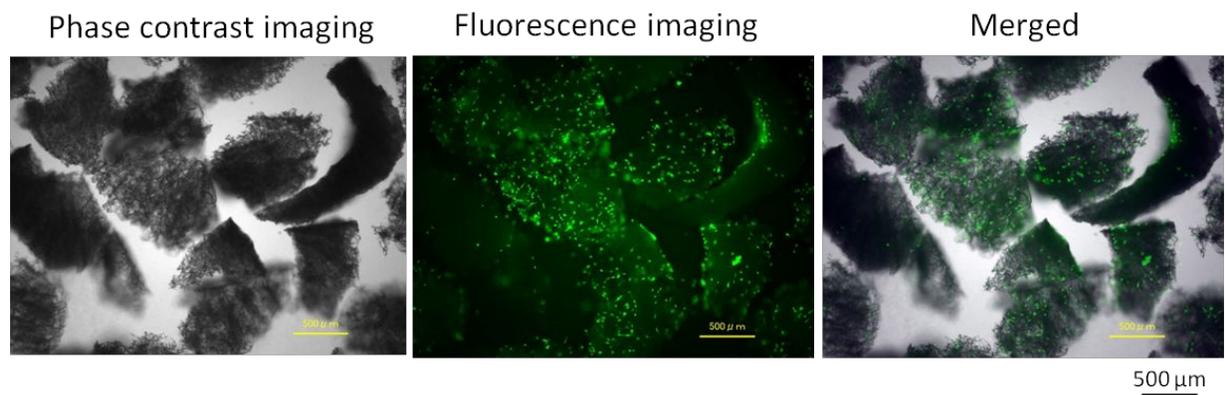


Figure S5. Protein composition of demineralized bone matrix allograft (Puros DBM, Zimmer Biomet, Warsaw, IN, USA).

A total of 78 proteins were identified, including 7 collagen molecules. Collagen type I, alpha 1 and alpha 2 chains were the predominant proteins. Other minor components were ECM molecules and bone metabolism-related proteins. BMP was not detected.

Protein	Gene name	PSMs
Collagen alpha-2(I) chain	COL1A2	2096
Collagen alpha-1(I) chain	COL1A1	1779
Collagen, type I, alpha 1, isoform CRA_a	COL1A1	456
Collagen alpha-1(III) chain	COL3A1	36
Collagen alpha-5(IV) chain	COL4A5	42
Collagen alpha-1(XII) chain	COL12A1	1
Collagen alpha-1(XI) chain	COL11A1	1 (filtrate)

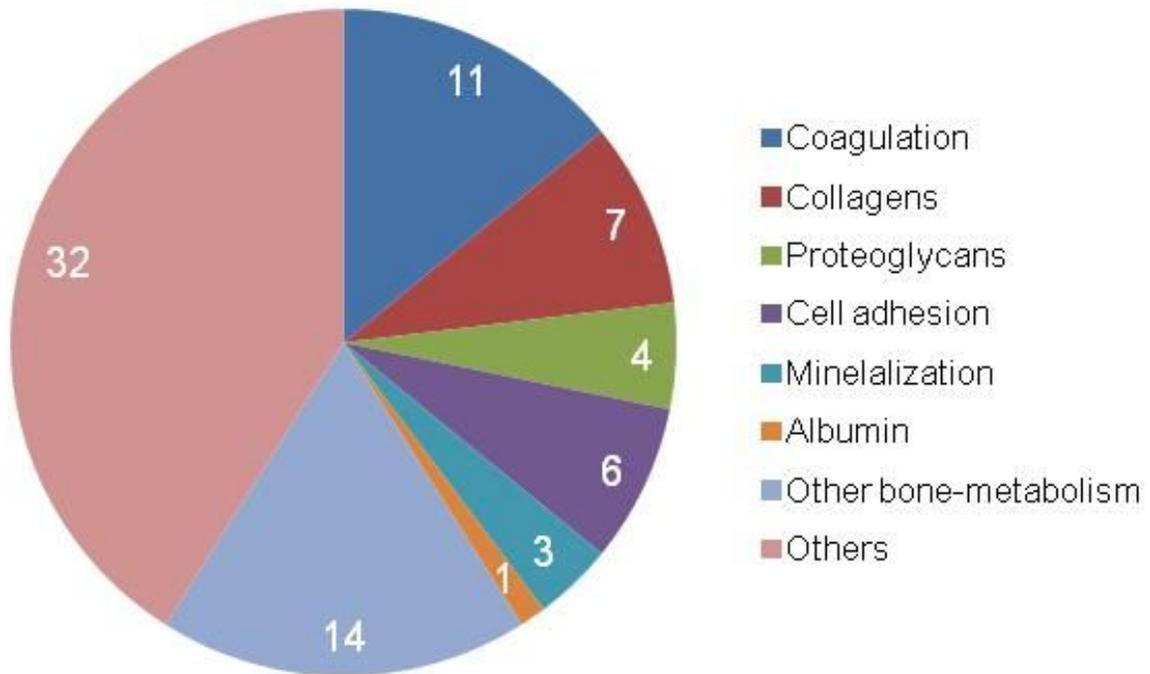


Figure S6. Time course bone volume measurements of individual animals implanted with DBM with chip or RCPCh1 Bone Graft.

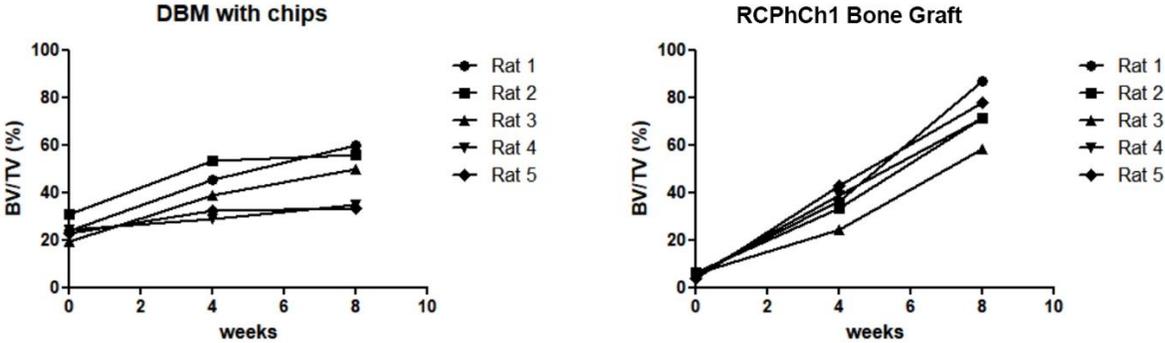


Figure S7. Tooth extraction wound healing in dogs. One week after tooth extraction of distal root of 3rd premolar, gingival tissue was closed. The extraction socket was clearly identified in microCT images.

