Supplementary information

Induction of mesenchymal stem cell differentiation by co-culturing with mature cells in double-layered 2-methacryloyloxyethyl phosphorylcholine polymer hydrogel matrices

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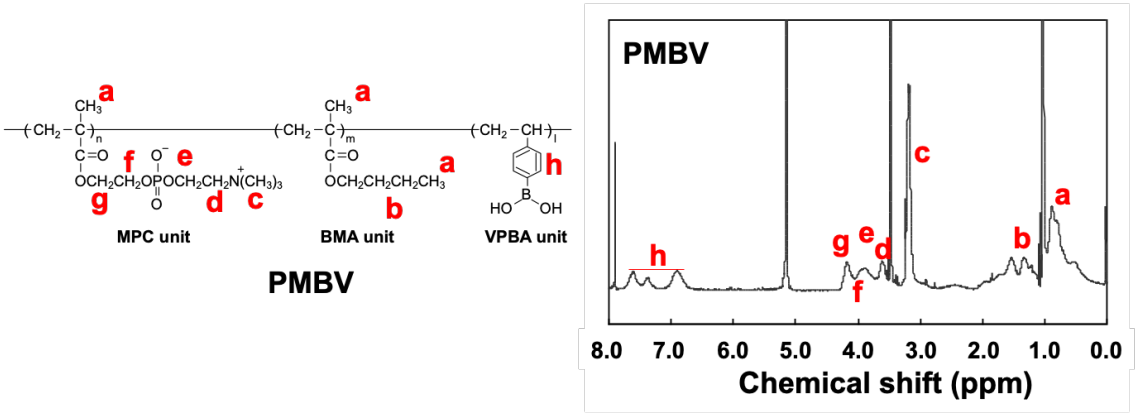

Fig. S1 Representative ^{1}H-NMR spectrum of PMBV-2 in d6-ethanol at 25 °C.

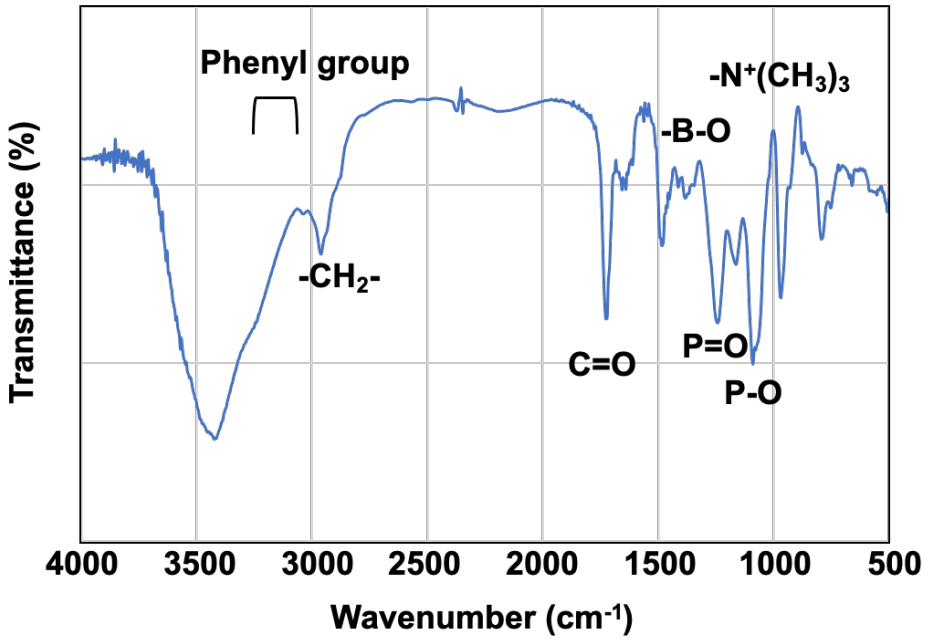

Fig. S2 Representative FT-IR spectrum of PMBV-2.

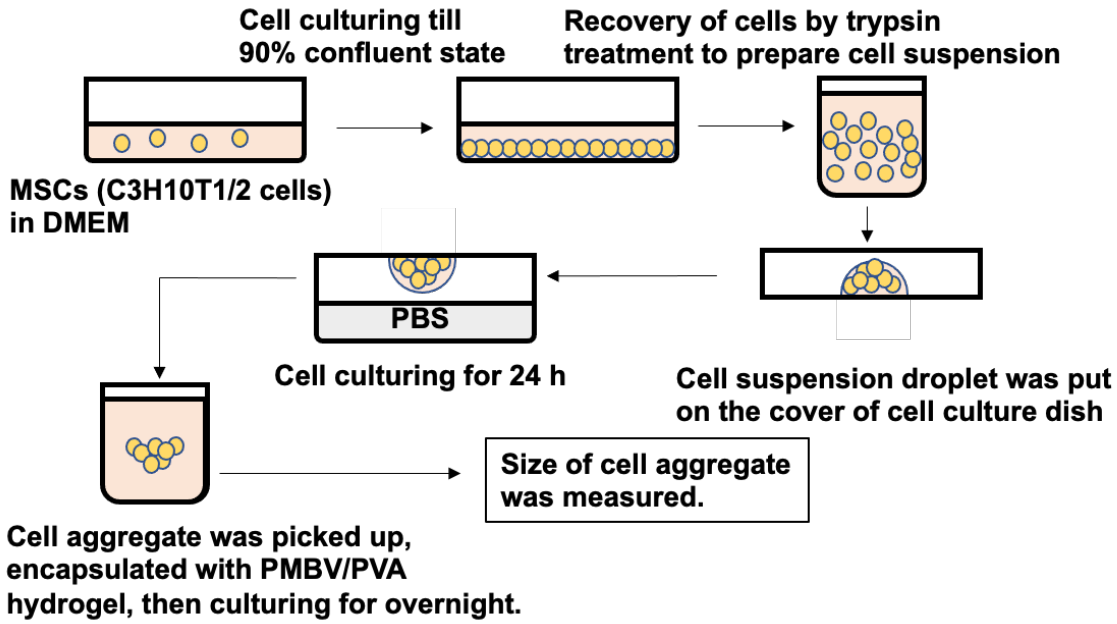

Fig. S3 Procedure of cell aggregate formation by hanging drop method.

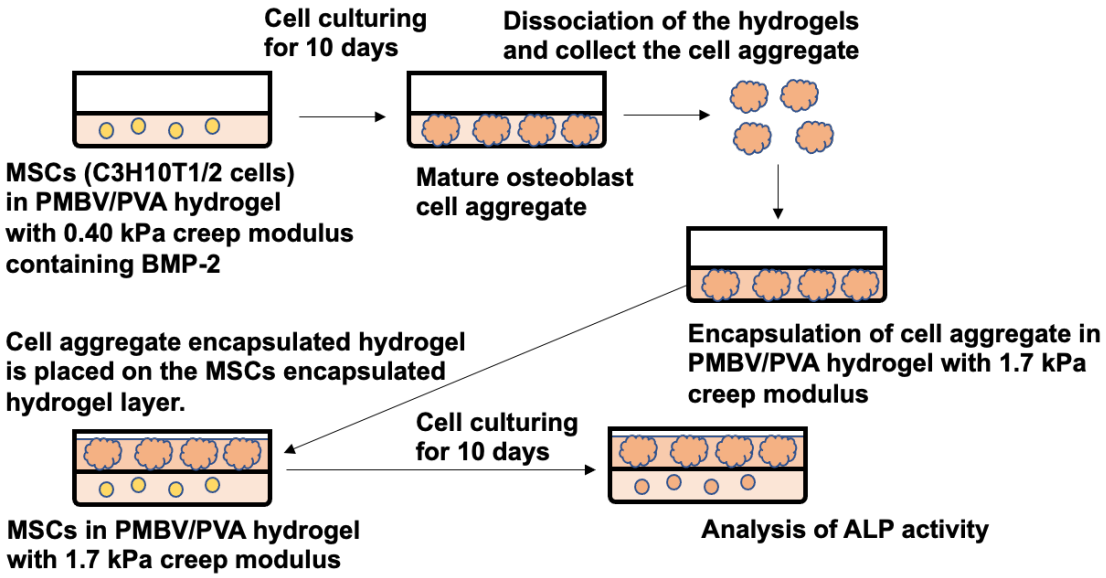

Fig. S4 Schematic of the protocol for the preparation of the double-layered PMBV/PVA hydrogel system containing undifferentiated and differentiated C3H10T1/2 cells. The C3H10T1/2 cells in the PMBV/PVA hydrogel differentiated by the addition of BMP-2 and formed mature osteoblast cell aggregates. The cell aggregates could recover by dissociation of the PMBV/PVA hydrogel. The cell aggregates were encapsulated in the PMBV/PVA hydrogel. The hydrogel was integrated on the other hydrogel containing undifferentiated C3H10T1/2 cells. The ALP activity was analyzed to evaluate the differentiation of cells.

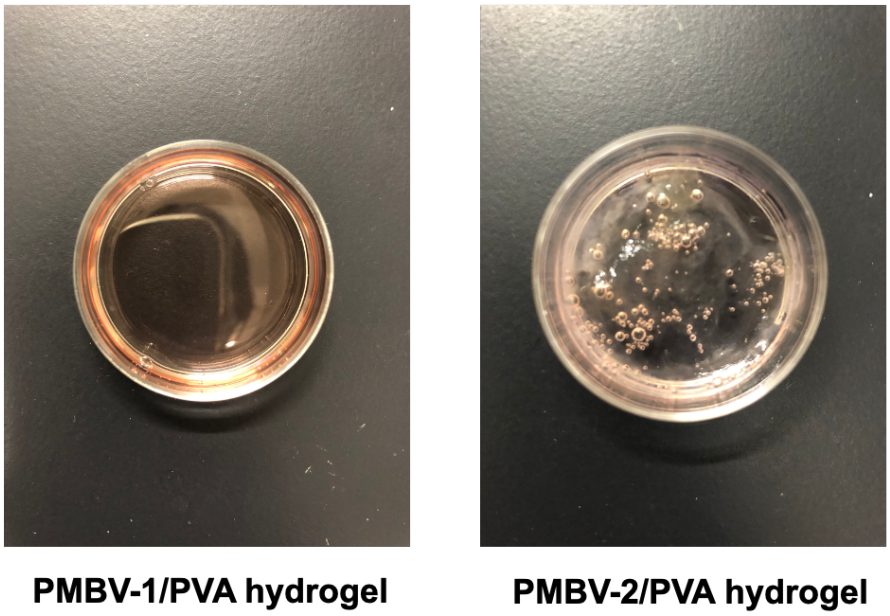

Fig. S5 Optical image of the PMBV/PVA hydrogels. Concentrations of PMBV was 5.0 wt% and PVA was 2.5 wt% in DMEM. Two polymer solutions were mixed together and the solution was pipetting gently several times at room temperature.

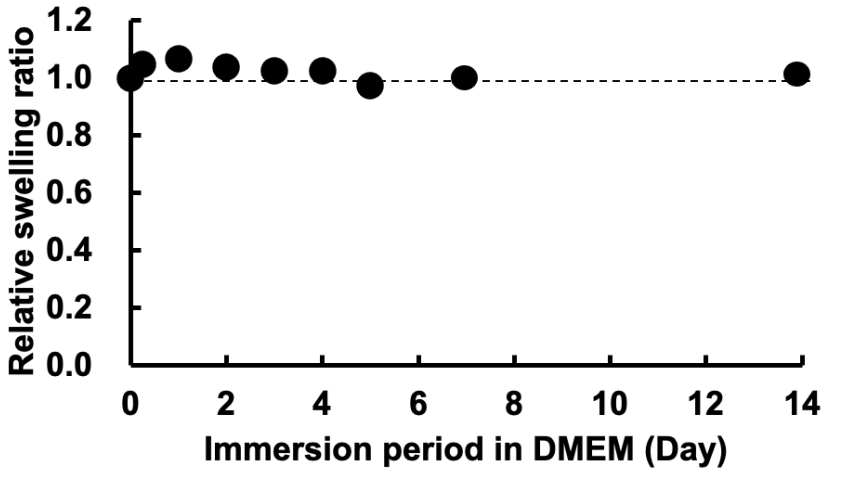

Fig. S6 Swelling stability of the PMBV/PVA hydrogel stored in DMEM at room temperature. Concentrations of PMBV was 5.0 wt% and PVA was 2.5 wt% in DMEM for preparation of PMBV/PVA hydrogel.

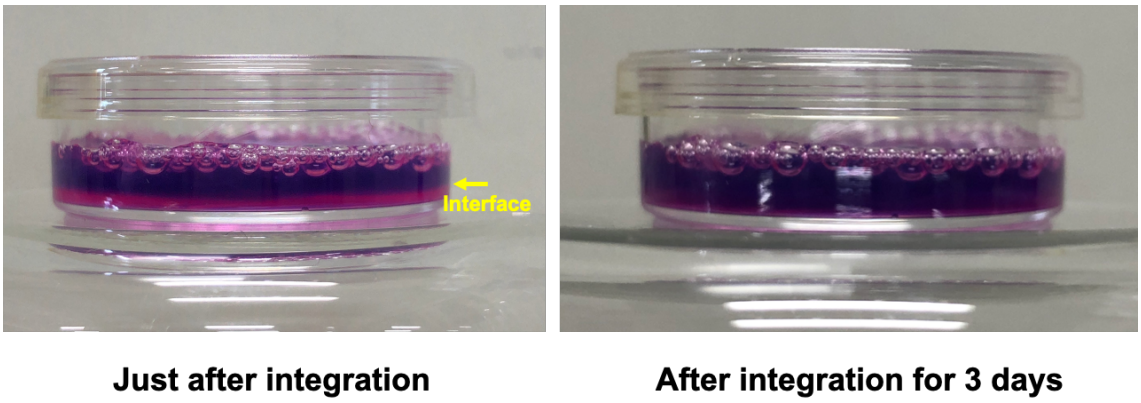

Fig. S7 Adhesion of the PMBV/PVA hydrogel for the preparation of the double-layered hydrogels. Two hydrogel layers were observed by staining the upper layer of the hydrogel immediately after the double-layered hydrogel layer was prepared. The yellow allow indicates the initial interface between two hydrogel layers. After three days, the border of the two hydrogel layers became unclear.

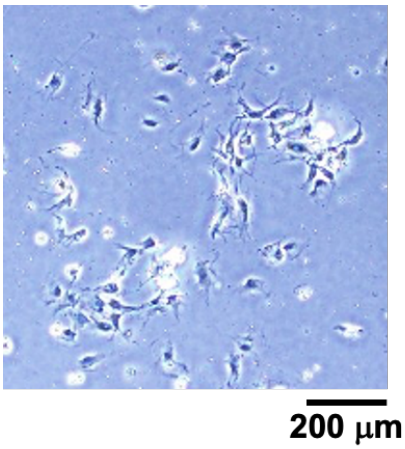

Fig. S8 Phase-contrast microscopy image of differentiated C3H10T1/2 cells stained by a conventional APL staining method.