# Enhancement of mesenchymal stem cell differentiation by co-culturing with mature cells in a double-layered phospholipid polymer hydrogel matrix

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## Background

### ◆ Cell engineering with stem cells

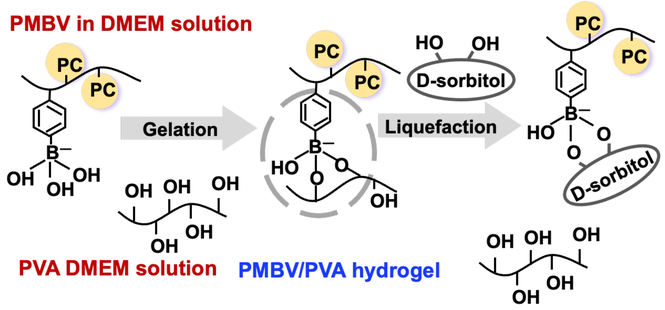
In stem cell-based regenerative medicine, it is necessary to culture the stem cells in three dimensions to produce cell aggregates and then induce differentiation.

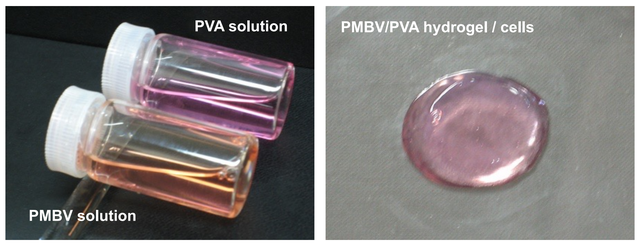
In addition, cells may communicate with each other via various bioactive molecules such as cytokines, and cell-aggregates are good for diffusion of these bioactive molecules due to cells contact closely in the aggregate.

Thus, to understand the effects of the co-culture of cell aggregates on differentiation is necessary.

Cell proliferation and differentiation depend on the surrounding circumstances. When cell culture is carried out in 3-dimensionally(3D), suitable polymer hydrogel matrices are required.

### ◆ Spontaneously forming hydrogel for 3D cell culture







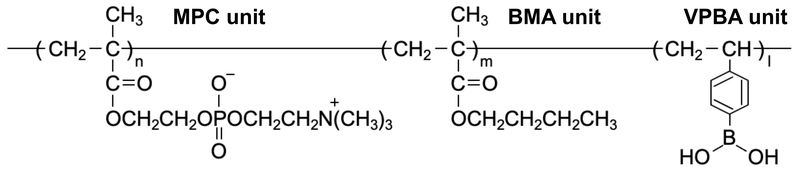
When two water-soluble polymers, poly[2-methacryloyl-oxyethyl phosphorylcholine (MPC)-*co*-*n*-butyl methacrylate (BMA)-*co*-*p*-vinylphenyl boronic acid (VPBA)] (PMBV) and poly(vinyl alcohol) (PVA) are mixed together, PMBV/PVA hydrogel is formed spontaneously under cell culture condition. This hydrogel can be dissolved reversibly by addition of D-sorbitol.

T. Konno, K. Ishihara, Biomaterials (2007)
K. Ishihara, Biomaterials (2020)

## Objective

We analyzed the effects of mature cells on stem cell differentiation by adhering two PMBV/PVA hydrogel layers encapsulated in different states of the cells (stem cells and mature cells). This is important to clarify the characteristics of the cell-growth environment when stem cells are used as a cell source.

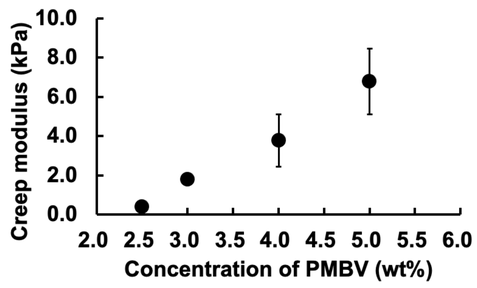
## Synthesis




PMBV was synthesized by a conventional radical polymerization. Composition of MPC/BMA/VPBA = 0.73/0.21/0.06, Mw = 25 kDa.

## Mechanical property of PMBV/PVA hydrogel

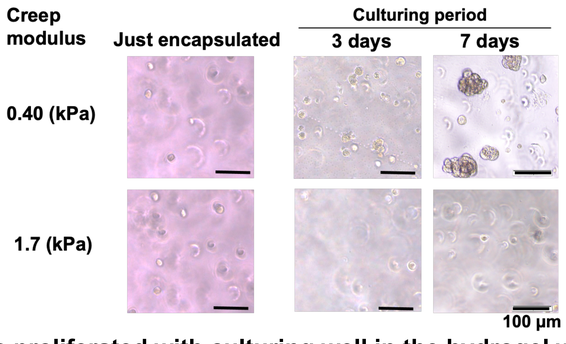
### ◆ Creep modulus of PMBV/PVA hydrogel




The creep modulus increased linearly with PMBV concentration. This is suitable for regulating cell behavior after encapsulation.

### ◆ MSC culture in the PMBV/PVA hydrogel

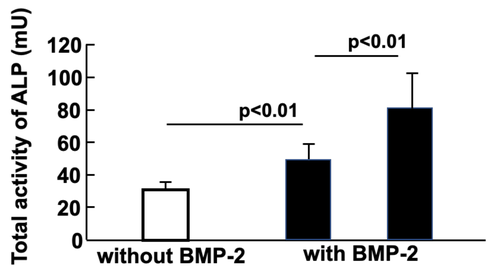
C3H10T1/2 cells were used as MSCs.

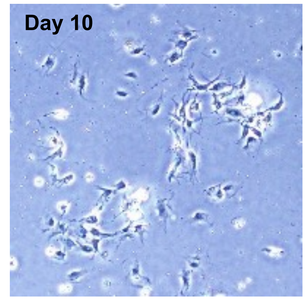



The cells proliferated with culturing well in the hydrogel with low moduli. Small cell aggregates were observed on day 3. The size of the aggregates increased on day 7.

## Differentiation of MSCs in PMBV/PVA hydrogel

### ◆ Differentiation of MSCs by BMP-2

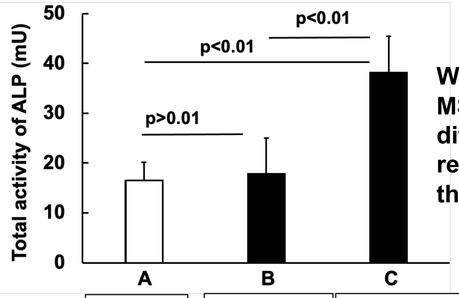


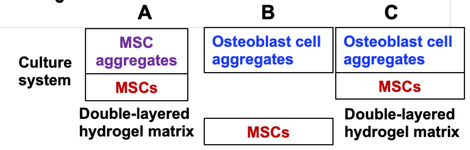

ALP staining of cells in PMBV/PVA hydrogel with 1.7 kPa moduli.

Total activity of alkaline phosphatase (ALP) based on the differentiation of MSCs stimulated by BMP-2 was increased in PMBV/PVA hydrogel.

### ◆ Differentiation of MSCs by mature cells (Osteoblast)




When mature cell aggregates and MSCs were encapsulated in different two hydrogel layers, respectively, and adhered them, the MSCs differentiated efficiently.




A: Double-layered hydrogels containing MSC aggregates and MSCs
B: Two hydrogels containing osteoblast cell aggregates and MSCs, respectively
C: Double-layered hydrogels containing osteoblast aggregates and MSCs

## Conclusion

Mature cells influenced the differentiation of MSCs when they are existed closely. This is good information to enhance tissue regeneration using stem cells. One of the polymer hydrogels as a matrix of the cells had an important role for the enhancement to control diffusion of cytokines secreted from mature cells.