Cellular microenvironment tunable phospholipid polymer hydrogel for cell proliferation control

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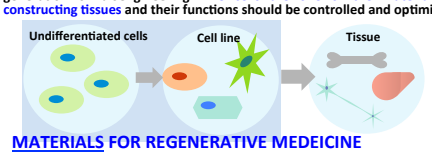
Background

The regenerative medicine starts to play an important role in the new generation of bioengineering. The cells are one of the materials for constructing tissues and their functions should be controlled and optimized.



MATERIALS FOR REGENERATIVE MEDEICINE

- Defined quality
- Unified quality among group of cells (>10^6 cells)
- Reproducibility of the same quality

Aim and Strategy

Control of the signal response of cells through its physical property of microenvironment

Creation of 3D matrix with no chemical interactions

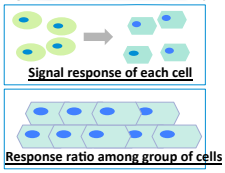
Large number of cells | Solute, oxygen permeabil | Diffusion of signal molecules

Physically controllable cellular micro-environment

Cellular micro-environment is equivalent among each cells

Possible outcomes

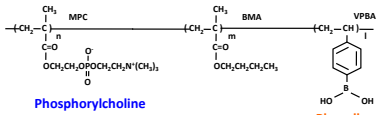
High Differentiation efficiency



Same manipulation to large number of cells are possible
No chemicals will be used to control cell function
The existing knowledge on differentiation signal could be applied with better response

Phospholipid Polymer Hydrogel

Poly(MPC-*co*-BMA-*co*-VPBA) (PMBV) hydrogel



Phosphorylcholine — Phenylboronic acid

MPC : 2-methacryloyloxyethyl phosphorylcholine BMA : *n*-butyl methacrylate
VPBA : *p*-vinylphenylboronic acid

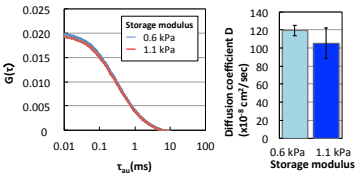
Synthetic table of PMBV

Abb.	Mole fraction In feed MPC / BMA / VPBA	Mole fraction In copolymer MPC / BMA / VPBA	Mw (x 10^4)	Mw / Mn
PMBV631	60 / 30 / 10	57 / 35 / 8	4.3	1.4
PMBV622	60 / 20 / 20	60 / 24 / 16	1.2	1.9
PMBV802	80 / 0 / 20	84 / 0 / 16	2.1	1.6

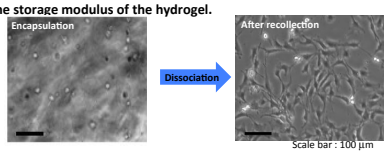
Mole fraction of monomer units in polymer was determined by ^{1}H-NMR (C$_2$D$_5$OD)
Molecular weight of the polymer was determined by GPC (Methanol / Water = 7/3, [LiBr] = 10mM, [Sorbitol]= 1mg/mL)

Phosphorylcholine

High water content hydrogel without interaction with biological molecules



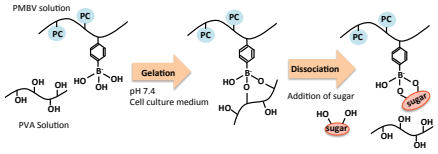
The diffusion of the protein inside the hydrogel was not affected by the storage modulus of the hydrogel.

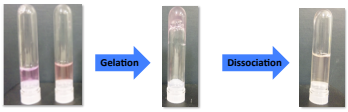


Cells were separately distributed inside PMBV hydrogel. The cells adhered and proliferated after the dissociation of hydrogel and recollection.

Phenylboronic acid

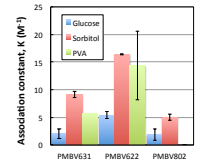
Cell encapsulation and recollection in mild condition





The chemical bond between phenylboronic acid and diols makes it possible for the hydrogel to dissociate. This enables both analysis and utilization of the encapsulated cells after recollection from the hydrogel.

Association constant of phenylboronic acid increased with butyl methacrylate units



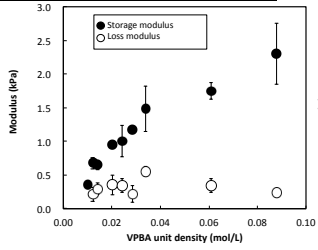
The Association constant between the phenylboronic acid moiety of various PMBV polymers and Glucose, Sorbitol, and PVA was measured.

Glucose : included in DMEM (trans-diol)
Sorbitol : used to dissociate the hydrogel (cis-diol)
PVA : used to form hydrogel

Association constant has good relationship with what has observed during gelation and dissociation : PMBV forms hydrogel by mixing with PVA in DMEM solution, and dissociated with sorbitol solution.
This result also illustrates the importance of n-butyl methacrylate, the hydrophobic unit for binding with PVA. It is hypothesized that aggregation of PMBV leads to the increase in association constant of phenylboronic acid.

Physical property of PMBV hydrogel

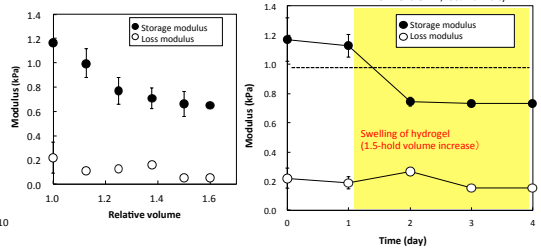
Storage modulus was controlled by VPBA density



The storage modulus was controlled by the initial boronic acid density of the hydrogel.
H. Oda *et al*, *Biomaterials*, 34, 5891(2013)

Storage modulus changed by swelling hydrogel

Environment : 37℃,100%humidity



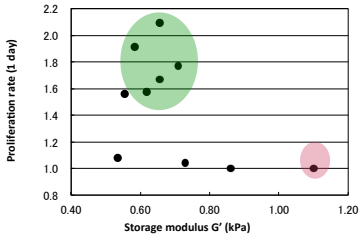
The storage modulus changed by swelling the hydrogel with DMEM. The storage modulus was stable before and after the swelling. This indicated that decrease of storage modulus was done without deformation of the three dimensional matrix.

The storage modulus of PMBV hydrogel was controlled before and after the formation of 3D matrix.

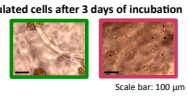
Reaction of Encapsulated Cells

Cellular proliferation in PMBV hydrogel

Mouse MSC (C3H10T1/2) was encapsulated in the hydrogel. (5.0 x 10^5 cells/mL)

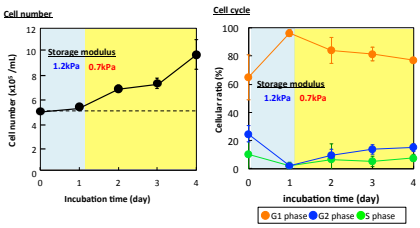


Encapsulated cells after 3 days of incubation



Scale bar: 100 μm

The proliferation of the encapsulated cells changed by the initial storage modulus of the PMBV hydrogel. When the storage modulus was between 0.6 to 0.8 kPa, the cells proliferated. When the storage modulus was more than 1.0 kPa, the cellular proliferation was suppressed and the cells were converged into G1 phase.
H. Oda *et al*, *Biomaterials*, 34, 5891(2013)

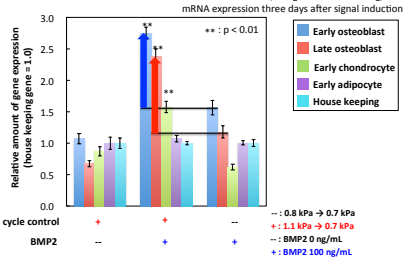


The cell proliferation is known to be suppressed when the cells were encapsulated in the hydrogel with storage modulus higher than 1.0 kPa. The cell cycle also converges to G1 phase. When the storage modulus of the PMBV hydrogel was lowered to 0.78 kPa by swelling the hydrogel by cell culture medium, the cells started its proliferation from its suppressed state. The cell cycle also started to go back to the ratio of the normal state.

The proliferation of the encapsulated cells were controlled through its physical environment.

Differentiation induction using PMBV hydrogel

Cell : Murine MSC lineC3H10T1/2 Signal : BMP2 (100ng/mL)
mRNA expression three days after signal induction



Improvement of differentiation efficiency was observed with cell cycle controlled cells

Conclusion

Suppression and restart of encapsulated stem cell proliferation through its environment significantly improved the differentiation.
The use of PMBV hydrogels to control the cell condition during signal induction will lead to high quality supply for new generation of bioengineering.

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