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Review Article

Application of a Thermo-Reversible Gelation Polymer, Mebiol Gel, for Stem Cell Culture and Regenerative Medicine

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Abstract

Recent studies have revealed the possible utility of a three-dimensional culture system using a thermo-reversible gelation polymer, Mebiol Gel. It is a purely synthesized biocompatible copolymer composed of thermoresponsive polymer blocks [poly(N-isopropylacrylamide-co-n-butyl methacrylate) poly(NIPAAm-co-BMA)] and hydrophilic polymer blocks (polyethylene glycol [PEG]). Mebiol Gel is characterized by its temperature-dependent dynamic visccoelastic properties. Mebiol Gel is used as a biocompatible scaffold for three-dimensional culture without any toxicity. Representative biological scaffolds for three-dimensional culture, i.e. type I collagen and Matrigel, interact with cells and affect cellular functions, but Mebiol Gel hardly showed such effects. Because of its innertness, Mebiol Gel enables clonal expansion of single stem cells. Application of Mebiol Gel to tissue defects in animal models revealed that Mebiol Gel enhanced tissue regeneration with activation of stem cells and prevention of inflammation. Thus, Mebiol Gel is suitable for preparation of cells for transplantation and is useful for direct application to promote regeneration of damaged tissues *in vivo.*

Key words; Thermo-reversible gelation polymer; Mebiol Gel; Stem cells; Three-dimensional culture; Clonal expansion

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Introduction:

Recent progress in stem cell biology has opened up the possibility of new cell-based therapeutic approaches in regenerative medicine. To apply the cell-based therapies to patients with various diseases, including heart diseases, diabetes, and neural degenerative disorders, preparation of cells for tissue regeneration will be one of the highest hurdles to overcome. [1,2] Many different types of cells have been cultivated for this purpose, among which stem cells of various origins are the most promising materials because of their high propagation capability and diverse differentiation potentials. [3]

In general, cells that compose solid tissues *in vivo* can only grow in an anchorage-dependent manner *in vitro*. [4, 5] Thus, colony formation in three-dimensional culture matrices can be used as a selection tool for cells with a capacity of anchorage-independent growth. [6] Stem cells possess normal cell properties, form solid tissues *in vivo*, and grow with attachment to culture substrates in a monolayer culture. However, many types of stem cells, including neural stem cells[7] and mammary gland cells[8], have been shown to grow in suspension. Three-dimensional culture systems can support growth of stem cells without a niche. [9, 10]

We previously reported the utility of a three-dimensional culture system using a thermo-reversible gelation polymer, Mebiol Gel, to isolate neural and skin stem cells.[11, 12] In this concise review, we focused on the application of Mebiol Gel for stem cell culture and regenerative medicine.

Mebiol Gel, a thermo-reversible gelation polymer:

A thermo-reversible gelation polymer, Mebiol Gel, is a copolymer composed of thermoresponsive polymer blocks [poly(N-isopropylacrylamide-co-n-butyl methacrylate) poly(NIPAAm-co-BMA)] and hydrophilic polymer blocks (polyethylene glycol [PEG]).[13,14] Mebiol Gel is characterized by its temperature-dependent dynamic visc oelastic properties.[15,16,17] The thermoresponsive blocks are hydrophilic at temperatures below the sol-gel transition temperature and are hydrophobic at temperatures above the sol-gel transition temperature. The hydrophobic interaction results in formation of a homogenous three-dimensional polymer network in water. The sol-gel transition temperature can be controlled by altering the chemical composition of NIPAAm-co-BMA and PEG. Cells or tissues can be embedded in liquid Mebiol Gel solution at lower temperatures and cultured three-dimensionally in a hydrogel state at 37^{o}C.

A number of new *in vitro* and *in vivo* applications of Mebiol Gel have been reported. For example, Mebiol Gel has been used for wound dressing,[18] microcapsules for pancreatic islets,[19] a drug delivery system,[20] and three-dimensional culture matrices for various cells.[21,22,23] Extracellular matrices, including collagen and Matrigel, have been used as scaffolds for clonal expansion of cells in three-dimensional culture. Materials from biological sources, however, cannot be absolutely free from contamination with unknown substances, including pathogens. Mebiol Gel is a purely synthesized biocompatible polymer that has no biological contaminants.

Biological effects of Mebiol Gel on culture cells:

Biological effects of Mebiol Gel have been extensively studied.[14-18] These studies demonstrated that Mebiol Gel was not toxic to cells but suppressed the growth of fibroblasts, which usually grow in an anchorage-dependent manner *in vitro*. Madhavan et al. reported that Mebiol Gel continuously supported the growth of animal cell lines without showing any toxicity.[22] In addition, Mebiol Gel is biologically inert. Hishikawa et al. reported that collagen gel by itself altered the gene expression profile of mesenchymal stem cells but that Mebiol Gel did not.[24]

JSRM/ 006010200003/Apr 05, 2010.

We compared the effect of Mebiol Gel with that of representative scaffolds, i.e., type I collagen and Matrigel.[25] The neuroblastoma cell line SH-SY5Y has been reported to differentiate into neuronal cells *in vitro*.[26] In type I collagen gel or in Matrigel, the cells showed an elongated shape and formed many processes (Figure 1C, 1D). In Mebiol Gel, however, the colonies that formed were completely round, and no interaction between cells and the matrix was indicated (Figure 1E, 1F). Our data indicated that Mebiol Gel is a suitable scaffold for three-dimensional culture of certain types of cells without unexpected interaction.

Figure 1

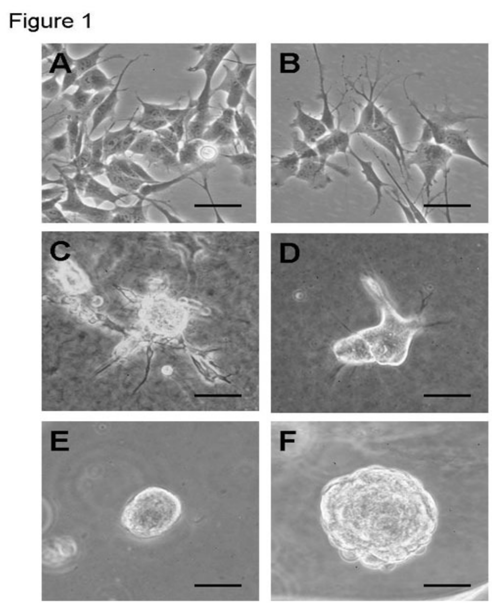

Figure 1: Effects of various scaffolds on cells in culture. SH-SY5Y neuroblastoma cells were cultured on an uncoated dish (A), on a type I collagen-coated dish (B), in a type I collagen gel at day 4 (C), in Matrigel at day 3 (D), and in Mebiol Gel at day 4 (E) and at day 7 (F). Bars, 50 ?m.

Clonal expansion of a single stem cell in Mebiol Gel:

Clonal expansion of stem cells from primary culture has great significance because it is the only way to determine the range of differentiation potential and self-renewing capacity of individual cells. In our previous study, we clonally propagated neural stem cells in Mebiol Gel. Neural stem cells formed single-cell-derived homogeneous spheroids.[11] We also succeeded in isolation of multipotent stem cells from mouse embryonic skin using Mebiol .[12]

Embryonic stem cells and induced pluripotent stem (iPS) cells were also shown to form spheroids in Mebiol Gel in a clonal manner. When mouse iPS cells (iPS-MEF-Ng-20D-17)[27] were inoculated in Mebiol Gel, spheroids were formed from single cells (Figure 2A). The spheroid-forming iPS cells stably expressed a stem cell marker, Nanog (Figure 2B).

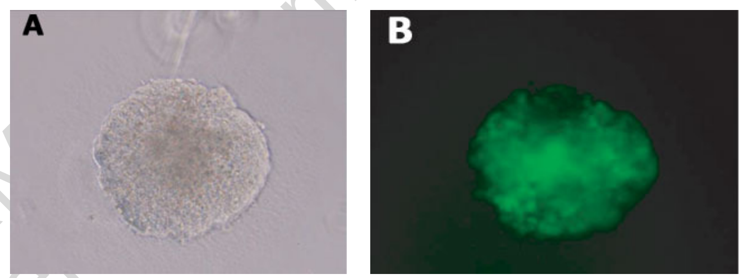

Figure 2

Figure 2. Spheroid formation of mouse induced pluripotent stem (iPS) cells in Mebiol Gel. Single iPS cells were cultivated in Mebiol Gel for 7 days (A). A stem cell marker, Nanog, was expressed in spheroid-forming cells (B). Bars, 100 mm.

These results indicate that Mebiol Gel is a potent tool for isolation and propagation of stem cells of a broad variety of origins.

Application of Mebiol Gel for regenerative medicine:

Mebiol Gel has been experimentally used as an artificial pancreas and wound dressing material in the treatment of skin defects and has been proved to be effective.[18, 19]

Nagaya et al. reported that liver regeneration was accelerated by Mebiol Gel treatment.28 When the surgical defect was covered with Mebiol Gel, fibrosis was suppressed and proliferation of hepatocytes was enhanced. In the process of regeneration, Mebiol Gel

JSRM/ 006010200003/Apr 05, 2010.

provoked marked neovascularization, which is a prerequisite for tissue regeneration. Furthermore, hepatic stem cells were activated during the liver regeneration process.[29]

Sudha et al. reported that human corneal limbal epithelial cells could be cultivated in Mebiol Gel and that the cells showed both limbal and corneal phenotype.[30] Sitalakshmi demonstrated that transplantation of limbal epithelial cells grown in Mebiol Gel could reconstruct the surgically damaged corneal epithelium in a rabbit model.[31] Since Mebiol Gel is a synthetic and biodegradable polymer, it rarely induces adverse immune response, contrary to future application of allogeneic biomaterials such as human amniotic membrane.

These observations demonstrated that Mebiol Gel provides suitable environments for tissue regeneration without adverse inflammatory responses.

Conclusion:

In conclusion, three-dimensional culture of stem cells using Mebiol Gel has the following advantages: (1) it enables clonal expansion of stem cells from single cells, (2) it is simple and easy without the need for a sophisticated apparatus such as a cell sorter, and (3) s ince Mebiol Gel is a purely synthesized copolymer, it is free from biological contamination. In addition, Mebiol Gel provides suitable environments for tissue regeneration *in vivo*. Thus, Mebiol Gel is suitable for preparation of cells for transplantation and is useful for direct application to promote regeneration of damaged tissues *in vivo* .

References:

1. Atala A. Engineering tissues, organs and cells. J Tissue Eng Regen Med. 2007; 1: 83-96.

2. Yu D, Silva GA. Stem cell sources and therapeutic approaches for central nervous system and neural retinal disorders. Neurosurg Focus. 2008; 24: E11

3. Polak JM, Mantalaris S. Stem cells bioprocessing: an important milestone to move regenerative medicine research into the clinical arena. Pediatr Res. 2008; 63: 461-466.

4. Chiarugi P. From anchorage dependent proliferation to survival: lessons from redox signalling. IUBMB Life. 2008; 60: 301-307.

5. Chiarugi P, Giannoni E. Anoikis: a necessary death program for anchorage-dependent cells. Biochem Pharmacol. 2008; 76: 1352-1364.

6. Thullberg M, Strömblad S. Anchorage-independent cytokinesis as part of oncogenic transformation? Cell Cycle. 200; 7:984-988.

7. Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J Neurosci. 1996; 16: 7599-7609.

8. Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev. 2003; 17: 1253-1270.

9. Dellatore SM, Garcia AS, Miller WM. Mimicking stem cell niches to increase stem cell expansion. Curr Opin Biotechnol. 2008; 19: 534-540.

10. Liu H, Collins SF, Suggs LJ. Three-dimensional culture for expansion and differentiation of mouse embryonic stem cells. Biomaterials. 2006; 27: 6004-6014.

11. Yang XZ, Kataoka K, Medina R, Yamamoto K, Than SS, Miyazaki M, Huh NH. A novel three-dimensional culture system for isolation and clonal propagation of neural stem cells using a thermo-reversible gelation polymer. Tissue Eng Part C. 2009; 15: 615-623.

12. Medina RJ, Kataoka K, Takaishi M, Miyazaki M, Huh NH. Isolation of epithelial stem cells from dermis by a three-dimensional culture system. J Cell Biochem. 2006; 98: 174-184.

13. Yoshioka H, Mikami M, Mori Y. Preparation of poly(N-Isopropylacrylamide)-b-poly(ethylene glycol) and calorimetric analysis of its aqueous solution. J Macromol Sci Pure Appl Chem. 1994; A31: 109-112.

14. Yoshioka H, Mikami M, Mori, Y. A synthetic hydrogel with thermoreversible gelation. I. Preparation and rheological properties. J Macromol Sci Pure Appl Chem. 1994; A31: 113-120.

15. Yoshioka H, Mikami M, Mori Y. A synthetic hydrogel with thermoreversible gelation. II. Effect of added salts. J Macromol Sci Pure Appl Chem. 1994; A31: 121-125.

JSRM/ 006010200003/Apr 05, 2010.

16. Yoshioka H, Mori Y. A synthetic hydrogel with thermoreversible gelation, III: an NMR study of the Sol-Gel transition. Polym Adv Technol. 1994; 5: 122-127.

17. Yoshioka H, Mori Y, Tsukikawa S, Kubata S. Thermoreversible gelation on cooling and on heating of an aqueous gelatin poly(N-isopropylacrylamide) conjugate. Polym Adv Technol. 1998; 8: 155-158.

18. Yoshioka H, Mori Y, Kubota S. Wound dressing of newly developed thermogelling thermoreversible hydrogel. Jpn J Artif Organs. 1998; 27: 503-506.

19. Shimizu S, Yamazaki M, Kubota S, Ozasa T, Moriya H, Kobayashi K, Mikami M, Mori Y, Yamaguchi S. In vitro studies on a new method for islet microencapsulation using a thermoreversible gelation polymer, N-isopropylacrylamide-based copolymer. Artif Organs. 1996; 20:1232-1237.

20. Arai T, Joki T, Akiyama M, Agawa M, Mori Y, Yoshioka H, Abe T. Novel drug delivery system using thermoreversible gelation polymer for malignant glioma. J Neurooncol. 2006; 77: 9-15.

21. Tsukikawa S, Matsuoka H, Kurahashi Y, Konno Y, Satoh K, Satoh R, Isogai A, Kimura K, Watanabe Y, Nakano S, Hayashi J, Kubota S. A new method to prepare multicellular spheroids in cancer cell lines using a thermo-reversible gelation polymer. Artif Organs. 2003; 27: 598-604.

22. Madhavan HN, Malathi J, Joseph JR, Yuichi M, Abraham JK, Yoshioka H. A study on the growth of continuous culture cell lines embedded in Mebiol gel. Curr Sci. 2004; 87: 1275-1277.

23. Yasuda A, Kojima K, Tinsley KW, Yoshioka H, Mori Y, Vacanti CA. In vitro culture of chondrocytes in a novel thermoreversible gelation polymer scaffold containing growth factors. Tissue Eng. 2006; 12: 1237-1245.

24. Hishikawa K, Miura S, Marumo T, Yoshioka H, Mori Y, Takato T, Fujita T. Gene expression profile of human mesenchymal stem cells during osteogenesis in three-dimensional thermoreversible gelation polymer. Biochem Biophys Res Commun. 2004; 317: 1103-1107.

25. Benton G, George J, Kleinman HK, Arnaoutova IP. Advancing science and technology via 3D culture on basement membrane matrix. J Cell Physiol. 2009; 221: 18-25.

26. Påhlman S, Mamaeva S, Meyerson G, Mattsson ME, Bjelfman C, Ortoft E, Hammerling U. Human neuroblastoma cells in culture: a model for neuronal cell differentiation and function. Acta Physiol Scand Suppl. 1990; 592: 25-37.

27. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature. 2007; 448: 313-317.

28. Nagaya M, Kubota S, Suzuki N, Tadokoro M, Akashi K. Evaluation of thermoreversible gelation polymer for regeneration of focal liver injury. Eur Surg Res. 2004; 36: 95-103.

29. Nagaya M, Kubota S, Suzuki N, Akashi K, Mitaka T. Thermoreversible gelation polymer induces the emergence of hepatic stem cells in the partially injured rat liver. Hepatology. 2006; 43: 1053-1062.

30. Sudha B, Madhavan HN, Sitalakshmi G, Malathi J, Krishnakumar S, Mori Y, Yoshioka H, Abraham S. Cultivation of human corneal limbal stem cells in Mebiol gel--A thermo-reversible gelation polymer. Indian J Med Res. 2006; 124: 655-664.

31. Sitalakshmi G, Sudha B, Madhavan HN, Vinay S, Krishnakumar S, Mori Y, Yoshioka H, Abraham S. Ex vivo cultivation of corneal limbal epithelial cells in a thermoreversible polymer (Mebiol Gel) and their transplantation in rabbits: an animal model. Tissue Eng Part A. 2009; 15: 407-415.

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