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Synthesis and evaluation of PEG hydrogel incoorporating two dimensionally dispersed cell spheroid.

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ABSTRACT

The cell spheroid having high differentiation ability is biologically interesting as a promissing technology for tissue engineering. In the present study, we've succeeded in controlling the size of cell spheroids which were two-dimensionally aligned on the micropatterned substrate. To use the spheroids for tissue engineering as an implantable construct, we designed the biocompatible hydrogels with photo-crosslinkable polymer, based on end-acrylated multi-armed poly(ethylene glycol). These gels' storage elastic modulus G' was determined from rheologycal test. As a result, the gels' modulus covered between 100 and 2500Pa by changing the different concentrations and types of macromers. The permeability of these hydrogels was determined using the two-chamber cells. In use of sodium benzoate as probe molecules, the probe passed through all the gels, while no clear effect in the different types of monomers was observed. After photoencapsulation of the chondrocyte spheroids, the multi-array formation of spheroids were maintained in the hydrogel. Biochemical analysis demonstrated that aggrecan increased for 1week on the hydrogels and the viability of the cell. Therefore, noble cell spheroid sheet was established using PEG hydrogel. These findings suggest the gel may have high utility as an implant multi-arrayed cell spheroids.

1. INTRODUCTION

The cell spheroids have been recently expected to be used in tissue engineering because of their high functionality and long term viability. In our laboratory, we succeeded in fabricating a micro fabricated PEG-Brush surface which could control the size and pattern of spheroids [1].However the multi-arrayed spheloids were constructed on only hard materials such as glass and cell culture dish.

In this study, we tried to design the PEG hydrogel to implant the spheroid into the body and characterized the mechanical properties and permeability of PEG hydro-gel

2. MATERIAL AND METHOD

PEG Hydrogel Preparation. The end-acrylated poly (ethylene glycol)(PEG) which were ~MW,20000 in the form of a chain or 3,4, and 8 arms(Fig.1) were respectively dissolved and well mixed in 1ml of the photoinitiator solution (0.1%IRUGACURE 2959 in milliQ water) at 2.5, 5.0, 10, 20% w/v. The resulting solution was subsequently crosslinked in cylindrical molds with UV lamp(254nm, 3mW/cm^2, 3min, UV Cross Linker, FUNA).

Rheologycal test. The hydrogels were investigated on a strain-controlled rheometer(ARES, Rheometrics Scientific) with parallel plate geometry. Data for torque, normal force, and torsional displacement were recorded by data acquisition soft-ware(RSI Orchestrator, Rheometrics Scientific). The top platen was lowered to contact the sample which was wiped to put off extra water until 0.05N was applied.The dynamic time sweep was perfomed at 1Hz and 1% for 3min. The storage(G') and loss(G') moduli were determined frome the average data. The Rheologycal test were all performed with n=3.

Permeation experiment. Permeation experiment was performed using a two-chamber cell system. The each chambers were filled with 200ml of PBS and the system

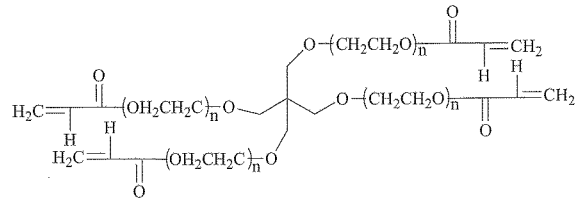


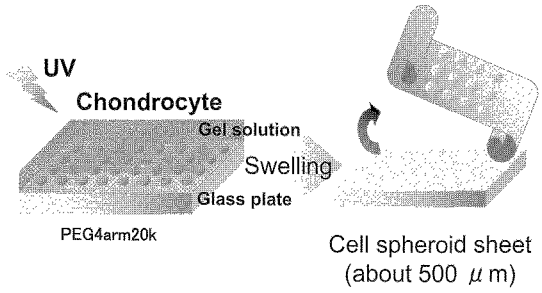
Fig.1 The molecular frame of PEG 4arms

was kept at 37℃ in constant-temperature bath. To fix the soft gel between two chamber, the gels were immobilized on the gel-bond film, which was EVAL film®(kuraray) silane coupled by 3-(Trimethoxysilyl)propyl methacrylate, 98%(ALDRICH).To immobilize the gel on the film, the solution was crosslinked with UV with the film under the cylindrical molds[2].

The predetermined amount (40mg) of concentrated solution of sodium benzoate(MW,144.1, Kanto Chemical) was poured into donor cell. Then the concentration of the sodium benzoate was continuously measured by UV absorbance using pump and photodiode array.

Transferring and encapsulating cell spheroids. Multi-arrayed cell spheroids were prepared as described elsewhere[1]. In brief, the cells were cultured on the glass which has patterned surface of hydrophilic and hydrophobic by using PEG polymer and photolithography. In second process, PEG 4arms MW,20000 and Irugacure 2959 as photoinitiator were dissolved in PBS to form 10% and 0.1%, respectively, and then the solution were passed through the 0.22μm sterilizing filter. The cytocompatibility of photoinitiator is confirmed previously[3]. After dipping the solution on the glass on which cell spheroids were, cell spheroids were encapsulated usinf a long–wave ultraviolet lamp(365nm,As one) at an intensity of ~1mW/cm^2 for 10min. The cell-polymer constructs were cultured for 1days in medium and peeled from glass (Scheme).Swollen gel encapsulate the cell spheroids and cut off the adhesion between the gel and the glass.

Morphology and histology of chondrocyte spheroids on the gel. The cell morphology have been observed for 1weeks using microscope. Histological characterization and organization of the chondrocyte were evaluated micros-copically on upper surface that were stained with Safranin-O[4].



Scheme. encapsulation of spheroids with gel

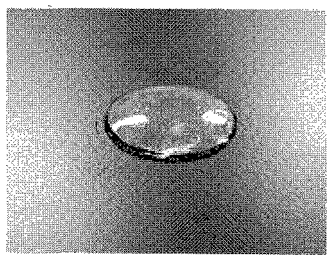
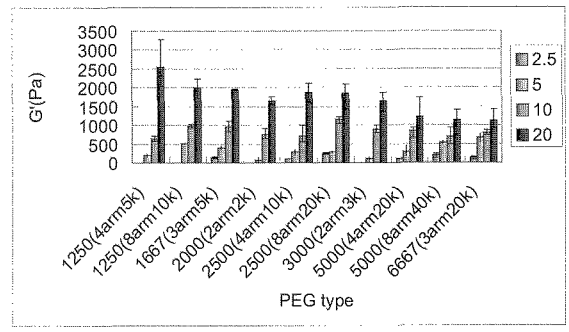


Fig.1 The PEG hydrogel



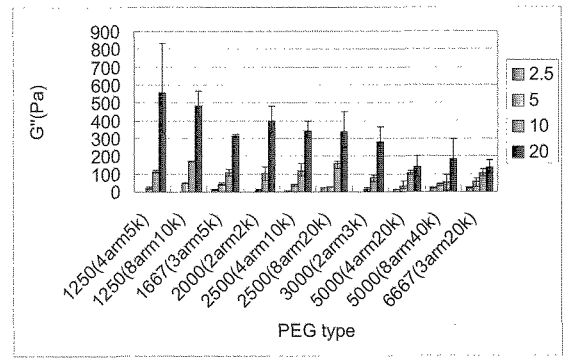


Fig.2 Storage modulus G',and loss modulus G" for the crosslinked hydrogel samples at 4 different concentration of monomer

Cell viability (live/dead assay). After 1week of culture on the glass and 1week of culture on the gel, the cell viability of chondrocyte in the hydrogel was assessed with Live/ Dead Viability/Cytotoxicity kit(Funakoshi), based on simultaneous determination of living and dead cells with two probes, calcein AM for intracellular esterase actibity and propidium lodide(PI) for plasma-membrane integrity. Briefly, the cultured cells on the gel were washed with PBS, and the working solution(2 μl of calcein AM and 3 μl of PI in 1ml PBS) was added directly to these cells. After 15min incubation at 37℃, the stained cells were observed under a fluorescence microscope.

3. RESULT AND DISSCUSSION

Hydrogel Synthesis. Monomer concentration from 2.5% to 20%w/v were confirmed to crosslink uniformly within the molds(Fig.1). All were homogeneous and transparent gels.

PEG-based hydrogels are of particular interest because of their biocompatibility, hydrophilicity, and ability to be customized by varying chain length or chemically adding biological molecules[5]. Moreover, by conjugating collagen

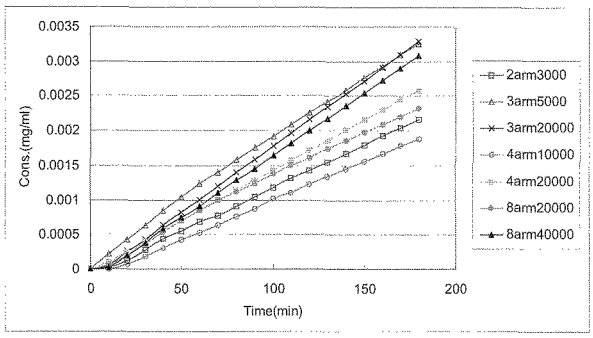


Fig.3 The elution time course of the sodium benzoate through 7 types PEG hydrogels made in 20% concentration.

and hyaluronic acid, chemically or non-chemically, adhesion properties for cell and mechanical, biochemical, physiological properties of hydrogels could be simply changed[6]. So this hydrogel have capability for being applied to spheroid scaffold.

Mechanical Properties. The storage modulus G' of the hydrogels showed concentration and type of monomer dependence, increasing from 100 to 2500Pa, and the loss modulus G'' from 10 to 550Pa(Fig.2).The reason why the storage modulus G' and the loss modulus G'' of PEG which had short arms was higher than those of PEG which had long arms seems to be due to crosslink density. For example, because each chain of PEG 4arms MW,5000 was shorter than that of PEG 3arms MW,20000, the crosslink density of PEG 4arms MW,5000 was higher than PEG 3arms MW,20000. Therefore, the modulus of PEG 4arm MW,5000 was higher than PEG 3arm MW,20000 ($p<0.05$,$n=3$).

Tissue cells usually respond to the stiffness of their substrate, and then they changed their morphology and migration[7]. There was a report that chondrocyte encapsulated within the relatively firm hydrogel (360 kPa) generated more ECM than soft hydrogel or too firm hydrogel[8]. These indicate that controlling the gel's mechanical properties by changing the concentration and type of monomer control the cell's property. From this result, the stiffness of the hydrogel could be controlled by changing the different concentration and type of monomer and respond to different cells type.

Diffusive permeability. Fig.3 shows the elution time course of the sodium benzoate. As a result, probe molecule could pass through each hydrogels for 180min.In this case using sodium benzoate as probe molecule, no clear effect in defferent types of monomers was observed.

Mesh sizes of PEGDM(MW,3400) reported by S.J.Bryant and K.S.Anseth[8] are from 140 to 40 Å, depending on macromer concentrations. In this study, the mesh size of PEG hydrogels isn't totally different from those values. Because the sodium benzoate as the probe molecule was small enough to pass through all gels, no clear effect of the gel type of monomer was observed.

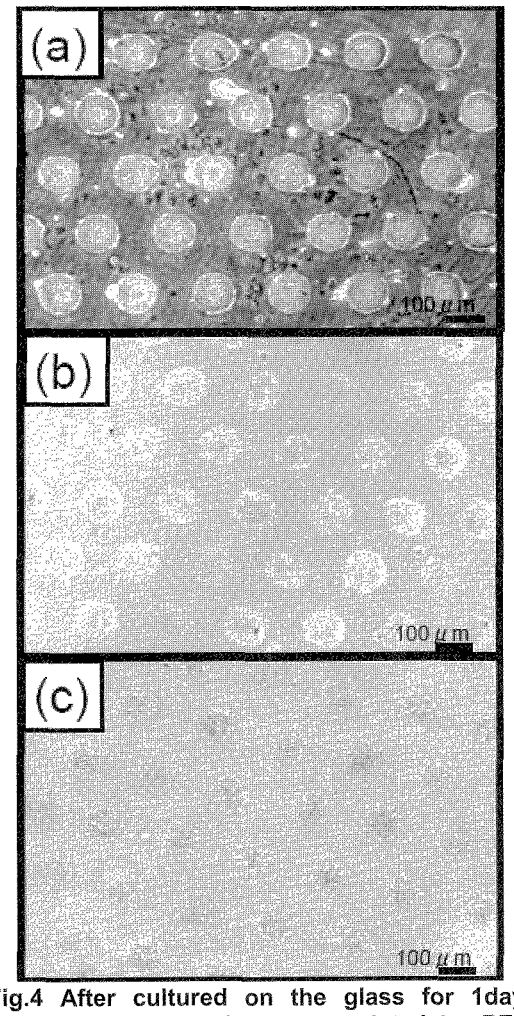


Fig.4 After cultured on the glass for 1day, chondrocyte spheroids encapsulated by PEG hydrogel. (a)the spheroids on the encapsulated day, (b)after cultured on the gel for 3days, and(c)for 1week

From the view point of the cell metabolism, gel's permeability is very important factor as scaffold. From this experiment, it was indicated that the substance having low molecular like oxygen and glucose could pass through this gel.

Cell spheroid encapsulation in PEG hydrogel. Cell spheroids were transferred from the glass to PEG4arms MW,20000 10% hydrogel. From the result of temporal culture, the cell spheroids kept the array formation for 1week.

The spheroids size is very important for cell function because the cells in the center of too large spheroid die due to those necrosis[1]. In addition, multi-array formation of spheroids shows higher performance than cell monolayer in per unit area. As a result, it suggests that this scheme

suggests the ability of cell spheroids sheet with highly differentiated function.

Histology. After the cells were cultured on the glass for 1week and then on the gel for 1week, respectively, safranin-O staining demonstrated an extracellular matrix consisting mainly of glycosaminoglycans(Fig.5). These results suggestthat the cell spheroids encapsulated with PEG hydrogel keep high differentiation ability without loss of cell function due to the transformation.

Live/dead cell assay for viability of chondrocyte spheroids encapsulated within PEG hydrogel. Fluorescence microscope image was studied using photoencapsulated chondrocyte spheroid in PEG4arms MW,20000 10% and cutured both on the glass for 1week and on the gel for 1 week. This result shows that the viability of many cells(green) composing spheroid can be confirmed. Though there are some dead cells(red), in comparison with spheroids without encapsulation in hydrogel, some cells in the center of cell spheroid become necrosis due to shortage of nutrient(no data).

Because scheme was very dynamically method to collect the cell spheroids, there is fear that PEG hydrogel damage the cell adhering on the glass. Therefore, it was important to confirm the cells viability encapsulated with PEG hydrogels. From the result, many cells viability was comfirmed. Therefore, to transfer the cell to PEG hydrogel, this scheme has high utility to implant multi-arrayed cell spheroid.

4. CONCLUSION

In conclusion, the hydrogel formed by photo-crosslinkable polymer, based on end-methacrylated multi-armed poly(ethylene glycol) was successfully prepared. By changing the concentration and type of monomer, we succeeded in controlling the mechanical properties. This indicate that we can control the cell's property with this gel. In the case using sodium benzoate as probe molecule, probe molecule could pass through each hydrogels, however, no clear effect of the different concentration and type of monomer was observed. To use hydrogel swelling behavior, the cell spheroids on the glass were transferred to PEG hydrogel. Moreover the cell's functionality and viability were confirmed by safranin-O and Live/Dead staining. These findings suggest the PEG hydrogel may have high utility to implant multi-arrayed cell spheroids and become noble cell sheet.

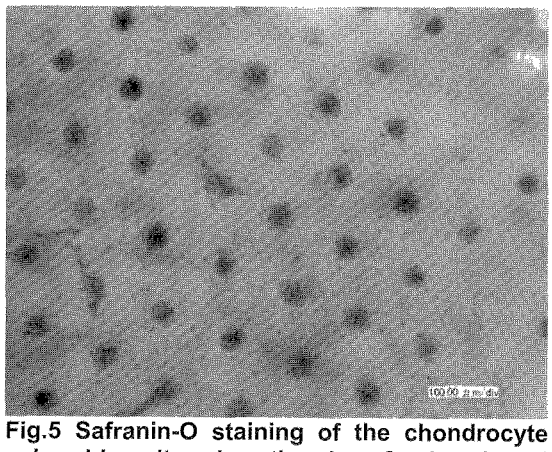


Fig.5 Safranin-O staining of the chondrocyte spheroids cultured on the glass for 1week and on the gel for 1week.

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