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Thermo-Sensitive PLGA-PEG-PLGA Tri-Block Copolymer Hydrogel as Three-Dimensional Cell Culture Matrix for Ovarian Cancer Cells

Naizhen Zhou[1], Ke Hu[2], Zhaobin Guo[1], Qiying Zhang[1], Junsong Chen[1], Tianzhu Zhang[1,3,*], and Ning Gu[1,3,*]

[1] *State Key Laboratory of Bioelectronics, Jiangsu Key Laboratory for Biomaterials and Devices, National Demonstration Center for Experimental Biomedical Engineering Education, School of Biological Sciences and Medical Engineering, Southeast University, Nanjing 210096, China*
[2] *Key Lab of Clinical and Medical Engineering, Department of Biomedical Engineering, School of Basic Medical Sciences, Nanjing Medical University, Nanjing 211166, China*
[3] *Collaborative Innovation Center of Suzhou Nano-Science and Technology, Suzhou Key Laboratory of Biomaterials and Technologies, Suzhou 215123, China*

Thermo-sensitive hydrogels which could encapsulate cells and provide a three dimensional (3D) microenvironment have great potential in building new cell culture models *in vitro*. In this study, a thermal responsive hydrogel based on PLGA-PEG-PLGA tri-block copolymers was developed as matrix for 3D ovarian cancer cell culturing. The gelation of PLGA-PEG-PLGA tri-block copolymer was concentration-dependent. SEM images showed the pores were suitable for the formation of 3D cell structures. Cell morphological results showed that large aggregates of ovarian cancer cells (HO8910) were formed after cultured for 10 days. Therefore, hydrogel based on PLGA-PEG-PLGA tri-block copolymers hold potential as *in vitro* cell culture matrix for ovarian cancer cells.

Keywords: Thermo-Sensitive Hydrogel, PLGA-PEG-PLGA, 3D Cell Culture, Ovarian Cancer Cell.

1. INTRODUCTION

Despite the three-dimensional structure of tissues *in vivo*, the researches on the structures, functions and pathology of human tissues frequently relies on the two-dimensional (2D) model *in vitro* and animal model. Since the structure of monolayer *in vitro* model is quite different from the cell microenvironment *in vivo*, cell behaviors and functions, such as cell–cell interaction and cell-matrix interaction, are greatly affected. Moreover, animal model often fail to repeat the human characteristic because of species differences. Three-dimensional (3D) culture of tumor cell lines has been advocated as the alternative.[1–4] It is simple and practicable and has the advantage of simulating the cell microenvironment *in vivo*.

Matrices for 3D cell culture mimic one or more properties of the extracellular matrix (ECM) and tumor microenvironment *in vivo*.[1] The 3D cell culturing matrices are generally composed of porous structures with diameter less than 300 nm, which can provide enough space for the growth of cells. The cancer cells can form 3D aggregates or spheroids inside the matrix. According to the main component, 3D cell culture matrices can be divided into two main categories: matrix based on natural materials and matrix based on synthetic materials. Matrices based on natural materials can provide a biological environment, but the mechanical performance of materials is commonly poor and the batch-to-batch discrepancy cannot be completely eliminated. Natural materials are usually used to form hydrogel composites.[5,6] Synthetic scaffolds are polymers like Polyethylene glycol (PEG), Polylactide (PLA), Poly(lactide-co-glycolide) (PLGA/PLG) which are biocompatible, biodegradable and easy to reproduce.[7–9] Among these materials, the thermogelling synthetic copolymer hydrogels with a sol–gel transition exhibit lower critical solution temperature (LCST) behavior, which is meaningful for a 3D cell culturing matrix. When the sol–gel transition temperature of smart

*Authors to whom correspondence should be addressed.

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hydrogel is between 5 °C and 37 °C, the matrix has advantages in further separation of materials and cell aggregates. Thermo-sensitive hydrogel based on PLGA-PEG-PLGA tri-block copolymers has been used for delivery of proteins and water-insoluble drugs.[10] The proper LCST and good biocompatibility of PLGA-PEG-PLGA tri-block copolymers make it a good choice for *in vitro* cell culture matrix.

In the current study, the thermogelling hydrogel based on PLGA-PEG-PLGA tri-block copolymer was developed and applied as cell culture matrix. The ovarian cancer cell (HO8910) was cultured inside the hydrogel with porous structures. The results of cell morphological characterizations proved that this smart hydrogel provided a proper microenvironment for the proliferation of ovarian cancer cells.

2. MATERIALS AND METHODS

2.1. Materials

PLGA-PEG-PLGA tri-block copolymer was purchased from Shandong Daigang Bio-tech Co. Ltd. Ovarian cancer cell line (HO8910) was obtained from Shanghai Cellular Institute of China Scientific Academy. DMEM culture medium and fetal bovine serum (FBS) were obtained from Gibco. Penicilin and streptomycin were obtained from Nanjing Simcere.

2.2. Characterization of PLGA-PEG-PLGA Tri-Block Copolymers

A Bruker DMX300 spectrometer was used for ^{1}H NMR measurements in $CDCl_3$ to determine the chemical structure and composition of the PLGA-PEG-PLGA tri-block copolymers. The molecular weights of copolymers and their molecular weight distributions were determined using a Waters 515 Gel Permeation Chromatography (GPC) apparatus with Wyatt Technology Optilab rEX refractive index as a detector. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1.0 mL/min at 35 °C, and polystyrene standards were used as the calibration sample. The lower critical solution temperature of PLGA-PEG-PLGA was characterized with an ultraviolet spectrophotometer (Shimadzu UV-3600, Japan), and the sol–gel transition temperature was characterized with inverted tube test.

PLGA-PEG-PLGA tri-block copolymer was dispersed in OD water with the assistance of ultrasonic, and the pH of this sol was adjusted to 7.0 with 1 M NaOH. The sol was heated with water bath and formed hydrogel inside a frozen storage tube. After pre-freezing with liquid nitrogen, the hydrogel was freeze-dried and the porous structures of hydrogel were characterized with SEM.

2.3. *In Vitro* Cell Culture on PLGA-PEG-PLGA Tri-Block Copolymer Scaffold

2 mL of PLGA-PEG-PLGA tri-block copolymer sol was sterilized with vacuum high-pressure steam sterilization (120 °C, 2 h). 60 μL of PLGA-PEG-PLGA tri-block copolymer solution was added in every well of 96-well plates with sterile burette by manual pipetting on ice. 100 μL of cell dispersion containing 5% PLGA-PEG-PLGA tri-block copolymers solution was added in every well to make the total cell score (TCS) to reach about 3,000. Then the plates were inoculated in the incubator for 60 minutes for the formation of hydrogel. The cultures were maintained in an incubator at 37 °C with a humidified atmosphere of 5% CO_2. The medium was changed every 2–3 days. The cell morphology was characterized with a BX63 Olympus microscope.

3. RESULTS AND DISCUSSION

3.1. Thermo-Sensitivity and Morphology of PLGA-PEG-PLGA Tri-Block Copolymers

PLGA-PEG-PLGA tri-block copolymer is a thermo-sensitive polymer. At the temperature below its lower critical solution temperature, it is hydrophilic and soluble in water. However, it collapses and precipitates after heating above its LCST. Under appropriate conditions, it forms a hydrogel with the induction of thermo.[11] Unlike LCST, the sol–gel transition temperature changes with many factors, especially the concentration of tri-block copolymers in water.[12, 13] Characterized with ultraviolet spectrophotometer, the LCST of PLGA-PEG-PLGA tri-block copolymer was 26.2 °C. The sol–gel transition temperature of the

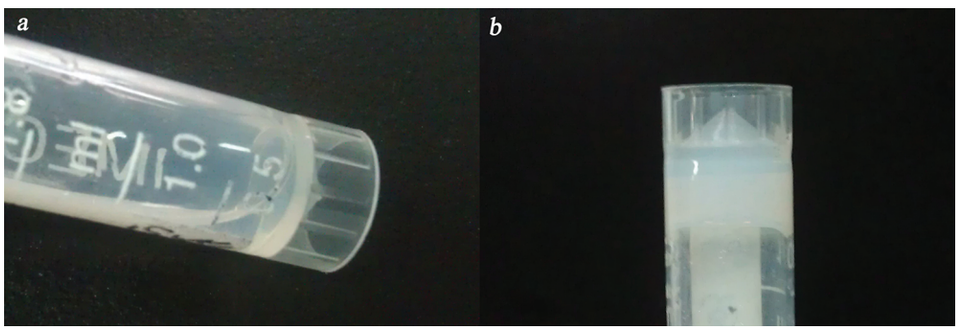

Figure 1. Photographs of sol (a)–gel (b) transition of PLGA-PEG-PLGA tri-block copolymers hydrogel (30 wt%).

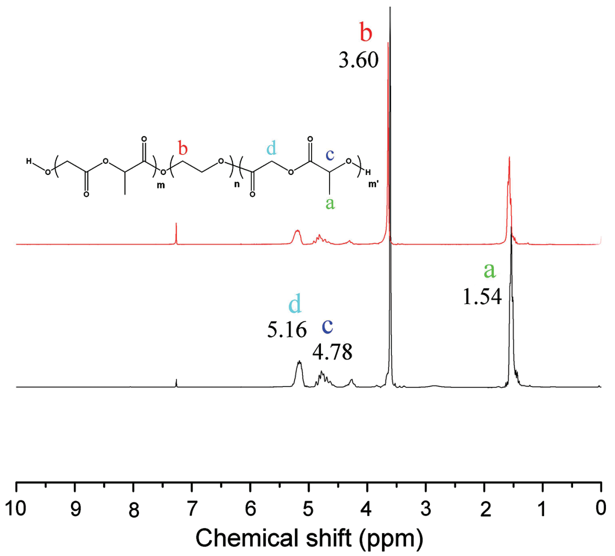

Figure 2. [1]H-NMR spectra (300 MHz, $CDCl_3$) of PLGA-PEG-PLGA tri-block copolymer before (black) and after (red) sterilization.

30 wt% PLGA-PEG-PLGA triblock copolymer solution was measured via the test tube inverting method with a temperature increment of 0.5 °C per step.[14] However, the sol–gel transition was observed until the concentration of PLGA-PEG-PLGA tri-block copolymer raised to 30 wt% (Fig. 1). When heated to 37 °C, the tri-block copolymer still maintained the gel state, indicating that this thermo-sensitive hydrogel was capable of being matrix for cell culture at the incubation temperature.

The [1]H NMR and the GPC spectra of the PLGA-PEG-PLGA tri-block copolymer were shown in Figures 2 and 3. The structure of PLGA-PEG-PLGA tri-block copolymer was of no difference before and after sterilization. Compared with the molecular weight (Mw) of PLGA-PEG-PLGA tri-block copolymer before sterilization, which was 6400, the Mw after sterilization was decreased slightly with the value of 4700. This might be due to the degradation of tri-block copolymer caused by sterilization. When encapsulated inside the hydrogel, cells need enough space to grow and form 3D structures. SEM images of freeze-dried PLGA-PEG-PLGA tri-block copolymer hydrogel showed the diameter of pores was about 200~300 μm which was largely enough for cells to proliferate and aggregate (Fig. 4).

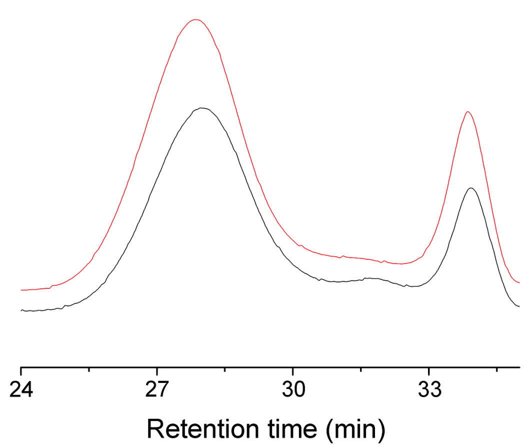

Figure 3. GPC spectra of PLGA-PEG-PLGA tri-block copolymer before (red) and after (black) sterilization.

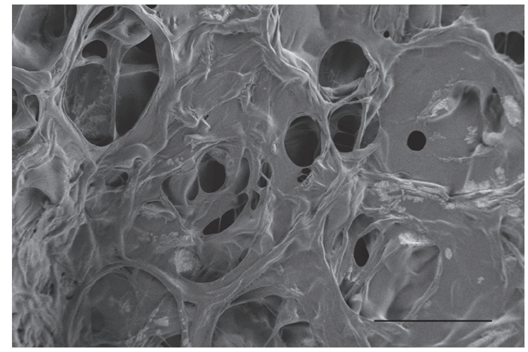

Figure 4. SEM images of PLGA-PEG-PLGA tri-block copolymer hydrogel (freeze-dried). Scale bar = 100 μm.

3.2. Cellular Study on PLGA-PEG-PLGA Tri-Block Copolymer Scaffold

Different from the complex demands of the equipment for tissue and organ culture in bioengineering, this PLGA-PEG-PLGA tri-block copolymer based matrix is simple, efficient, low cost, and accords with the demands of cancer cell culturing in 3D. Due to the good hydrophilicity of PEG chain segment, the interface of PLGA-PEG-PLGA tri-block copolymer hydrogel was hydrophilic and cells could not adhere on the hydrogel. Based on this anti-cell adhesive interfacial property, cells could not form cell-matrix interaction and were forced to form cell–cell interaction and aggregates. The cell morphological results obtained with optical microscope showed that 10 days after inoculation, the ovarian cancer cells continued to grow and form large multicellular aggregates (Fig. 5),

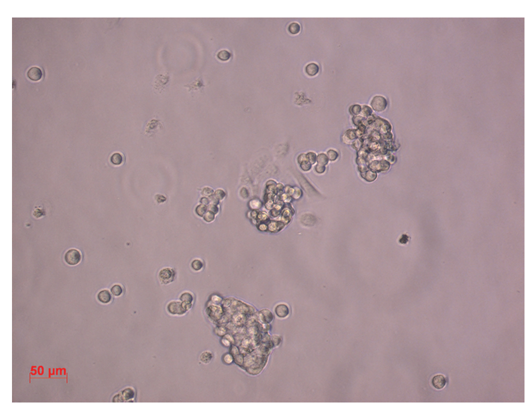

Figure 5. Optical microscope images of HO8910 cells cultured on PLGA-PEG-PLGA tri-block copolymer hydrogel after 10 days. Scale bar = 100 μm.

which was similar with results when HO8910 cell cultured in widely used commercial materials Matrigel. We believe this thermo-sensitive tri-block copolymer hydrogel has potential in building novel *in vitro* cell model.

4. CONCLUSIONS

In summary, the ovarian cancer cells (HO8910) were successfully cultured inside the thermo-sensitive hydrogel based on PLGA-PEG-PLGA tri-block copolymers. The SEM results showed that porous structures with proper size of this hydrogel were suitable for cell to grow and form 3D structures. Cell morphological results showed ovarian cancer cells grew into large cell aggregates after 10 days. More comparative tests for this thermo-sensitive hydrogel on ovarian cancer cells 3D culturing will be carried out in further.

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