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The controllable preparation of porous PLGA microspheres by the oil/water emulsion method and its application in 3D culture of ovarian cancer cells

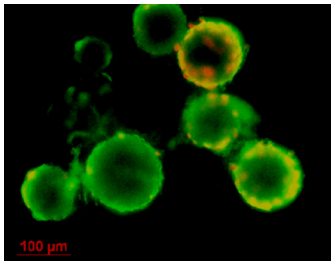
Tianzhu Zhang[a,b,*], Qiying Zhang[a,b], Junsong Chen[c], Kun Fang[a], Jun Dou[c], Ning Gu[a,b,*]

[a] Jiangsu Key Laboratory for Biomaterials and Devices, State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Sipailou 2, Nanjing 210096, China
[b] Suzhou Key Lab of Biomedical Materials and Technology, Research Institute of Southeast University in Suzhou, Ren Ai Road 150, Suzhou Industrial Park, Suzhou 215123, China
[c] Department of Pathogenic Biology and Immunology, Medical School, Southeast University, Dingjiaqiao 87, Nanjing 210009, China

HIGHLIGHTS

- Ovarian cancer cell line HO8910 can better grow on PLGA microspheres.
- Collagen I coated PLGA microsphere promoted the adhesion and growth of HO8910 cells.
- HO8910 cells grew on PLGA microspheres increased expression of E-cadherin.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we prepared surface-smooth and porous poly(lactic-*co*-glycolic acid) (PLGA) microspheres with different diameters, different sizes of pores and different densities of pores using a simple oil/water emulsion method and varying the preparation conditions, including the molecular weight of PLGA, the components of the PLGA and the addition of porogen. The surface-smooth nonporous and porous PLGA microspheres were also further modified with collagen I. The characteristics of these PLGA microspheres, including original and collagen I-coated PLGA microspheres, were evaluated in a three-dimensional (3D) culture of ovarian cancer HO8910 cells. The HO8910 cells can growth better on original porous and collagen I-coated PLGA microspheres, and express remarkably E-cadherin. These results indicate that porous PLGA microspheres and collagen-coated PLGA microspheres are promising candidates as ovarian cancer cell culture microcarriers for pathological study and high-throughput antitumor drug screening.

1. Introduction

Poly(lactic-*co*-glycolic acid) (PLGA) is a kind of biodegradable and biocompatible synthetic polymer. It is used in numerous applications in biomedical fields [1–3]. Porous PLGA microspheres (beads or microparticles) are widely used as drug-releasing carriers [4–7], ultrasound contrast agents [8] or three-dimensional (3D) cell culture scaffolds, especially for tissue engineering or

* Corresponding authors at: Jiangsu Key Laboratory for Biomaterials and Devices, State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Sipailou 2, Nanjing 210096, China.
Tel.: +86 025 83272476; fax: +86 025 83272460.
E-mail addresses: zhangtianzhu@seu.edu.cn, zhangtianzhuglq@sina.com (T. Zhang), guning@seu.edu.cn (N. Gu).

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regenerative medicine [9,10] and tumor research [11,12]. Depending on their purpose, different methods have been tried for the fabrication of porous PLGA microspheres. Of these, the water/oil/water ($W_1/O/W_2$) multiple emulsion solvent evaporation technique and the oil-in-water (O/W) single emulsion solvent evaporation technique are frequently used methods [13–15].

To accommodate most types of mammalian cells, the size of the pores in PLGA microparticles has usually been designed to be larger than 20 μm [16]. Chung et al. prepared highly open porous PLGA microcarriers using the $W_1/O/W_2$ double-emulsion method [17]. By finely tuning gas foaming conditions, they obtained porous PLGA microcarriers with a mean size of approximate 175 μm and an average pore diameter of approximate 29 μm for cell cultivation and injectable delivery for cartilage tissue engineering. Using a fluidic device, Xia et al. prepared uniform PLGA/gelatin beads with controllable pore sizes for NIH-3T3 fibroblast cell culture [18]. Their results indicated that beads with large pores can lead to high cell viability. Besides their application in normal cells culture, PLGA microspheres also are increasingly employed in 3D cultures of tumor cells, a promising technique for in vitro pathological and physiological study of cancer cells [19,20]. For example, PLGA/PLA microspheres were used to culture breast cancer MCF-7 cells to check the antidrug capability of cancer cells [21,22]. To better promote the adhesion and proliferation of cancer cells, the morphology of PLGA microspheres usually needs to be finely designed. It is extremely important to tailor the morphologies and surface components of PLGA microspheres to the specific application.

Epithelial ovarian cancer is often an aggressive disease, and 3D culture studies aiming at understanding the metastasis and multidrug resistance of ovarian cancer are becoming increasingly important and challenging because of the underlying complex mechanism [23–28]. So far, to our knowledge, PLGA microspheres have not been used in 3D cultures of ovarian cancer cells. Using a single O/W emulsion method and adjusting the molecular weight (M_η) of the PLGA and the ratio of LA:GA in PLGA, we prepared PLGA microspheres with small pores of approximately 3–5 μm, and then performed preliminary 3D culture of ovarian cancer HO8910 cells with these porous PLGA microspheres as carriers.

2. Materials and methods

2.1. Materials

PLGA (M_η = 20 K, 50 K; LA:GA = 50:50, 65:35, 75:25) was purchased from Jinan Daigang Biomaterial Co., Ltd. For the convenience, six different PLGA samples is denoted as $PLGA_{20K}$ (50:50), $PLGA_{20K}$ (65:35), $PLGA_{20K}$ (75:25), $PLGA_{50K}$ (50:50), $PLGA_{50K}$ (65:35), and $PLGA_{50K}$ (75:25), respectively, where the subscription is M_η of PLGA and the value in the parentheses after PLGA is the ratio of LA:GA. 2-Methylpentane (d = 0.6530 g/mL) and polyvinyl alcohol (PVA) ($M_w \approx$ 31 K, 86.7–88.7 mol% hydrolysis) were purchased from Sigma–Aldrich chemical company. Dichloromethane (AR) was acquired from Sinopharm Chemical Reagent Shanghai Co., Ltd. The human ovarian cancer cell line HO8910 was obtained from the cell bank of the Chinese Academy of Sciences in Shanghai. Collagen I gel was purchased from Trevigen Inc. Other reagents are commercially available.

2.2. Fabrication of porous PLGA microspheres

A total of 0.3000 g PLGA and 2-methylpentane (0.2 mL or 0.0 mL) was first dissolved in 3.0 mL of dichloromethane to obtain an oil phase. With a syringe equipped with a 21G needle, the PLGA solution in CH_2Cl_2 was added drop wise into 100.0 mL of aqueous solution containing 1 wt% of PVA with a mechanical stirring speed of 500 rpm. The volume ratio (V/V) of water to CH_2Cl_2 was 10:1. At a constant stirring speed, the CH_2Cl_2 in the oil phase was evaporated slowly at room temperature for 6 h. The microspheres obtained were then washed with distilled water three times and collected by centrifugation at 5000 rpm for 10 min. The final product was dried under vacuum at 40 °C for 24 h.

2.3. Surface aminolysis and collagen I coating of the PLGA microspheres

The surface aminolysis and subsequent collagen I coating of the PLGA microspheres were carried out according to previously reported methods [29,30]. Briefly, the PLGA microspheres were first immersed in an excess of 6% (V/V) hexanediamine/*n*-propanol solution at room temperature, with stirring for 8 min. The residuary solution was then removed through filtrating, and the surface-aminolysed PLGA microspheres obtained were extensively washed with deionised water. To transfer $–NH_2$ groups into –CHO groups, the aminolysed PLGA microspheres were again immersed in 1% glutaraldehyde solution at room temperature for 4 h. After washing fully, the microspheres were immersed in 50 μg/L of collagen I in a 3% acetic acid solution at 4 °C for 24 h. After filtering and washing fully, collagen I-coated PLGA microspheres were obtained.

2.4. Scanning electron microscopy (SEM) analysis of the morphology of the PLGA microspheres

The morphology and the pore size of the PLGA microspheres were examined using a scanning electron microscope (SEM) (Zeiss Ultra Plus, Germany). The optical images were used to measure the diameter of PLGA microspheres.

2.5. Water contact angle measurement

The sessile drop method was used for contact angle measurements at 20 ± 1.5 °C using a commercial contact angle meter (CAM 200, KSV Instruments Ltd., Finland) [31–33]. Ultrapure water droplets (10 μL) were placed at six different positions on one flat PLGA film, and then the average value was obtained. The standard deviation of contact angle was ±1°. The fabrication of flat PLGA films was carried out according to the reported method [29,34].

2.6. HO8910 cell culture with the PLGA microspheres (3D on-top assay)

The 3D on-top assay was employed as the culture method [19]. Cell dispersion with a concentration of 5×10^4 cells/mL was first obtained. A given weight of the original PLAG microspheres (control sample, without any treatment) and the collagen I-coated microspheres was sterilized by 75% ethanol solution and UV irradiation. After washing with PBS (pH = 7.4), 20 mg of the microsphere dispersion were added into each well of a 24-well culture plate and incubated at 37 °C for 30 min to obtain a uniform PLGA microsphere layer. Then, 100 μL of HO8910 cell suspensions were added slowly on the microsphere layer, followed by the subsequent addition of culture medium consisting of Dulbecco's Modified Eagle Medium, 10% heated inactivated fetal bovine serum (FBS), 1% penicillin and streptomycin admixture. The 24-well culture plate was maintained in an incubator at 37 °C with a humidified atmosphere of 5% CO_2. The culture medium was replaced every 2 d.

2.7. Cell viability detected by MTT assay

After the HO8910 cells were cultured for 7 d, a cell viability assay was carried out. When the microspheres were suspended uniformly in the medium via pipette blowing, 30 μL of the suspension

(containing 6 mg of microspheres) were taken out and placed into a 96-well culture plate, Then, 10 μL of CCK-8 solution were added in each well, the cells were continually incubated for another 4 h, and the absorbance at 450 nm was recorded under a microplate reader (168-100D, Bio-Rad 550).

2.8. Cell distribution and morphologies

After the HO8910 cells were cultured for 7 d, the culture medium was removed with a pipette, and then washed with PBS twice. The fluorescein diacetate (FDA)–propidium iodide (PI) solution (10 μL 100 μg/mL FDA and 10 μL PI/well) was added and incubated for 10 min in the dark. A fluorescence microscope (Axioskop40, Zeiss, Germany) with an activated laser wavelength of 488 nm and 535 nm was used to directly observe viable and dead cells. After culturing for 7 d, the culture medium was removed with a pipette, and the microspheres were washed with PBS three times. Then, the cells were fixed for 2 h with 2.5% glutaraldehyde at 4 °C. To dehydrate the cells, they were sequentially treated with a series of ethanol solution (30%, 50%, 75%, 90% and 100% each time for 15 min at room temperature). Finally, drying was performed under vacuum, and the cells were observed with SEM (Zeiss Ultra Plus, Germany).

2.9. Analysis of E-Cadherin expression

After the HO8910 cells were cultured for 7 d, the culture medium was removed with a pipette and washed with PBS twice. Then, 100 μL of 3.9% formaldehyde were added and incubated for 10 min to fix cells. Cells were washed with PBS twice, and 100 μL of 0.1% Triton-100 were added and incubated for 20 min to increase the permeability of the plasma membrane to the antibodies. After washing with PBS twice, 50 μL of 1:10 dilution E-cadherin antibody solution were added into the plate and incubated for 60 min, followed by washing with PBS twice. Then, 100 μL of Hoechst 33342 (Invitrogen) were added and incubated for 15 min in the dark at room temperature.

3. Results

3.1. Preparation of nonporous microspheres and porous microspheres

First, $PLGA_{20K}$ (65:35) was used, and no porogen 2-methylpentane was added. As a result, surface-smooth PLGA microspheres were formed, on which no pores were found. The average diameter of the $PLGA_{20K}$ (65:35) was 176 μm. The optical image, SEM image and size distribution are shown in Fig. 1. The addition of porogen 2-methylpentane leads to the formation of porous PLGA microspheres (Fig. 2).

The two main factors that exert a strong influence on the morphologies of porous PLGA microspheres, the components of PLGA and the M_η of PLGA, were investigated here. Influence of the ratio of LA:GA on the size and the density of pores in the PLGA microspheres were firstly studied. When 0.2 mL of 2-methylpentane was added, porous microspheres were subsequently formed. There are three kinds of $PLGA_{20K}$: $PLGA_{20K}$ (50:50), $PLGA_{20K}$ (65:35) and $PLGA_{20K}$ (75:25). When 0.2 mL of porogen 2-methylpentane was added to 3 mL of the oil phase, porous microsphere were clearly visible after the evaporation of the oil phase (Fig. 2A–C). When the ratio of LA:GA was 50:50, 65:35 and 75:25, the pore size was 3.4 μm, 5.1 μm, and 4.6 μm, respectively, and the pore density (the number of pores per 100 μm^2) was 1.72, 1.91, and 2.47, respectively. Therefore, in $PLGA_{20K}$, increasing the LA component not only resulted in bigger pores but also a higher pore density. The M_η of PLGA also can influence the size and the density of the pores in the PLGA microspheres. Under the same preparation conditions, when the M_η of PLGA was changed from 20 K to 50 K, that is to say, $PLGA_{50K}$ was used to replace $PLGA_{20K}$, both the pore size and the pore density of the $PLGA_{50K}$ microspheres were smaller and lower than that of $PLGA_{20K}$. When the LA component in $PLGA_{50K}$ was increased, the pore size and the pore density in the $PLGA_{50K}$ microspheres decreased on the contrary. When the ratio of LA:GA was 50:50, 65:35 and 75:25, the size of the pores was 4.4 μm, 3.9 μm, and 3.2 μm, respectively, and the pore density was 1.66, 0.88 and 0.72, respectively (Fig. 2D–F). Besides the influence from PLGA itself, the amount of porogen also can influence the size and density of pore in microspheres, the more amount of porogen will lead to bigger size and higher density of pores. In our case, the amount of porogen is fixed, so the influence of amount of porogen can be excluded here. The size and the density of the pores of the $PLGA_{20K}$ and $PLGA_{50K}$ microspheres are summarized in Table 1.

3.2. Surface modification of $PLGA_{20K}$ (65:35) microspheres with collagen I

For the nonporous $PLGA_{20K}$ (65:35) microspheres, after the aminolysis, the smooth surface became rougher, and further coating with collagen I did not lead to any obvious change in the morphologies (Fig. 3A_1, B_1, C_1). However, for the porous $PLGA_{20K}$ (65:35) microspheres, the aminolysis completely destroyed the surface structure and uncovered the inner porous structure of the microspheres. Similarly, further coating with collagen I did not lead to the complete recovery of the smooth surface (Fig. 3A_2, B_2, C_2). The reason probably is the insufficient amount of collagen I being grafted on the $PLGA_{20K}$ (65:35) microspheres. However, as discussed in Section 3.3, when the HO8910 cells were cultured on the surface-smooth or porous $PLGA_{20K}$ (65:35) microspheres, a tiny amount of collagen I grafted on the $PLGA_{20K}$ (65:35) microspheres led to rather different culture effects on these cells.

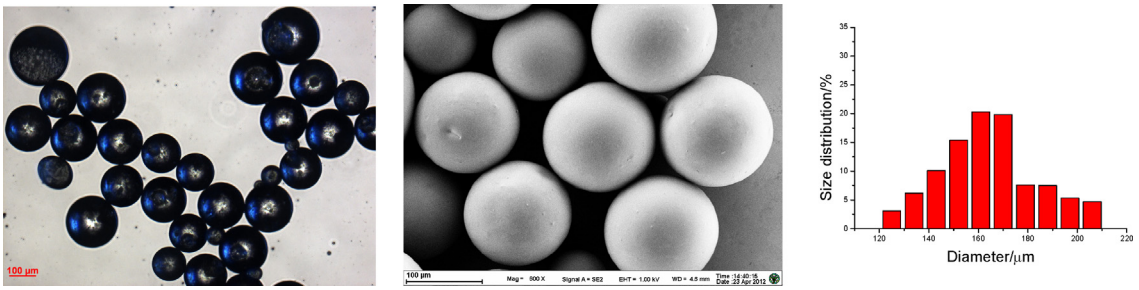
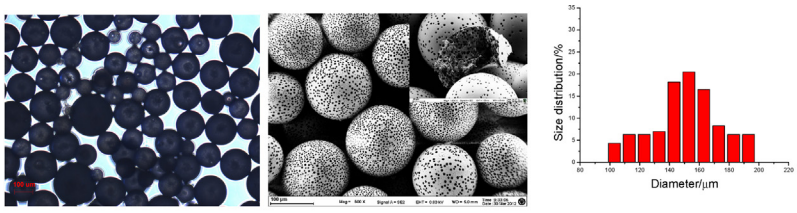
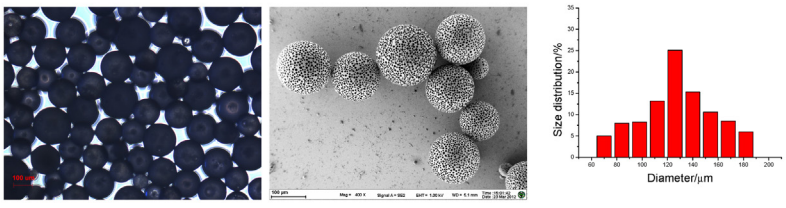


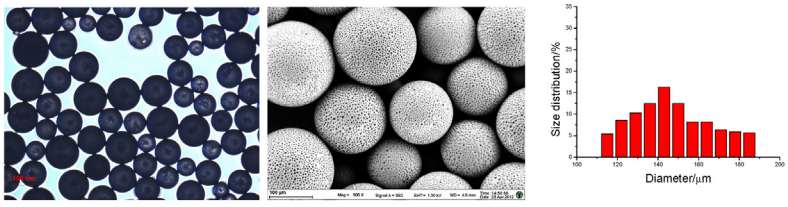
Fig. 1. The optical images, SEM images, and size distribution diagram of nonporous $PLGA_{20K}$ (65:35) microspheres (from the left to the right: optical image, SEM image, and size distribution diagram).



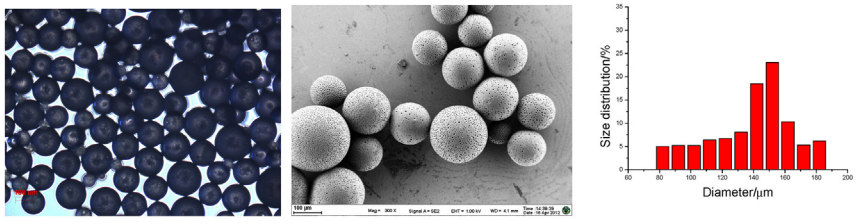
A. $PLGA_{20K}$ (LA:GA = 50:50)



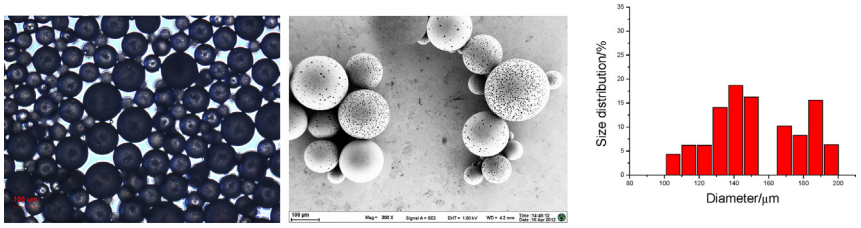
B. $PLGA_{20K}$ (LA:GA = 65:35)



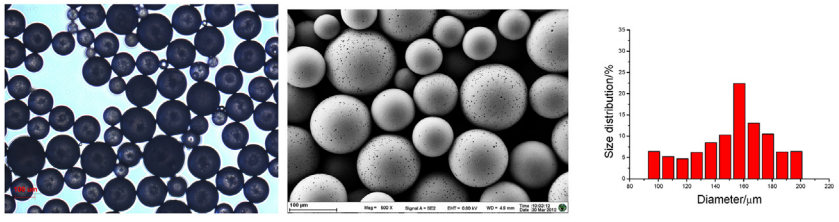
C. $PLGA_{20K}$ (LA:GA = 75:25)



D. $PLGA_{50K}$ (LA:GA = 50:50)



E. $PLGA_{50K}$ (LA:GA = 65:35)



F. $PLGA_{50K}$ (LA:GA = 75:25)

Fig. 2. The influence of the ratio of LA:GA on the morphologies of porous $PLGA_{20K}$ (LA:GA = 50:50, 65:35, 75:25) microspheres (mean diameter: 143 μm; 137 μm; 151 μm) and porous $PLGA_{50K}$ (LA:GA = 50:50, 65:35, 75:25) microspheres (mean diameter: 156 μm; 147 μm; 132 μm) (from the left to the right: optical image, SEM image, and size distribution diagram).

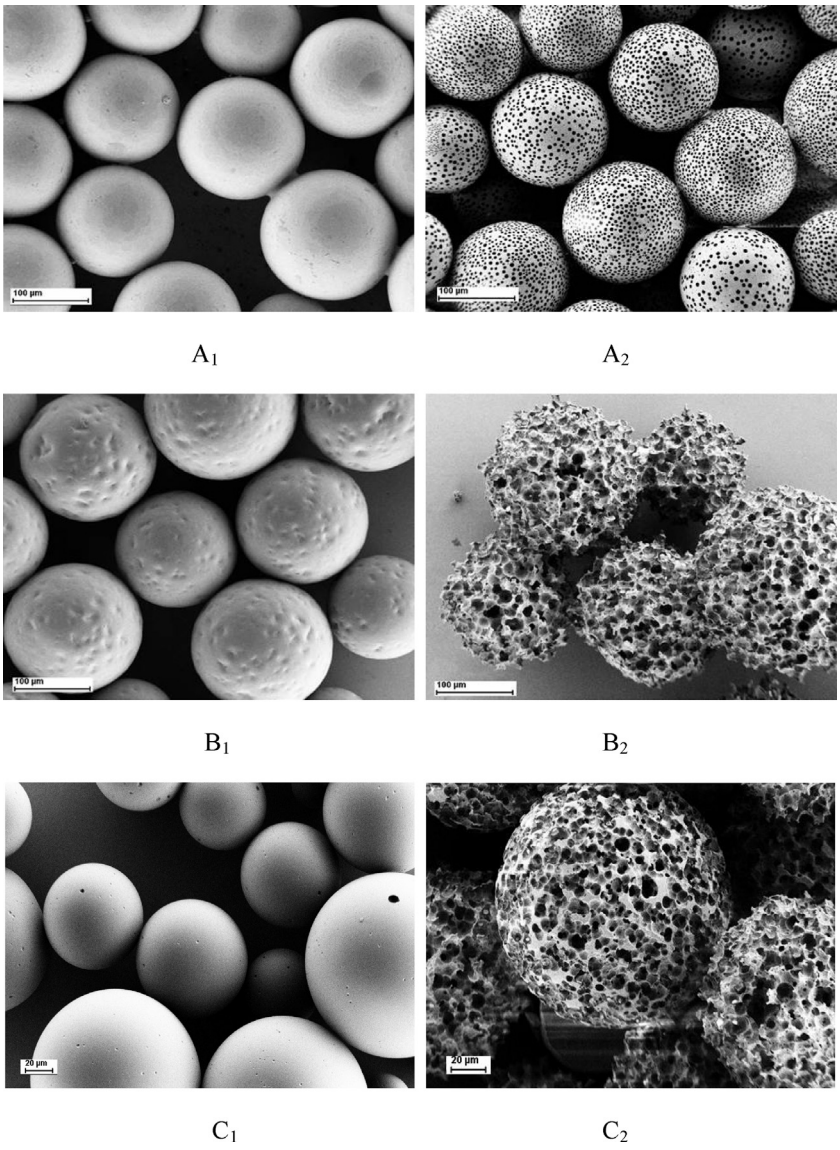


Fig. 3. The SEM images of original (A_1, A_2), aminolyzed (B_1, B_2), and collagen-coated (C_1, C_2) $PLGA_{20K}$ (65:35) microspheres. Nonporous microspheres (A_1, B_1, C_1) and porous microspheres (A_2, B_2, C_2). Scale bars are 100 μm in A and B; 20 μm in C.

3.3. Proliferation of HO8910 cells on the PLGA microspheres

During a 7 days culture period, the adhesion and the proliferation of the HO8910 cells were investigated. Fig. 4 presents optical images of the HO8910 cells cultured on the surface-smooth and porous $PLGA_{20K}$ (65:35) microspheres and the comparative adhesion of the cells. For the original $PLGA_{20K}$ (65:35) microspheres, there was no obvious adhesion of the cells to the surface-smooth microspheres (Fig. 4A_1), whereas there was visible adhesion of the cells to the porous microspheres (Fig. 4B_1). For the collagen I-coated $PLGA_{20K}$ (65:35) microspheres, the adhesion of the cells to both the surface-smooth and porous microspheres was significantly greater than that of the original microspheres. In particular, as shown in Fig. 4A_2 and B_2, the PLGA microspheres were enveloped in the HO8910 cells, and the HO8910 cells were clearly adhered on the edge of the microspheres.

Table 1
The mean pore size and the mean pore density on the microspheres with the different molecular weight and composites.

LA:GA	Mean pore size (μm)		Mean pore density[a]	
	$PLGA_{20K}$	$PLGA_{50K}$	$PLGA_{20K}$	$PLGA_{50K}$
50:50	3.4	4.4	1.72	1.66
65:35	5.1	3.9	1.91	0.88
75:25	4.6	3.2	2.49	0.72

Preparation conditions: oil:water is 10%; the volume of 2-methylpentane is 0.2 mL. The stirring speed is 500 rpm

[a] Unit is number/100 μm^2.

Fig. 5 shows the SEM images of the $PLGA_{20K}$ (65:35) microspheres covered with the HO8910 cells. In Fig. 5A_1 and B_1, only a few HO8910 cells were adhered on the original $PLGA_{20K}$ (65:35) microspheres, whereas the adhesion of tumor cells to the porous $PLGA_{20K}$ (65:35) microspheres was slightly better than that on the surface smooth $PLGA_{20K}$ (65:35) microspheres. The collagen I-coated $PLGA_{20K}$ (65:35) microspheres were almost completely covered with HO8910 cells (Fig. 5A_2 and B_2), showing the ability of collagen I to enhance the adhesion of the cells.

For further comparison of the proliferation of the HO8910 cells on the $PLGA_{20K}$ (65:35) microspheres, the number of

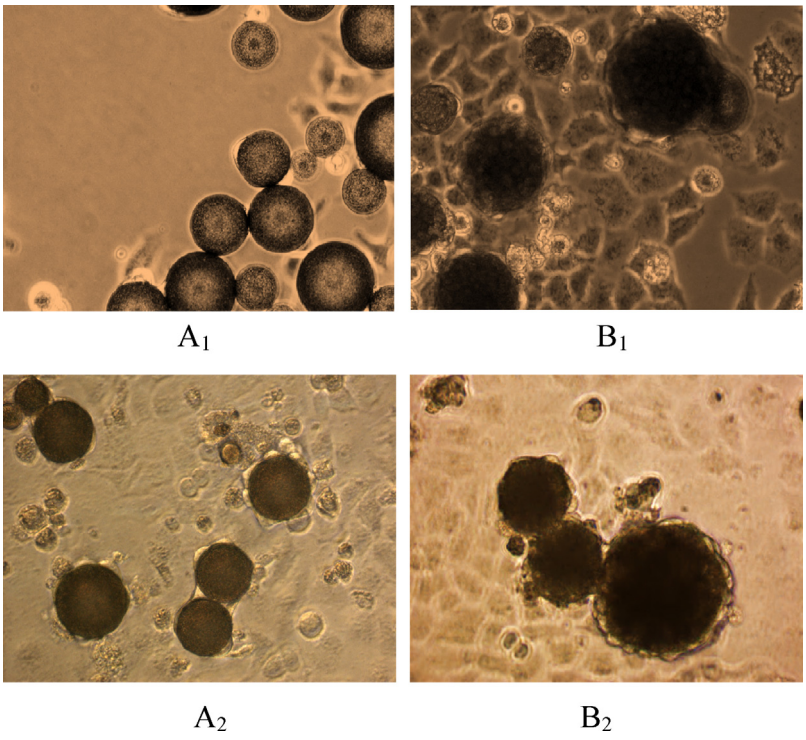


Fig. 4. The optical images of HO8910 cells cultured on smooth nonporous or porous $PLGA_{20K}$ (65:35) microspheres (culture conditions: 12-well plates, 30,000 cells/well, 1 mg microspheres/well). The culture period is 7 days. (A_1) Original nonporous PLGA microspheres; (B_1) original porous PLGA microspheres; (A_2) collagen I coated nonporous PLGA microspheres; (B_2) collagen I coated PLGA microspheres (100×).

cells adhered on the microspheres at the first day, third day and seventh day was examined quantitatively. Fig. 6A shows the kinetics of the growth of the HO8910 cells on the PLGA microspheres.

Groups 1, 2, 3 and 4 in Fig. 6 represent the original smooth $PLGA_{20K}$ (65:35) microspheres, the porous $PLGA_{20K}$ (65:35) microspheres, the collagen I-coated smooth $PLGA_{20K}$ (65:35) microspheres and the collagen I-coated porous $PLGA_{20K}$ (65:35)

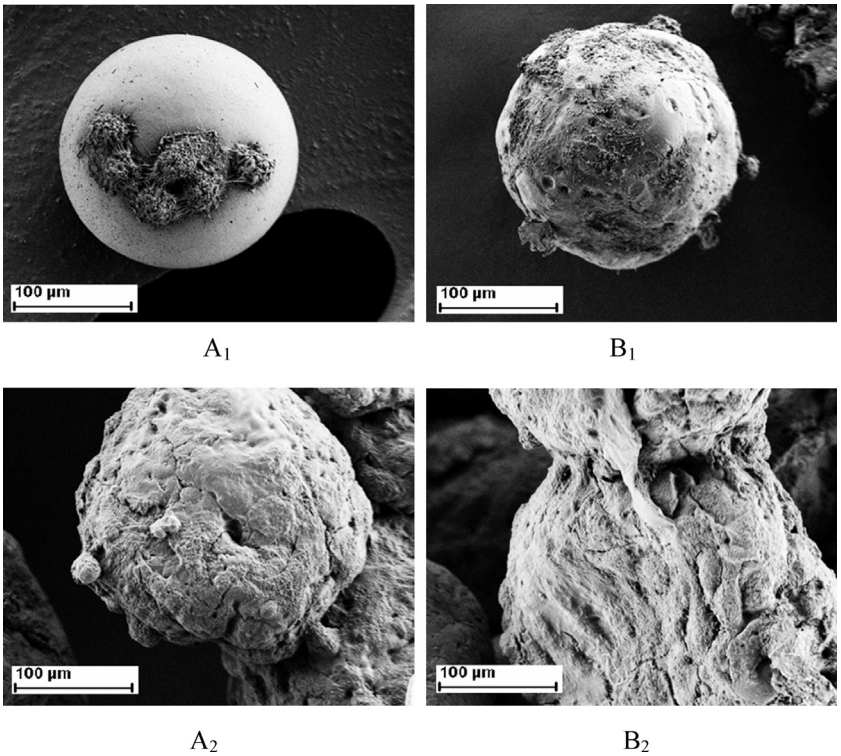


Fig. 5. SEM images of $PLGA_{20K}$ (65:35) microspheres covered with HO8910 cells. The culture period is 7 days. (A_1) Original nonporous PLGA microsphere; (B_1) original porous PLGA microspheres; (A_2) collagen I coated nonporous PLGA microspheres; (B_2) collagen I coated porous PLGA microspheres.

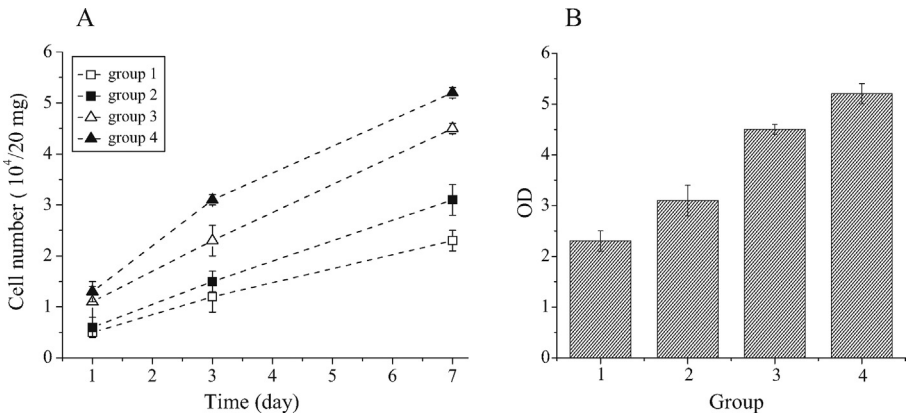


Fig. 6. The proliferation of HO8910 cells on $PLGA_{20K}$ (65:35) microspheres. (A) The number of HO8910 cell increases with the culture time on the different PLGA microsphere (group 1, original nonporous PLGA microspheres; group 2, original porous PLGA microspheres; group 3, collagen I coated nonporous PLGA microspheres; group 4, collagen I coated porous PLGA microspheres). (B) OD values at the seventh day.

microspheres, respectively. For group 1 and group 2, the number of cells on the porous microspheres was larger than that on the smooth microspheres. For group 3 and group 4, the number of cells adhered on the collagen I-coated porous microspheres was remarkably larger than that on the smooth $PLGA_{20K}$ (65:35) microspheres. Fig. 6B shows the optical density (OD) values at 450 nm at the seventh day. On the whole, the number of cells adhered on the collagen I-coated porous PLGA microspheres was greater than that on the collagen I-coated nonporous PLGA microspheres.

In Fig. 7, the green area is the viable cells stained with FDA, and the red area is the dead cells stained with PI. For group 1 and group 2, there was no obvious difference in either the fluorescent intensity or the distribution of the cells. A similar situation was found for group 3 and group 4, with no obvious difference in either the fluorescent intensity or the distribution of the cells. However, when the original microspheres and the collagen I-coated microspheres were compared in group 1 and group 3 and in group 2 and group 4, it can be found that the collagen I grafted on the nonporous microspheres and the porous microspheres promoted the proliferation of HO8910.

3.4. *E-cadherin expression of HO8910 cells on the PLGA microspheres*

Fig. 8 shows the comparison of the E-cadherin expression of the HO8910 cells cultured on the different PLGA microspheres. In group 1 and group 2, the fluorescent intensity of E-cadherin was lower because of the lower number of the adhered cells. After the PLGA spheres were coated with collagen I, the fluorescent intensity of E-cadherin was obviously increased in group 3 and group 4 compared with that in group 1 and group 2. At the same time, due to the absence of the quantitative fluorescent intensity in Fig. 8, so we only can make a qualitative comparison, instead of quantitative comparison, in Fig. 8. Fig. 9 shows the results of a control experiment, namely in 2D culture, where no E-cadherin expression was found.

4. Discussion

To obtain different kinds of PLGA microspheres, we systematically optimized the preparation conditions. The results indicated that the ratio of LA:GA in the PLGA and the M_η of the PLGA microspheres remarkably influence the formation of pores in the microspheres, including the size of the pores and the pore density.

For $PLGA_{20K}$ and $PLGA_{50K}$, the hydrophobicity, characterized by the water contact angle shown in Table 2, increased with the ratio of LA to GA. For $PLGA_{20K}$, when LA:GA was 50:50, 65:35 and 75:25, the water contact angle was 83°, 83°, and 84°, respectively. The case is similar for $PLGA_{50K}$. The ratio of LA to GA in the PLGA dictates their hydrophobicity and correspondingly influences their solubility capability in CH_2Cl_2. When the LA component was increased, the solubility capability in CH_2Cl_2 were enhanced, namely, PLGA with higher LA component can be more easily solubilized in hydrophobic CH_2Cl_2.

Table 2
The relation of molecular weight and the ratio of LA:GA of PLGA with the water contact angle.

LA:GA	Water contact angle (°)	
	$PLGA_{20K}$	$PLGA_{50K}$
50:50	83	72
65:35	83	74
75:25	84	88

The M_η of PLGA partially reflects its mechanical strength. During the formation of porous microspheres, M_η of PLGA has a significant effect on the formation of pores on the microspheres. For $PLGA_{20K}$, both mean pore size and pore density increase with the LA component. However, for $PLGA_{50K}$, the contrary tendency was found, namely, both mean pore size and pore density decrease with the increasing LA component. On the whole, the pore size and the density of the pores on the PLGA microspheres prepared with a lower M_η are larger due to its weaker mechanical strength, because the porogen can occupy the bigger space in PLGA with weaker mechanical strength.

According to the results of the statistical analysis, $PLGA_{20K}$ is more apt to form porous microspheres than $PLGA_{50K}$. Therefore, nonporous and porous $PLGA_{20K}$ (65:35) microspheres were chosen as carriers in the 3D culture of the HO8910 cell line. Although PLGA microspheres are not transparent, their excellent cytocompatibility means that they are ideal 3D cell culture carriers, both for tissue engineering and for tumor studies.

For the original $PLGA_{20K}$ (65:35) microspheres, according to the optical images, the porous structure can lead to more HO8910 cells adhering on the microspheres compared with the nonporous structure (Fig. 4A_1 and B_1). One possible reason is that the tumor cells have more pesudopods, which enables the tumor cells to adhere to the porous microsphere surface. Under the same condition, the smooth microspheres were not very favorable for the adhesion of tumor cells.

The collagen I is a main component of ECM, and collagen I include RGD sequence which can remarkably promote the adhesion of cancer cells, it was widely utilized in cell culture [35]. After

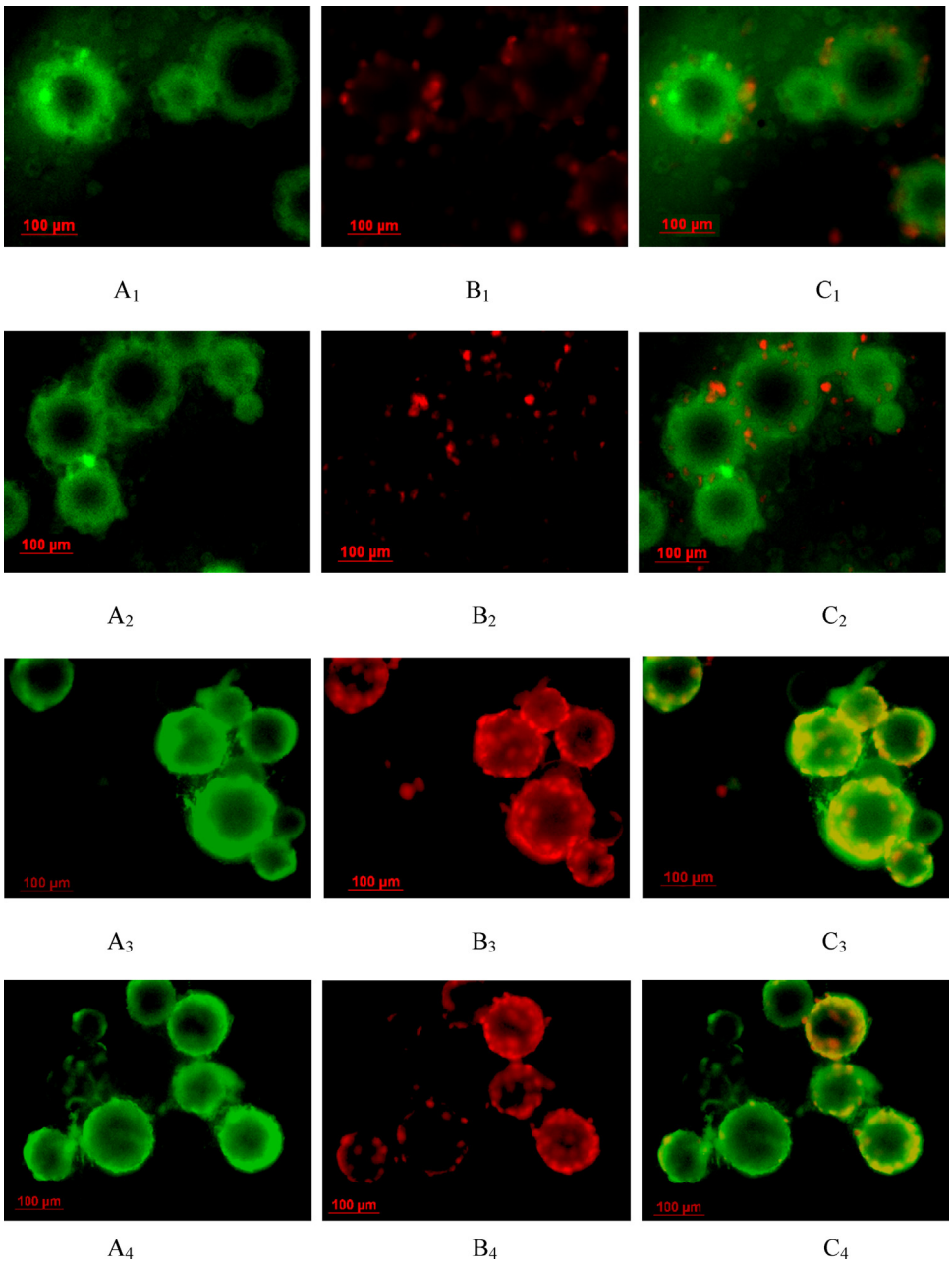


Fig. 7. The fluorescent images of HO8910 cells cultured on smooth nonporous and porous $PLGA_{20K}$ (65:35) microspheres stained with FDA and PI (culture conditions:12-well plates 30,000 cells/well, 1 mg microspheres/well). The culture period is 7 days. (Row 1) Original smooth nonporous PLGA microspheres; (Row 2) original smooth porous PLGA microspheres; (Row 3) collagen I coated smooth nonporous PLGA microspheres; (Row 4) collagen I coated smooth porous PLGA microspheres. (A) Stained with FDA; (B) stained with PI; (C) overlap of A and B.

coating with collagen I, the adhesion of the HO8910 cells on either the smooth or the porous $PLGA_{20K}$ (65:35) microspheres was obviously enhanced (Fig. 4A_2 and B_2). The adhesion difference in the HO8910 cells on the PLGA microspheres was further verified by the SEM observations. As integrin on the membrane surface of cancer cells can effectively combine with the RGD peptide sequence of collagen I, the adhesion difference in the HO8910 cells caused by the different morphologies of the PLGA microspheres was not found in the collagen I-coated PLGA microspheres, because the PLGA microspheres were almost completely covered by the HO8910 cells, both in the collagen I-coated smooth PLGA microspheres and in the collagen I-coated porous PLGA microspheres.

The expression of epithelial E-cadherin in epithelial tissues has been well studied [36,37]. Generally, the epithelial E-cadherin can generate strong cell cohesion, while loss of epithelial E-cadherin and increase of N-cadherin means partial or incomplete epithelial-mesenchymal transition (EMT). For example, in hepatoma cell lines HepG2, the compact spheroid also exhibited a six-fold increase in E-cadherin expression above that of monolayer cells [38].

In our experiment, it is worth noting that HO8910 cells did not express E-cadherin when cultured in the 2D model. In Fig. 9, through staining with Hoechst, the nucleus of viable cells was clearly displayed, but no obvious E-cadherin expression was found. The 3D culture with the PLGA microspheres promoted the expression of E-cadherin and consequent formation of HO8910 aggregates. The possible reason is that, in 2D model, cells are platode, and lost the contact with other cells and ECM, therefore 2D culture limited the express of cytoskeletal actin E-cadherin [39].

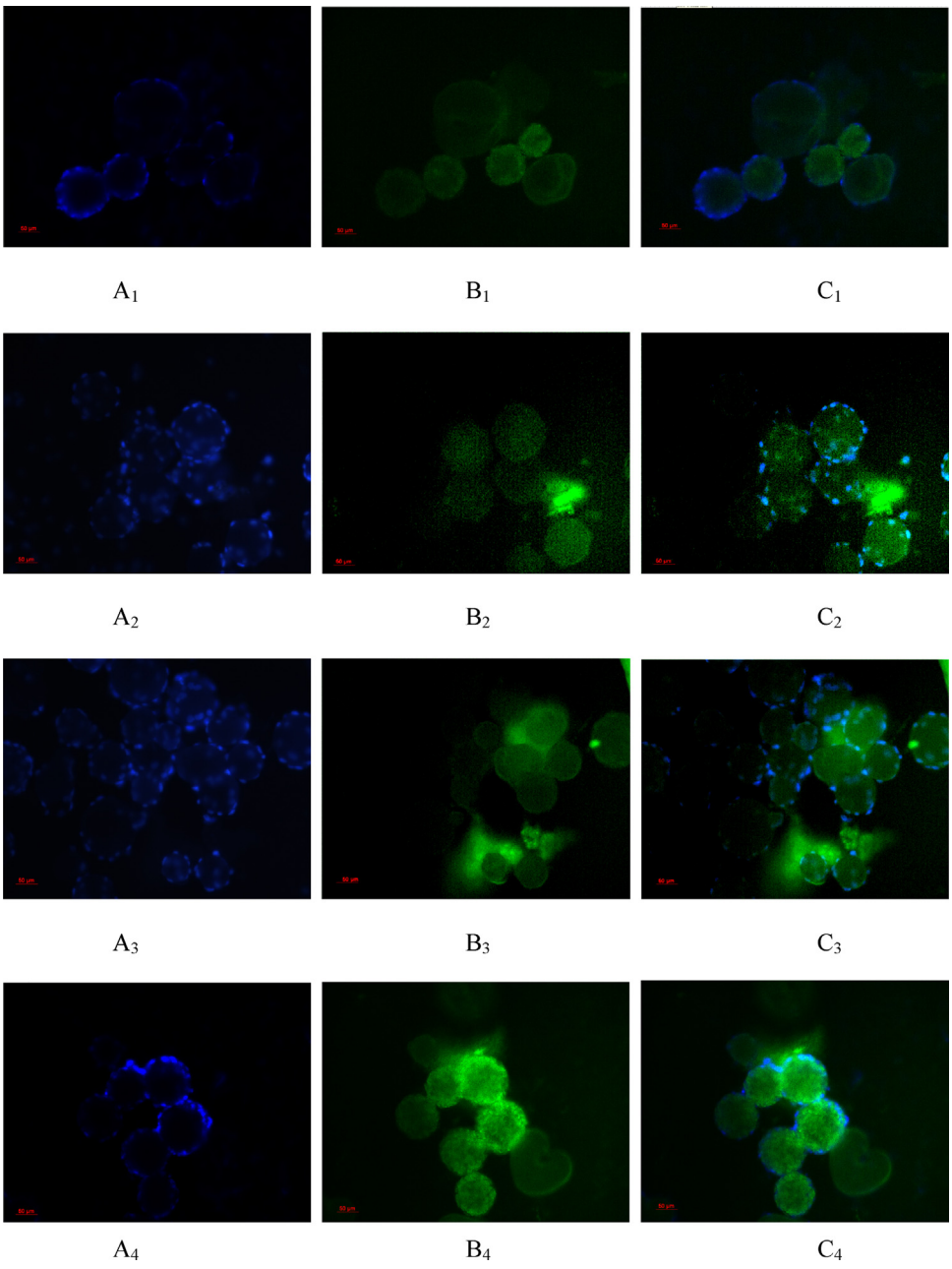


Fig. 8. The fluorescent images of HO8910 cells cultured on smooth nonporous and porous $PLGA_{20K}$ (65:35) microspheres (culture conditions: 12-well plates, 30,000 cells/well, 1 mg microspheres/well). The culture period is 7 days. (Row 1) Original smooth nonporous PLGA microspheres; (Row 2) original smooth porous PLGA microspheres; (Row 3) collagen I coated smooth nonporous PLGA microspheres; (Row 4) collagen I coated smooth porous PLGA microspheres. (A) stained with Hoechst; (B) E-cadherin marked with the green fluorescent antibody; (C) overlap of A and B.

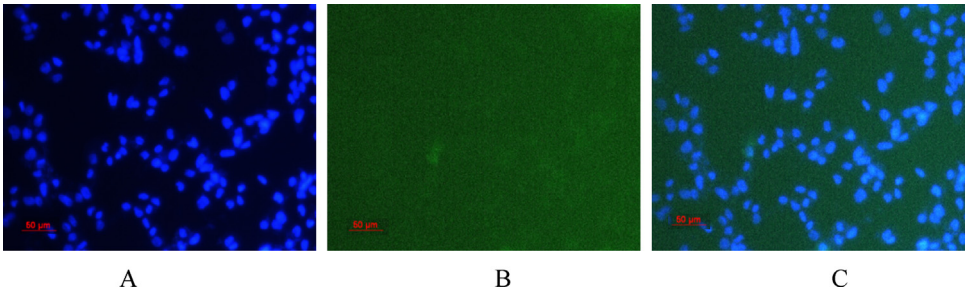


Fig. 9. The fluorescent images of HO8910 cells cultured in a two-dimensional model. The culture period is 7 days. (A) Stained with Hoechst; (B) E-cadherin marked with green fluorescent antibody; (C) overlap of A and B.

The 3D results in the current study depict normal cell-cell interactions found in vivo and closely resemble the situation in vivo.

5. Conclusions

In conclusion, the influence of the addition of porogen, the M_η of the PLGA microspheres and the LA component of the PLGA microspheres on the size and the porosity of the microspheres were systematically investigated. Under the same emulsion conditions, PLGA with an M_η of 20 K was more easily processed into porous microspheres than PLGA with an M_η of 50 K. In other words, the $PLGA_{20K}$ microspheres contained a higher density of pores and pores with larger diameter than $PLGA_{50K}$. In the 3D cell culture, the porous PLGA microspheres better supported the adhesion and the proliferation of the ovarian cancer cell line HO8910 than did the smooth PLGA microspheres. The promotion effect of the collagen I coating of the PLGA microspheres on the adhesion and the proliferation of the HO8920 cells was remarkable. E-cadherin, an important epithelial cell marker of HO8910 cells, was only present in the 3D culture model and not in the 2D model. $PLGA_{20K}$ (65:35) microsphere is a kind of promising carrier in 3D culture of HO8910 cells for further pathological research and high-throughput antitumor drug screening.

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