Inhibiting Cell Viability and Motility by Layer-by-Layer Assembly and Biomineralization

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ABSTRACT: Herein, we proposed a drug-free strategy named cell surface shellization to inhibit the motility of SKOV-3 and HeLa cells. We alternately deposited two- or three-layer cationic polyelectrolyte (PE) and anionic PE films on the surface of SKOV-3 and HeLa cells. Then, a mineral shell (calcium carbonate, CaCO3) was formed on the surface of polymer shells via electrostatic force and biomineralization. The CCK-8 assay results and live/dead staining showed that the surface shells strongly aggravated the cytotoxicity. The monolayer scratch wound migration assay results and immunofluorescence staining results showed that the shells, especially the mineral shells, could efficiently inhibit the migration of SKOV-3 and HeLa cells without any anticancer drugs. The immunofluorescence results of the three small G proteins of the cells showed that the immunofluorescence intensity in SKOV-3 did not change. Preliminary results from our laboratory showed an increase in MMP-9 secreted by cancer cells after coating with films or mineral shells. It suggests that mechanisms that inhibit cell migration are related to the MMP signaling pathway. All the results indicated that shellization (films or nanomineral shells) but not limited to calcification can be used as one of the tools to change the function of cells.

1. INTRODUCTION

Traditional cancer therapy tactics are limited to surgical resection, radiotherapy, and chemotherapy. Although these conventional methods could eliminate the tumor tissue masses or even kill the cancer cells, they also bring many severe side effects to the patients. In addition, some tumors tend to invade adjacent normal tissues or spread to new sites by micrometastasis before a definite diagnosis or therapy. Thus, it is more difficult to prevent or inhibit the metastasis of cancer cells than the viability. In clinics, the most frequently used method is blocking the metastasis signaling pathway of cancer cells via administrating molecular targeted therapeutic drugs. The drug toxicity is reduced when there are inevitably some side effects such as drug resistance. Therefore, finding new therapies has become a hot spot in cancer research. Fortunately, advanced nanomaterial technology has contributed greatly to achievements in cancer treatment. As is known, the key to cancer treatment is controlling or changing the state or cycle of the cancer cells. Thus, we can modify the surface of cancer cells with materials and affect the cell function indirectly without any expensive drugs in vitro.

Recently, inspired by the formation of natural shells such as diatoms, molluscs, fungi, radiolarians, and eggs, many living microorganism have been encapsulated with thin functional coats or shells via layer-by-layer assembly (LbL) and controlled cell calcification or biomineralization. In addition, other cells or tissues such as platelets, mammalian cells (red blood cells, HeLa cells, NIH 3T3 fibroblasts, stem cells, and Jurkat cells), islets, and even zebraﬁsh are also successfully encapsulated with thin functional coats or shells. It is proved that the surface shellizations or modiﬁcations can alter the cell behavior. However, previous studies mostly focus on whether they could synthesize novel materials using the organism as a template or the cells could gain new functions especially protecting or safeguarding, defending the cells from proteolytic attack by trypsin and other toxic compounds.

According to the findings, an attractive hypothesis suggested that cells could be inhibited via a shellization-based strategy. Currently, a novel drug-free tumor therapy strategy designated as cancer cell targeting calcification, which was originated from biomimetic pathological mineralization, has been proved to work for the tumor-bearing mouse model. Being consistent with the above research, we also...
verified the calcification method to be efficient. Unlike the direct calcification they used, we colligated with LbL assembly and biomineralization on the cell surface in the study. Because the density of electronic charge on the surface of cells is relatively low, direct calcification or mineralization is not satisfied. LbL assembly, applied for the fabrication of multicomponent films on solid supports via repeating alternant precipitation of cationic or anionic polyelectrolyte (PE) on the surface, could increase the electronic charge density and facilitate the biomineralization process. In the study, we proposed that shellization tactics (films or nanomineral shells) are effective but not limited to calcification in cell surface engineering fields.

Herein, we alternately deposited two- or three-layer cationic PE, such as poly(diallyldimethylammonium chloride) (PDDA-MAC or PDDA), polydopamine (PDA), and ε-polylsine (ε-PL), or anionic PE, such as poly(styrene sulfonate) (PSS) and polyglutamic acid (Glu), films on the surface of three kinds of cells (human ovarian cancer cell line SKOV-3, human breast cell line MDA-MB-231, and human cervical cancer cell line HeLa) (Scheme 1). In the following, a mineral shell, such as calcium carbonate (CaCO₃), was formed on the surface of polymer shells via electrostatic force and biomineralization. Finally, the influence of the shell on the viability and metastasis of cells was investigated.

### 2. RESULTS AND DISCUSSION

Scanning electron microscopy (SEM) was used to characterize the morphology of native cells (HeLa and SKOV-3), multilayer-coated cells, and cells@CaCO₃ (or CP) after 24 h of drying at room temperature. Figure 1 shows that the surface of native cells was smooth and cells can be fully spread. However, the surface typically becomes rough, porous, and round when coated with the PE multilayers (PDDA/PSS). When we use different types of PEs such as PDA (PDA/PSS) or e-PL/Glu, the morphology of the cells varies. For the PDA/PSS pair, the cells become elongated but spread well (Figure 1B). On the other hand, for the e-PL/Glu pair (Figure 1C), SEM observations clearly suggested a nanometer-sized meshwork morphology of the e-PL/Glu films on the cell surface. It seemed to be similar to the fibrous structure of the natural extracellular matrix. Moreover, the morphology of cells coated with polylsine is incomplete. It may have a destructive effect on the cellular structure of polylsine. This result was consistent with the previous study. From Figure 1D, it can be seen that the crystalline forms of the shells are mainly calcite and vaterite.

In addition, after in situ precipitation of CaCO₃ or calcium phosphates (CaP) (Figure S3) on the LbL-treated cell surface, it can be seen that the cells were fully enclosed by the mineral phase and covered by numerous flakelike nanocrystals. Energy-dispersive X-ray mapping with SEM indicated that the component elements of the mineral shell around the cell surface were C, O, Ca, and P (Figure S3C). These mineral phases are confirmed as CaCO₃ or amorphous calcium phosphate. Using two other pairs of polymers, PDA/PSS and ε-PL/Glu, a similar modification can also be obtained. It suggests that LbL treatment can effectively regulate in situ mineralization on the surface of cells.

To verify the inhibition effects of encapsulation or shellization on cells, we should exclude the cytotoxicity of the LbL films themselves. In other words, the LbL films should possess certain cellular compatibility. Before the fabrication of the LbL films on the cell surface, we evaluated the cytotoxicity of each solution by the CCK-8 counting kit assay as described previously. The anionic polymer (PSS) showed over 75% viability (Figure 2C), although the cationic polymer solution PDDA showed a greater cytotoxicity (<50% viability) (Figures 2A,B and S8). PDA and ε-PL showed a high cell viability (>80%) even with a cationic charge (Figure 2D,E). These results were all consistent with the study of Kadowaki et al. According to the study, the cytotoxicity mechanism of the cationic PE may be related to the aggregation and electrostatic interaction of the cationic polymers with anionic proteins or polysaccharides on the cell membrane. A preliminary test in our laboratory indicated that there was a low current impulse when the two kinds of polymers came in contact with each other (data not shown). Certainly, the cytotoxicity depends on the charge density, concentration, molecular weight, and conformational flexibility of the cationic PE.

Another cytotoxicity test in our case was based on a live/dead staining by means of calcine AM and ethidium homodimer. Dead or late apoptotic cells appear red, while the living cells appear green in the fluorescence microscope. The live/dead staining depicted in Figures S4 and S5 indicated that for all the two tested PDDA/PSS concentrations, the cells were dead even only applying a one-layer PDDA/PSS film, while for the untreated cell layer, no dead or apoptotic cells were counted. In addition, the number of dead cells increases with the layers of PEs. The most of number of dead cells appeared in (HeLa-PDDA/PSS)@CaCO₃ or (SKOV-3-PDDA/PSS) @CaCO₃. It indicated that the mineral shell on the cell surface strongly aggravated the cytotoxicity.

For the PDA/PSS film-treated cells (Figures 3A and 4A), no dead or apoptotic cells were observed in the image until the number of PE layers reached three. However, when coated with the mineral shell on the surface of one PE layer, the number of dead cells is even more than that of three PE layers (cells@(PDA/PSS)3).

For the ε-PL/PSS film-treated cells (Figures 3B and 4B), no dead cells were observed when coated with two PE layers, while many dead cells appeared when coated with the mineral shell on the surface of one PE layer.

From the results mentioned above, we can summarize that PDDA films were toxic to the cells but PDA and ε-PL were not. Therefore, PDA and ε-PL can be good candidates to...
prepare LbL films on the cell surface. We mainly adopted PDA and ε-PL when evaluating whether PEs affect cell migration as follows.

A monolayer scratch wound migration assay was applied to study the cellular motilities in the presence of PE shells and mineral shells. In Figures 5A,C, 6A,C, S6, and S7, the migration distance of PDA/PSS-treated SKOV-3, HeLa, and MDA-MB-231 cells is shorter than that of untreated cells after 1 day. Additionally, the width of the scratches of one PDA/PSS layer plus CaCO₃ shell-treated cells is the widest. It is indicated that the migration of cells is inhibited by the outer shells. Obviously, there was no significant difference between the migration distance of PDA/PSS- and (PDA/PSS)₂-treated cells until incubated for 3 days. On the third day, there were

![Figure 1. SEM images of SKOV-3 cells coated with different PE films after 24 h of incubation. (A) Coating with PDDA/PSS; (B) coating with PDA/PSS; (C) coating with ε-PL/Glu; and (D) X-ray diffraction patterns of CaCO₃ shells on the surface of SKOV-3 cells.](https://dx.doi.org/10.1021/acsomega.0c00846)
almost no scratches for the untreated cells and one PDA/PSS layer-treated cells. At the same time, the scratches of (PDA/PSS)2-treated cells and (PDA/PSS)@CaCO3 shell-treated cells were visible clearly. The image also showed that the width of the (PDA/PSS)@CaCO3 shell-treated cells almost unchanged with the increase of incubated time. We can conclude that the even only one layer of PE shell could inhibit the migration of cells in 1 day. However, the inhibition level will decrease with the increase of time because of the degradation of the PE in the solution. The number of layers could slow down the decreasing tendency. Fortunately, the mineral shell can maintain the inhibition regardless of the increase of time.

For the ε-PL/PSS-treated cells, the width of both HeLa and MDA-MB-231 cells almost unchanged with the increase of incubated time (Figures 5B,D, 6B,D, and S7). In addition, the number of layers shows no differences. However, for the SKOV-3 cells, the phenomenon was the same as that of the PDA/PSS layer-treated cells. The inhibition depends on the number of layers. This cause may be related to the diversities of anionic proteins or polysaccharides on the cell membrane. In addition, this is also related to what the cell secretes. In the case of a small number of layers (<3 layers), a PE is not enough to prevent this from happening. However, by adding a layer of PE or a layer of mineral shell, the different cells respond the same to the PE shell or mineral shells, and all migration is inhibited. Thus, the mineral shell can eliminate the diversities.

In order to further verify the inhibition mechanism, F-actin is investigated by immunofluorescence staining. F-actin was stained with Alexa Fluor 488 phalloidin labeling with FITC, and the nucleus was stained with Hoechst 33258. The results are shown in Figure 7. As can be seen from the image, the cytoskeleton of untreated cells is integrated and the morphology of the cells is fusiform, and the cell pseudopodia extend well, suggesting good adhesion. However, the cells encapsulated with two layers of PDA/PSS represent a spindle morphology (Figure 7A). In addition, as the number of layers increases, the cytoskeleton becomes more elongated, and two layers of PDA/PSS also stimulated the cells to put out more pseudopodia. When coated with CaCO3 shells, the cytoskeleton of SKOV-3 cells is not integrated. The CaCO3 shells result in a collapsed morphology. The actin filament is not observed in the center but on the edge just like a circular ring. Compared with the untreated and PDA/PSS-treated cells, the nucleus fluorescence of cancer cells, which are encapsulated with CaCO3y, is very weak. Moreover, the cells show signs of death.

It is indicated that the CaCO3 shells could induce cell apoptosis and even death. This result is exactly consistent with that of the live/dead staining mentioned above. In addition, current research also proved that CaCO3 nanostructures could
significantly inhibit the cell proliferation. They indicated that CaCO₃ could bind with 66 membrane proteins, leading to block the transport proteins such as Na and K-ATPase. Thus, this could modulate the cell cycle arrest in the G₀/G₁ phase and induce the apoptosis of cancer cells.

For the ε-PL/PSS-treated cells (Figure 7B), the cytoskeleton of the cells begins to contract and the morphology becomes long and thin with the increase of layers. It is indicated that the ε-PL/PSS films may affect cell adhesion. When coated with CaCO₃ shells, the results were the same as that of PDA/PSS–CaCO₃-treated cells. All of the results mentioned above demonstrated that the LbL films and the mineral shells affected cell adhesion and morphology. Certainly, the influence depends on the film components. As is known, mammalian cells do not have a robust cell wall or exoskeleton. Thus, the membrane is very susceptible to environmental changes because of its mechanical fragility. During the LbL assembly process, the cationic PE interacts or aggregates with anionic glycoproteins on the cell membrane with the electrostatic force. The electrostatic interactions can affect the morphology and adhesion of cells. Moreover, a multilayer of PE films especially coated with the nanomineral shells may interfere with the nutrient supply to the cells. Furthermore, it was reported that the mechanical properties (higher Young’s modulus) of the LbL films presumably induced the cell adhesion inhibition and low growth rate. Additionally, increased matrix stiffness has profound effects on tumor growth and metastasis. In this study, the shells on the surface of the cell change the matrix microenvironment including stiffness. Further work is still needed to verify the clear relationship between shells stiffness and cell migration velocity.

It is known that cell migration is related to the Rho family small GTPases. Rac 1, Rho A, and Cdc 42, which regulated lamellipodia, stress fibers, and filopodia, respectively, are regarded as the most important factors among the Rho family. Cdc 42 and Rac1 induce cell polarization and lamellipodium formation at the leading edge, respectively, whereas Rho A acts at the cell body to facilitate contraction. The immunofluorescence results of the three small G proteins, Rac 1, Rho A, and Cdc 42, of the cells are shown in Figures S9–S11. It revealed that the immunofluorescence intensity of three small G proteins in SKOV-3 almost did not change. Fluorescence results preliminarily indicated that this method (shellization) did not affect the expression of small G proteins in cells. In other words, the signaling pathway associated with small G proteins for cell migration has not been changed. It is indicated that the inhibition of cell migration by the shell is independent of this signaling pathway (small G protein). In addition, the shells outside the cells may affect cell migration from other pathways. Preliminary results from our laboratory showed an increase in MMP-9 secreted by cancer cells after...
coating with films or mineral shells. In the preliminary study, we have encapsulated the SKOV-3 cells with PDA and gelatin (GE) and CaCO₃. Then, we measured the content of MMP-9 secreted by cells by ELISA kit. The results are shown in Figure S12. As can be seen from the image, MMP-9 secreted by cells after coating with films or mineral shells increased significantly, especially the mineral shells. In addition, the amount of MMP-9 secreted by cells is dependent on the number of PE layers. The PE shell or CaCO₃ shell stimulates the cancer cells to secrete more MMP-9. On the one hand, the cell releases more MMP-9 to break the shells.

3. CONCLUSIONS

In summary, we used LbL assembly and biomineralization to prepare various nanometer-sized LbL films and CaCO₃ shells onto the surface of human cells. The cells with PDA/PSS and ε-PL/PSS films on the surface showed good cytocompatibility but not the PDDA/PSS films. However, the condensation of the cationic PE (PDA or ε-PL) and CaCO₃ onto the cell membrane affects the cell morphology, adhesion, and migration after incubation. We estimated that this method can be used as a good tactic for cell surface engineering.

4. EXPERIMENTAL SECTION

4.1. Cells and Regents. In the present work, we used ovarian carcinoma cell lines (SKOV-3), cervical cancer cells (HeLa), and breast ductal cancer cells (MDA-MB-231). SKOV-3 and MDA-MB-231 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). HeLa cells were obtained from the Institute of Biomedical and Applied Mechanics, Taiyuan University of Technology (Taiyuan, China). The cells were cultured in the RPMI 1640 (Gibco, USA) medium with 10% new-born calf serum (NBS, Sijiqing, China) and 1% penicillin–streptomycin solution under a CO₂ (5%) atmosphere at 37 °C.

PDDA (100 kD), poly(styrene sulfate sodium salt) (PSS, 70 kD), ε-poly(lysine hydrochloride) (ε-Lys, 5 kD), and dopamine hydrochloride (PDA, 0.2 kD) are all purchased from Aladdin Biochemical Technology Co. LTD., China.

4.2. Single-Cell Encapsulation. 4.2.1. LbL Assembly of the PE Inner Layer. All of the PE solutions (1.0 mg·mL⁻¹) for multilayer assembly were prepared by dissolution of a PE in a serum-free medium (RPMI 1640). The solutions were filtered through 0.22 μm Millipore polyvinylidene fluoride filters prior to use. The cell suspension (1 × 10⁶ cells/mL) was rinsed with phosphate-buffered saline (PBS) (10 mM, pH 7.2) and then incubated with a cationic PE solution (1 mg·mL⁻¹, 2 mL) such as PDDA, dopamine hydrochloride solution, and ε-PL at 37 °C for 10 min. After the rinsing step with PBS, the (cancer cells/cationic PE) suspension was treated with an anionic PE solution such as PSS solution (1 mg·mL⁻¹, 2 mL) following the same protocol. Subsequently, this procedure was repeated with alternating polycation and polyanion solutions until an appropriate coating was obtained on the surface of cancer cells.

Figure 4. Fluorescence microscopic images of various PE films or mineral shells prepared on the cancer cells after 24 h of incubation. The cells were stained with a calcein-AM/EthD-1 kit. Dead or late apoptotic cells appear red, while living cells appear green in the fluorescence microscope. (A) HeLa cells coated with PDA/PSS and (B) HeLa cells coated with ε-PL/PSS.
4.2.2. Mineralization Coating. The cells coated with a PE were incubated with the calcium chloride solution (0.33 mol·L⁻¹) for 10 min at 37 °C. Then, the cells were washed thoroughly with PBS (10 mM, pH 7.2) to remove unabsorbed calcium ions three times. An equal volume of sodium carbonate solution (NaCO₃, 0.33 mol·L⁻¹) was added into the calcium ion-treated cell suspension. After 10 min of reaction at 37 °C in an incubator, the mineralization cells were collected by centrifugation and washed with PBS.

4.3. Characterization. The morphology of cells encapsulated by a PE inner layer and coated with a calcium carbonate mineralization shell was characterized by SEM (JEOL JSM-7100F, Japan) equipped with an energy-dispersive system (OXFORD, X-MaxN, UK). The cells were fixed with 4% paraformaldehyde solution at 4 °C in a refrigerator for 12 h. Subsequently, the cells were dehydrated with ethanol in a series of concentrations of 30, 50, 70, 90, 95, and 100% (v/v) for 10 min. All specimens were sputter-coated with platinum before SEM observation.

4.4. Cell Viability Test. The CCK-8 assay was applied to evaluate the effect of the different PE solutions on cell viability. Cell suspensions at a density of 1 × 10⁴ were seeded (six wells for each) in 100 μL of RPMI 1640 in a 96-well plate (BD, USA) and incubated overnight (16 h) to allow for cell attachment. Then, the culture medium was added with 100 μL of PE suspensions with concentrations of 1.0, 0.01, and 0.0001 mg·mL⁻¹ per well. Then, the plate was cultured at 37 °C for 24, 72, and 120 h. Control cells were incubated with the culture medium 1640/PBS solution. Then, 20 μL of the CCK-8 solution was added into each well and incubated for 2 h at 37 °C. After incubation, the absorbance of solubilized formazan was measured at 450 nm with a microplate reader (IMark, Bio-Rad). All of the experiments were performed in triplicate.

4.5. Cell Migration Assay—Scratch Wound Migration Assay. A monolayer scratch wound migration assay was applied to study the cellular motilities after LbL assembly and mineralization. SKOV-3, HeLa, and MDA-MB-231 cells were used in the study. Cell suspensions (500 μL; 1 × 10⁵) were added per well until reaching the confluence and starved serum-free for 12 h. During the last 4 h, hydroxyurea was added to prevent further DNA synthesis at a final concentration of 6 mM·L⁻¹. Then, a uniform scratch was performed in the cell monolayer with a micropipette tip (200 μL, Corning, USA). Then, cell monolayers were washed with PBS (pH 7.2) and photographed with an inverted phase contrast microscope (Nikon, Japan). Then, the cells were treated with the LbL assembly procedure and calcium carbonate mineralization as described above. The control cell was incubated with 400 μL of the PBS solution (pH 7.2). After incubating for 24 and 72 h, the images of the wounds under static culture in an incubator condition of 5% CO₂ at 37 °C without serum were acquired by an inverted phase contrast microscope.

4.6. Fluorescence Assay. In order to further evaluate the influence of shells on the migration of cells, fluorescence staining (F-actin and live/dead staining) was applied and analyzed by a laser scanning confocal microscope (Leica TCS SP5, Germany). The cells were fixed with 4% paraformaldehyde (Sigma-Aldrich) and permeabilized with 0.5% Triton X-

Figure 5. Effects of various PE films or mineral shells on the migration of cell lines by the scratch wound healing assay. (A) Migration of SKOV-3 cells coated with PDA/PSS and CaCO₃ at 0, 1, and 3 d (white scale bar = 100 μm). (B) Migration of SKOV-3 cells coated with ε-PL/PSS and CaCO₃ at 0, 1, and 3 d (white scale bar = 100 μm). (C) Migration distance of SKOV-3 cells coated with PDA/PSS and CaCO₃ at 1 and 3 d. (D) Migration distance of SKOV-3 cells coated with ε-PL/PSS and CaCO₃ at 1 and 3 d. ** denotes a statistically significant difference between the samples calculated by a two-sample t-test.
100 for 10 min. For the F-actin staining, the Alex Fluor 488 phalloidin was incubated with the fixed cells for 30 min at room temperature. The cell nucleus was stained by Hoechst 33258 solutions at room temperature for 15 min. For the live/dead staining, the cell live/dead assay kit was applied according to the manufacturer’s protocol. The live/dead stock solution was prepared by adding 2.5 μL of calcein-AM and 10 μL of EthD-1 into 5 mL of PBS. The stock solution was added to the coated cells for 40 min at room temperature in a dark place. The cells were washed with PBS and sealed with a fluorescent antiquenching agent, and then, the cells were observed with an inverted fluorescence microscope (ECLIPSE Ti, Nikon, Japan).

4.7. Statistical Analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, Illinois) version 18.0 software. The data obtained in this study were reported as means + standard error and then statistically compared. To reveal the differences

Figure 6. Effects of various PE films or mineral shells on the migration of cell lines by the scratch wound healing assay. (A) Migration of HeLa cells coated with PDA/PSS and CaCO₃ at 0, 1, and 3 d (white scale bar = 100 μm). (B) Migration of HeLa cells coated with ε-PL/PSS and CaCO₃ at 0, 1, and 3 d (white scale bar = 100 μm). (C) Migration distance of HeLa cells coated with PDA/PSS and CaCO₃ at 1 and 3 d. (D) Migration distance of HeLa cells coated with ε-PL/PSS and CaCO₃ at 1 and 3 d. ** denotes a statistically significant difference between the samples calculated by a two-sample t-test.

Figure 7. Immunofluorescence analyses of the effects of various PEs films or mineral shells on the expression and distribution of F-actin. (A) SKOV-3 cells were coated with PDA/PSS and CaCO₃ and (B) SKOV-3 cells were coated with ε-PL/PSS and CaCO₃ for 24 h. F-actin was stained with Alexa Fluor 488 phalloidin labeling with FITC, and the nucleus was stained with Hoechst 33258 (white scale bar: 100 μm).
among the groups, one-way analysis of variance followed by Tukey’s test was used to reveal the differences among the groups. In all statistical evaluations, \( P < 0.05 \) was considered as statistically significant.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00846.

Physiochemical properties of polymers used in this study; chemical structures of the cationic and anionic polymers used in this study; SEM images of the HeLa cells coating with PDDA/PSS films after 24 h of incubation; SEM images of the HeLa cells coating with PDDA/PSS films and calcium phosphate shells after 24 h of incubation; fluorescence microscopic images of PEs films (PDDA/PSS) or mineral shells prepared on the SKOV-3 cells after 24 h incubation; fluorescence microscopic images of PEs films (PDDA/PSS) or mineral shells prepared on the HeLa cells after 24 h incubation; effects of PDDA/PSS films or mineral shells on the migration of cell lines by the scratch wound healing assay; effects of various PE films or mineral shells on the migration of MDA-MB-231 cell lines by the scratch wound healing assay; morphology of HeLa cells before and after coating with PDDA/PSS films and CaCO\(_3\) shells for 1, 3, and 5 days at 37 °C; immunofluorescence analyses of the effects of various PE films or mineral shells on the expression and distribution of RhoA after 24 h coculture; immunofluorescence analyses of the effects of various PE films or mineral shells on the expression and distribution of Cdc42 after 24 h coculture; concentration of MMP-9 or mineral shells on the expression and distribution of Cdc42 after 24 h coculture; concentration of MMP-9 or mineral shells prepared on the HeLa cells after 24 h incubation; effects of various PE films or mineral shells on the expression and distribution of Cdc42 after 24 h coculture; fluorescence microscopic images of PEs films or mineral shells on the expression and distribution of Cdc42 after 24 h coculture; concentration of MMP-9 and concentration of Rac1 of SKOV-3 cells coated with PDA/GE and CaCO\(_3\); and concentration of Rac1 of SKOV-3 cells coated with PDA/GE and CaCO\(_3\) (PDF)

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**Author Contributions**

Y.W. designed the experiments; Y.W., H.X., S.X., H.S., R.S., and L.Z. carried out the experiments; Y.W. analyzed the experimental results. D.H., L.Z., K.W., Y.H., and X.L. analyzed the data and made statistical calculations. D.H. provided some financial support. Y.W. and S.X. wrote the manuscript.

**Notes**

The authors declare no competing financial interest.

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