Biological responses of T cells encapsulated with polyelectrolyte-coated gold nanorods and their cellular activities in a co-culture system

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Abstract Currently, human T cell therapy is of considerable scientific interest. In addition, cell encapsulation has become an attractive approach in biomedical applications. Here, we propose an innovative technique of single-cell encapsulation of human T cells using polyelectrolytes combined with gold nanorods. We have demonstrated encapsulation of human Jurkat T cells with poly(sodium 4-styrenesulfonate) (PSS)-coated gold nanorods (PSS-GNRs). Other forms of encapsulation, using polyelectrolytes without GNRs, were also performed. After Jurkat T cells were encapsulated with poly(allylamine hydrochloride) (PAH) and/or PSS-GNRs or PSS, most cells survived and could proliferate. Jurkat T cells encapsulated with a double layer of PSS-GNR/PAH (PSS-GNR/PAH@Jurkat) showed the highest rate of cell proliferation when compared to 24-h encapsulated cells. With the exception of IL-6, no significant induction of inflammatory cytokines (IL-2, IL-1 β , and TNF- α) was observed. Interestingly, when encapsulated cells were co-cultured with THP-1 macrophages, co-cultures exhibited TNF- α production enhancement. However, the co-culture of THP-1 macrophage and

PSS-GNR/PAH@Jurkat or PSS/PAH@Jurkat did not enhance TNF- α production. No significant inductions of IL-2, IL-1 β , and IL-6 were detected. These data provide promising results, demonstrating the potential use of encapsulated PSS-GNR/PAH@Jurkat to provide a more inert T cell population for immunotherapy application and other biomedical applications.

Keywords Cell encapsulation · Polyelectrolytes · Gold nanorods · Biological activity · Human T cell therapy

Introduction

The transplantation of cells or tissues has been applied for treating various human diseases (Bhatia et al. 2005). Unfortunately, when using this technique, transplanted cells can be rejected by the immune system of the body. Immunosuppressant drugs are applied to solve this problem, but these drugs can cause many complications (Uluoglu et al. 2000). The encapsulation of cells has been developed to avoid immune rejection in cell/tissue transplantation. Furthermore, this technique can be used for other applications such as controlled delivery (Orive et al. 2014), regenerative medicine (Hunt and Grover 2010), and drug delivery (Gurruchaga et al. 2015). There are many approaches that have been used to encapsulate cells, such as, using alginate-poly cationic poly(L-lysine) (PLL) (Uluoglu et al. 2000), interfacial precipitation (Ai et al. 2003), and coacervation techniques (Kampf 2002). The layer-by-layer (LBL) technique is another attractive approach that can be used for cell encapsulation. This technique provides a well-defined surface, gentle conditions, nanoscale precision, and tuneable multilayer construction (Ai et al. 2003; Franz et al. 2010). Various types

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of mammalian cells, such as, human adipose mesenchymal stem cells (Granicka et al. 2014; Hachim et al. 2013), islet cells (de Vos et al. 2014), lymphocytes (Granicka et al. 2014), and human leukemia cells (Borkowska et al. 2014) have been encapsulated using this technique. The LBL encapsulation technique can also be applied for yeasts, fungi, and bacteria (Franz et al. 2010; Diaspro et al. 2002; Fakhrullin et al. 2009).

Human T lymphocytes play an important role in the immune system and have been used in immunotherapy (Juan et al. 2009; Zhao et al. 2016). Unfortunately, T lymphocytes from a donor can be activated or interact with cells in the immune system of a recipient, leading to adverse effects (Roncarolo and Battaglia 2007). Therefore, cell encapsulation of T lymphocytes might help mitigate this problem and increase the efficiency of immunotherapy in many diseases. Recently, gold nanorods (GNRs) have attracted growing interest in biological/biomedical applications. It is known that the surfaces of gold nanorods (GNRs) can be modified with polyelectrolytes through the LBL technique (Pissuwan et al. 2013; Pissuwan and Nii-dome 2015). This modification can reduce the toxicity of the cationic surfactant (cetyltrimethylammonium bromide; CTAB) that is commonly used to stabilize the surface of GNRs. Because of the excellent optical properties of GNRs, the combination of surface-modified GNRs, with LBL cell encapsulation, should provide substantial benefits. For example, GNRs allow for the use of a variety of characterization techniques including transmission and scanning electron microscopy, Raman spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), and spectroscopy. Furthermore, GNRs can be conjugated with various biological/chemical molecules. Therefore, rather than encapsulating cells with polymers alone, the deposition of GNRs on the surface layer of polymer-encapsulated cells can provide additional benefits. Firstly, the encapsulated cells that have GNRs fabricated on the layer of cells are able to be easily detected, or tracked, without being faced with photobleaching issues. Next, GNRs provide a high potential for a controlled release application. They can help control release of embedded therapeutic molecules after irradiating under a specific light exposure at tissue window wavelengths leading to heat energy build-up to break the capsule wall. In this work, PSS-coated gold nanorods (PSS-GNRs) were used. These types of GNRs are biocompatible and easy to conjugate with various biological molecules. Finally, the encapsulated T lymphocytes designed for therapeutic or diagnostic purposes can be used to target specific cells.

However, careful consideration is required before designing cells encapsulated with polyelectrolyte-coated gold nanorods for the applications mentioned above. It is necessary to investigate the biological responses of cells

after encapsulation. Therefore, the effect of combined encapsulation with polyelectrolytes and GNRs has been assessed to clarify the impact of the coating layer on the cell surface. Furthermore, the downstream effects of this technique on other cell types such as macrophages should be assessed using a co-culture system. For these reasons, we aimed to encapsulate human Jurkat T cells using the LBL technique. These cells acted as a template for encapsulating two polyelectrolytes: poly(sodium 4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH). The final layer on the encapsulated cells consisted of GNRs coated with PSS (PSS-GNRs). A characterization of encapsulated human Jurkat T cells was performed. To our knowledge, there are only a few reports regarding the encapsulation of T lymphocytes (Pandey et al. 2013) and no reports using GNRs to encapsulate T lymphocytes. We examined the biological responses of encapsulated cells. To examine the host response to encapsulated human Jurkat T cells, a co-culture system of human macrophages and encapsulated cells was also utilized. Our proposed model of cell encapsulation and information on biological cellular responses demonstrates the possibility of applying this technique for future biomedical applications.

Materials and methods

Preparation of poly(styrene sulfonate)-coated gold nanorods

Commercial GNRs (~ 40 nm in width and ~ 68 nm in length) were purchased from NanopartzTM, Loveland, USA. The preparation of PSS-GNRs was performed by slightly modifying a previously published procedure (Pissuwan et al. 2013). First, PSS-GNRs were prepared by mixing 600 μ L GNRs with 300 μ L PSS ($M_w = 70,000$ at a concentration of 2 mg mL⁻¹ dissolved in 0.5 mM NaCl; Sigma Aldrich, Louis, USA) and the mixture was shaken for 30 min on a shaker at room temperature. Following this, the mixture of PSS and GNRs was centrifuged at $9391 \times g$ for 10 min. Next, the pellet of PSS-GNRs was dispersed in Milli-Q water for use in further experiments. The morphology of the GNRs was examined by transmission electron microscopy (TEM).

Cell culture

Human Jurkat T cells (ATCC) and THP-1 macrophages were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS) plus 1% penicillin/streptomycin. Cells were maintained at 37 °C in a 5% CO₂ incubator.

Cell encapsulation preparation

PAH ($M_w = 15,000$; Sigma Aldrich, Louis, USA) was dissolved in 18 mM CaCl_2 to have a stock concentration of 2 mg mL^{-1} . Jurkat T cells at a concentration of $1 \times 10^6 \text{ cells mL}^{-1}$ (in RPMI-1640 medium without FBS) were incubated with 0.05 mg mL^{-1} PAH (diluted from the stock solution using FBS-free RPMI-1640 medium) on a shaker for 5 min at room temperature. Thereafter, the free PAH was removed by centrifugation (6500 rpm, 5 min). The cells coated with PAH were named here as PAH@Jurkat cells. PAH@Jurkat cells were then incubated with 0.1 mg mL^{-1} PSS (dissolved in FBS-free RPMI-1640 medium) on a shaker for 10 min at room temperature. Next, the same process mentioned above was used to remove free PSS. To prepare Jurkat cells coated with PSS-GNRs (PSS-GNR/PAH@Jurkat cells), PAH@Jurkat cells were firstly prepared and then a $150 \mu\text{L}$ of FBS-free RPMI-1640 medium was added into cells. Following this step, $50 \mu\text{L}$ PSS-GNRs ($\text{OD}_{604\text{nm}} \sim 0.6$) was added into the cell mixture and incubated on a shaker for 15 min at room temperature. Next, cells were centrifuged to remove free PSS-GNRs. Finally, PAH@Jurkat and PSS-GNR/PAH@Jurkat cells were suspended in RPMI-1640 medium plus 10% FBS for use in further experiments.

Cell encapsulation characterization

The successful encapsulation of Jurkat T cells was confirmed using various techniques.

Fluorescence observation

Fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC) dyes (Sigma Aldrich, Louis, USA) were conjugated with PAH by following the protocol published by Winky et al. (2003). The conjugation of PAH to FITC (PAH-FITC) or TRITC (PAH-TRITC) was prepared by dissolving 2 mg FITC or TRITC dye in $250 \mu\text{L}$ dimethylsulphoxide (DMSO). The PAH solution was prepared by dissolving 250 mg PAH in 3 mL Milli-Q water. The pH of the PAH solution was adjusted to 8.0–8.5 using 1 M NaOH. The conjugation of PAH to FITC or TRITC dye was performed by mixing the prepared FITC or TRITC solution ($250 \mu\text{L}$) with 3 mL PAH solution (Winky et al. 2003). The mixture was then incubated overnight at room temperature in the dark. After incubation, the mixture was dialyzed to remove free dye molecules of FITC or TRITC by a 3.5 kDa molecular weight cut off membrane (Cellu Sep H1, Texas, USA). Finally, the conjugated PAH to dye molecules was lyophilized by a freeze dryer. Standards of PAH solution at different concentrations were prepared to measure the optical density at 212 nm. This

standard was used to calculate the amount of PAH in PAH-dye conjugates.

To confirm the encapsulation of Jurkat T cells into a PAH layer (PAH@Jurkat), unencapsulated Jurkat T cells (1×10^6) cells were added into a cell culture dish coated with 0.01% poly-L-lysine (PLL). Cells were then incubated in a cell incubator for 1 h and washed once with FBS-free RPMI 1640 medium. After washing, $500 \mu\text{L}$ PAH-FITC conjugates (containing 0.05 mg mL^{-1} PAH) were added into a dish and incubated in the dark for 5 min at 37°C . Thereafter, the solution of PAH-FITC conjugates was removed. Next, the cells attached to the dish were washed twice with FBS-free RPMI-1640. In the cell fixation process, the cells attached onto the dish were fixed with 3% paraformaldehyde in PBS for 15 min at 4°C and washed 2 times with cold PBS. Then, cold PBS was added. A green fluorescent signal was observed under the fluorescence microscope. To confirm whether the double layers of polyelectrolytes formed on the surface of encapsulated Jurkat cells (PSS/PAH@Jurkat cells), the preparation of Jurkat cells was prepared as mentioned above. For the process of encapsulation, cells were first encapsulated with 0.05 mg mL^{-1} PAH and then washed. After this, cells were encapsulated with 0.1 mg mL^{-1} PSS for 10 min at 37°C . Thereafter, the PSS/PAH@Jurkat cells were washed and stained with $500 \mu\text{L}$ PAH-TRITC conjugates (containing 0.05 mg mL^{-1} PAH) under dark conditions for 5 min at 37°C . Then, cells were washed and observed under a fluorescent microscope. All washing and encapsulating processes were performed using the same approach mentioned earlier. The confirmation of PSS-GNR/PAH@Jurkat cell preparation was performed using a similar process to that described for PSS/PAH@Jurkat cells. Cells stained with fluorescent dyes ($\sim 5 \mu\text{g mL}^{-1}$ for 5 min) alone were also prepared as a control sample.

Transmission electron microscopy (TEM)

PSS-GNR/PAH@Jurkat cells were fixed with 2.5% glutaraldehyde (in 0.1 M sodium cacodylate buffer (SCB) pH7.4 overnight). The post-fixation was performed by fixing encapsulated and unencapsulated cells with 1.0% osmium tetroxide (OsO_4) for 4 h at room temperature. Thereafter, the cell samples were dehydrated in 30, 50, 70, 80, and 90% ethanol, respectively. This process was performed twice (15 min soaking time at each concentration of ethanol). After this, cells were soaked in 100% ethanol 4 times for 15 min each time. Following dehydration, cells were embedded in araldite resin and cell samples were cut to a thickness of 90 nm and stained with 2% uranyl acetate followed by lead citrate. The samples were then observed under TEM at 80 kV (FEI Morgagni 268D, Netherlands). Unencapsulated cells were prepared as a control. To

observe the morphology of PSS-GNRs, a solution containing PSS-GNRs was dropped on a copper grid. The sample was dried and then observed under TEM.

Scanning electron microscope (SEM)

Encapsulated and unencapsulated cells were pre-fixed with 2.5% glutaraldehyde overnight. Following this, cells were fixed with 1% OsO₄ for 1 h for post-fixation. Next, cells were consequently dehydrated by soaking in 50, 60, 70, 80, 90, and 100% ethanol for 15 min (Heckman et al. 2007). The critical point drying (CPD) approach was used to dehydrate biological tissues before examination by SEM. This CPD technique can help avoid damaging the surface structure of cell samples. First, cell samples were dehydrated in ethanol. After dehydration, cell samples were then placed into a CPD chamber. Then, the chamber was sealed and cooled down to −10 °C. Next, the temperature was adjusted to 40 °C and the pressure was slowly increased to 80–120 kgf cm^{−2}. This condition is a CO₂ critical point. It is important to carefully monitor the temperature and the pressure to avoid sample damage. The samples were removed from the chamber when the pressure dropped to 0 kgf cm^{−2}. Finally, the cell samples were observed under SEM (Bray 2000).

Zeta potential measurement

Encapsulated cells (PAH@Jurkat cells, PSS@Jurkat cells, PSS/PAH@Jurkat cells, and PSS-GNR/PAH@Jurkat cells) at a concentration of 1×10^6 cells mL^{−1} were dispersed in FBS-free RPMI-1640 medium. Zeta potential values of encapsulated and unencapsulated Jurkat T cells and PSS-GNRs were determined by DLS (Malvern, Worcester, UK).

Gold element analysis by ICP-MS

Encapsulated and unencapsulated Jurkat T cells at a concentration of 1×10^6 cells per tube were lysed using 250 µL lysis buffer [10% tween-20 dissolved in phosphate buffer saline (PBS)]. Cells were sonicated for 30 min to destroy the cell membrane. After sonication, cells were digested in a digest buffer (3 mL 65% HCl mixed with 1 mL 6% H₂O₂) with overnight incubation in the dark inside a fume hood. The aqua regia (a 3:1 ratio of HCl to HNO₃) was prepared. Thereafter, 3 mL aqua regia was added into cell samples and incubated for 2 h (Kim et al. 2010). Finally, the cell samples were adjusted to a volume of 100 mL using Milli-Q water. The final concentration of aqua regia in the sample was 5% of the total volume of the sample. A standard gold chloride solution (containing 5% aqua regia) at concentrations of 0, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 µg L^{−1} (ppb) was prepared.

Cell viability

Different types of encapsulated Jurkat T cells at a concentration of 1×10^5 cells per well were added into a 96-well plate. Then, encapsulated cells were cultured for 24 h in a 5% CO₂ incubator. The cell viability of encapsulated cells was directly determined using a CellTiter-Glo luminescent cell viability assay (Promega, Madison, USA). The measurement was performed following the manufacturer's instructions. The luminescent signals were detected and used for calculation of the relative cell viability (%) expressed as a percentage relative to unencapsulated cells. The cell viability of Jurkat T cells after encapsulation was also measured immediately.

Cell proliferation

Different encapsulated cells were seeded into a 96-well plate (at a concentration of 1×10^4 cells per well). Cells were incubated in a cell incubator for 24, 48, and 72 h, respectively. Following the incubation, a CellTiter 96 AQueous one solution cell proliferation assay (Promega, Madison, USA) (20 µL) was used to detect cell proliferation. This process was performed following the manufacturer instructions. Unencapsulated cells were also prepared as a control.

Enzyme-linked immunosorbent assay (ELISA)

The release of proinflammatory cytokines (IL-6, IL-2, IL-1β, and TNF-α) was measured using an ELISA kit (Biolegend, San Diego, USA). Different encapsulated cells were seeded into a 96-well plate (1×10^5 cells per well). Cells were incubated for 5 and 24 h in a cell incubator. After incubation, the supernatant of cells was collected to analyze proinflammatory cytokines.

Co-culture system

A co-culture of THP-1 macrophages and encapsulated Jurkat T cells was prepared. The macrophages at a concentration of 5×10^4 cells per well were added into a 96-well plate and were cultured for 24 h in a 5% CO₂ incubator. After culturing of THP-1 macrophages, the encapsulated Jurkat T cells at a concentration of 5×10^4 cells per well were added into a 96-well plate containing cultured macrophage cells. The cells were co-cultured for 24 h. After co-culturing for 24 h, the supernatants were collected to analyze proinflammatory cytokines.

Statistical analysis

The statistical analysis was performed by using GraphPad Prism software Version 5.0 (GraphPad Inc.). ANOVA and

Tukey–Kramer tests were used for statistical significant analysis at $P \leq 0.01$.

Results and discussion

Confirmation of cell encapsulation

To prove the encapsulation of Jurkat T cells in PAH, PSS/PAH, or PSS-GNR/PAH, numerous techniques were utilized.

First, the zeta potentials of different encapsulated Jurkat T cells were measured. Measured cells were dispersed in FBS-free RPMI-1640 for all measurements. The zeta potential value of unencapsulated Jurkat T cells was -14.3 ± 0.2 mV. However, when cells were encapsulated with PAH (PAH@Jurkat cells), the zeta potential value changed to -9.7 ± 0.2 mV. The low negative number of zeta potential value confirms that the cationic polyelectrolyte (PAH) could form a layer on the surface of the Jurkat T cells. When PAH@Jurkat cells were encapsulated with PSS-GNRs (zeta potential $\sim -16.6 \pm 0.7$ mV), the zeta potential of PSS-GNR/PAH@Jurkat cells dropped to have a lower negative value at -11.3 ± 0.4 mV. The change in zeta potential value clearly indicates that Jurkat T cells were encapsulated in PAH or PSS-GNR/PAH. Our results were similar to previous works that investigated the zeta potential values of mammalian cells coated with different polymers (Zhao et al. 2016; Pandey et al. 2013; Bondar et al. 2012). As can be seen, PAH@Jurkat cells had a weak negative value of zeta potential (-9.7 ± 0.2 mV) and the zeta potential value altered to have a further negative value after encapsulating Jurkat T cells with PSS-GNRs. The mechanism underlying the interaction in this case is unclear. We shall not discuss this in further detail, but rather mention that the charge densities of

polyelectrolytes and the concentration of the ions could play a major role here. And, available results of this study strongly suggest that PSS-GNRs could modify the surface of PAH@Jurkat cells resulting in changing of zeta potential values. The binding of a weak negative charge on the cell surface to negatively charged polymers was also reported (Zhao et al. 2016). In the case of PSS/PAH@Jurkat cells, the zeta potential was more negative (-21.1 ± 0.4) than that of PSS-GNR/PAH@Jurkat cells (-11.3 ± 0.4). This could be caused by the amount of PSS formed on PSS-GNRs used in cell encapsulation, which should be less than that formed after using PSS alone. As expected, Jurkat T cells directly encapsulated in PSS had a similar zeta potential value (-14.6 ± 0.2) to unencapsulated cells (-14.3 ± 0.2). This implies that PSS could not bind well to Jurkat T cells through the same negative charge interaction of PSS and the cell membrane.

The presence of fluorescent dyes attached on PAH was used to confirm the cell encapsulation. There was no fluorescence signal detected in unencapsulated Jurkat T cells stained with FITC alone (Fig. 1a). However, FITC-positive cells were found in PAH-FITC@Jurkat cells (Fig. 1b). This indicates that cells were encapsulated with PAH. PSS/PAH@Jurkat cells stained with PAH-TRITC showed the red fluorescent signal (Fig. 1c). This confirms that PAH-TRITC could stain the cells through the PSS outer layer shielding on the Jurkat T cells. A similar result was also found in PSS-GNR/PAH@Jurkat cells (Fig. 1d). The negative control was prepared by staining PAH@Jurkat cells with PAH-TRITC. There was no presence of a red fluorescent signal. This result indicates that the same polyelectrolytes could prevent the binding between PAH and PAH-TRITC on the cell surfaces (Fig. 1e).

We also observed the morphology of Jurkat T cells by SEM. When compared to unencapsulated cells, the appearance of a smooth surface was detected on

Fig. 1 Images of unencapsulated Jurkat T cells stained with FITC alone (a), PAH-FITC@Jurkat cells (b), PSS/PAH@Jurkat cells stained with PAH-TRITC (c), PSS-GNR/PAH@Jurkat cells stained

with PAH-TRITC (d), and PAH@Jurkat cells with PAH-TRITC (e). The images were captured under bright field and fluorescent modes of a fluorescent microscope

Fig. 2 SEM images of unencapsulated (a) and encapsulated (PSS-GNR/PAH@Jurkat) Jurkat T cell (b)

encapsulated cells (PSS-GNR/PAH@Jurkat cells) (Fig. 2b). However, the surfaces of unencapsulated cells had abundant microvilli (Fig. 2a). Our results are consistent with the previous work published by Zhao et al. (2016) that used chitosan and alginate to conduct a conformal encapsulation of T cells.

All approaches used for confirming the encapsulation of Jurkat T cells showed that the cells were successfully encapsulated through a layer-by-layer technique. The cationic PAH was shielded on the negative charge of the Jurkat T cell membrane. The second layer of PSS or PSS-GNRs was formed surrounding each cell. Overall results from zeta potential measurement, fluorescent observation, and SEM demonstrated the formation of different encapsulated Jurkat T cells (PAH@Jurkat, PSS/PAH@Jurkat, and PSS-GNR/PAH@Jurkat cells).

Investigation of GNRs in PAH/PSS-GNR@Jurkat cells

The ICP-MS approach was used to confirm whether PAH@Jurkat cells could be shielded by PSS-GNRs. The TEM image of PSS-GNRs is shown in Fig. 3. The data from ICP-MS showed a significant presence of gold ($64.3 \pm 7.7 \mu\text{g L}^{-1}$) found in PSS-GNR/PAH@Jurkat cells (Fig. 3). The amount of gold observed provides evidence that PSS-GNRs (negatively charged surface) were shielded on the surface of PAH@Jurkat cells (positively charged cell surface) through the opposite charges of their surfaces. This result strongly indicates that a PSS-GNR layer formed on Jurkat T cells. The very small content of gold detected in PSS-GNR@Jurkat cells ($1.8 \pm 0.1 \mu\text{g L}^{-1}$) could occur due to non-specific binding of PSS-GNRs to cells.

Since the ICP-MS cannot perceive the difference between GNRs deposited on the cell surface and internalized GNRs, TEM was used to evaluate how GNRs were positioned on encapsulated Jurkat T cells. With TEM images, we found that PSS-GNRs were only located on the cell surface of Jurkat T cells encapsulated PAH layers (Fig. 4c, red arrow). This strongly indicates that PSS-

Fig. 3 The amount of gold detected in Jurkat T cells using the ICP-MS technique. *Significant difference in gold concentration at $P < 0.01$ compared with unencapsulated Jurkat T cells (Jurkat). Statistical analysis was performed by Tukey–Kramer test ($n \geq 3$). The TEM image shows the morphology of PSS-GNRs

GNRs only attached to the layer of PAH and did not internalize inside Jurkat T cells (Fig. 4c). It was reported by Fakhrullin et al. that the polyelectrolyte layer can act as a glue to attach metal nanoparticles and block nanoparticle translocation into the cells (Fakhrullin et al. 2012). As expected, there were no PSS-GNRs located on the cell surface, nor inside Jurkat T cells encapsulated with PSS/PAH (PSS/PAH@Jurkat cells; Fig. 4b) and unencapsulated Jurkat T cells (Fig. 4a). An excellent review article by Dykman and Khlebtsov (2014) has shown the effect of gold nanoparticles and their interaction with mammalian cells through cellular endocytosis. Our results here provide evidence that the PAH layer encapsulating on the cell surface might block the cellular uptake of PSS-GNRs through this endocytic pathway.

Cell viability and cell proliferation of unencapsulated and encapsulated Jurkat cells

To investigate whether the encapsulating layers affect the cell viability, we measured the cell viability at 0 and 24 h after cultivation of unencapsulated Jurkat T cells, PAH@Jurkat cells, PSS/PAH@Jurkat cells, and PSS-GNR/

Fig. 4 TEM images of unencapsulated Jurkat T cells (a), PSS/PAH@Jurkat (b), and PSS-GNR/PAH@Jurkat cells (c). PSS-GNRs are located (arrow) at the cell membrane of PSS-GNR/PAH@Jurkat cells

PAH@Jurkat cells, using the CellTiter-Glo assay. This technique measured viable cells from the content of adenosine-5'-triphosphate (ATP). After encapsulation, the cell viability of PAH@Jurkat cells, PSS/PAH@Jurkat cells, and PSS-GNR/PAH@Jurkat cells was measured immediately (0 h). The relative cell viabilities, based on that of unencapsulated cells of PAH@Jurkat cells, PSS/PAH@Jurkat cells, and PSS-GNR/PAH@Jurkat cells, were $\sim 94.4 \pm 0.3\%$, $90.8 \pm 1.2\%$, and $96.7 \pm 0.9\%$, respectively (Fig. 5a). Significant reductions ($P < 0.01$) in cell viability were observed in encapsulated PAH@Jurkat and PSS/PAH@Jurkat cells when compared with unencapsulated cells. The lowest cell viability was detected in PSS/PAH@Jurkat cells. The cell viability after 24-h cultivation of Jurkat T cells encapsulated with different forms was also measured. It showed that the cell viability of all encapsulated cells significantly decreased ($P < 0.01$) to around 8–15% depending on the encapsulation method.

Similar to the cell viability at 0 h, PSS/PAH@Jurkat cells at 24 h showed the lowest percentage of cell viability at $85.3 \pm 0.7\%$ (Fig. 5b). The cell viabilities of PSS-GNR/PAH@Jurkat and PAH@Jurkat were $87.0 \pm 0.5\%$ and

$91.5 \pm 0.6\%$, respectively (Fig. 5b). Although the results indicate that the metabolic activity of encapsulated cells was still active after culturing for 24 h, it seems that the number of layers could influence cell viability. Cells encapsulated with a layer of PAH (PAH@Jurkat cells) had a higher cell viability than cells encapsulated with two layers (PSS/PAH@Jurkat and PSS-GNR/PAH@Jurkat cells) after 24-h cultivation. The reason for this could be that there was a higher limitation of nutrient and waste diffusion through cells in cells encapsulated with two layers than that of encapsulation with one layer.

As reported in the review paper (Antipov and Sukhorukov 2004), a higher layer number could lead to a lower amount of penetrated molecules. When compared to the cell viability between PSS/PAH@Jurkat and PSS-GNR/PAH@Jurkat cells, there was no significant difference in cell viability ($P \leq 0.01$). Furthermore, cell proliferation was determined gain more information on the metabolic function of cells after encapsulation. Unlike cell viability assay that is normally used to determine the ratio of live and dead cells, it is well known that cell proliferation assay can be used to monitor the growth rate and metabolic activity of cells.

CellTiter 96[®] Aqueous One Solution cell proliferation assay was used to measure cell proliferation by measuring metabolic activity of cells through the reduction of MTS tetrazolium (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium compounds). We found that cell proliferation of PAH@Jurkat, PSS/PAH@Jurkat, PSS-GNR/PAH@Jurkat, and Jurkat T cells was increased when we increased the culturing time from 24 h to 48 and 72 h, respectively (Fig. 6). This indicates that the encapsulation of cells, using our approach here, could influence the growth rate of Jurkat cells. However, cell proliferation of unencapsulated Jurkat T cells was much higher than that of encapsulated cells.

The relative growth rates of unencapsulated Jurkat T cells at 48 and 72 h were increased ~ 1.4 - and 2.3 -fold, respectively, above unencapsulated Jurkat T cells cultured for 24 h. Around a 1.3 -fold increase in the proliferation rate of PSS/PAH@Jurkat and PSS-GNR/PAH@Jurkat cells cultured for 48 h was detected as compared to encapsulated Jurkat cells after 24 h culture. The proliferation rate of PAH@Jurkat at 48 h was ~ 1.1 -fold increased as compared to at 24 h culture. At 72-h culture, the increase in proliferation rate of all encapsulated Jurkat T cells (~ 1.4 – 1.6 -fold) was lower than that of unencapsulated Jurkat T cells (~ 2.3 -fold) when compared to 24-h culture of each cell condition (Fig. 6). The relative proliferating cell percentages of PAH@Jurkat, PSS/PAH@Jurkat, and PSS-GNR/PAH@Jurkat cells at 24 h significantly decreased from 100% (of unencapsulated Jurkat T cells) to $\sim 71.9 \pm 1.7\%$, $56.8 \pm 1.1\%$, and $50.2 \pm 1.5\%$,

Fig. 5 Cell viability of different encapsulated Jurkat T cells at 0 h (a) and 24 h (b) compared with unencapsulated Jurkat T cells. *Significant difference in cell viability at $P < 0.01$ compared with unencapsulated Jurkat T cells (Jurkat). Statistical analysis was performed by Tukey–Kramer test ($n \geq 9$)

Fig. 6 Cell proliferation of unencapsulated Jurkat T cells and encapsulated Jurkat T cells at 24, 48, and 72 h. The percentage of cell proliferation is a mean value of cell proliferation relative to the control (unencapsulated Jurkat T cells cultured for 24 h). $\neq$ Significant difference in cell proliferation at $P < 0.01$ compared with unencapsulated Jurkat T cells (Jurkat) at 24 h. $\#$ Significant difference in cell

proliferation at $P < 0.01$ compared with encapsulated PAH@Jurkat cells at 24 h. $\$$ Significant difference in cell proliferation at $P < 0.01$ compared with encapsulated PSS/PAH@Jurkat cells at 24 h. $*$ Significant difference in cell proliferation at $P < 0.01$ compared with encapsulated PSS-GNR/PAH@Jurkat cells at 24 h. Statistical analysis was performed by Tukey–Kramer test ($n \geq 6$)

respectively. A significant decrease in proliferation rate of PAH@Jurkat, PSS/PAH@Jurkat, and PSS-GNR/PAH@Jurkat cells cultured for 48 and 72 h was also detected as compared to unencapsulated Jurkat T cells ($P < 0.01$).

At 72 h, PSS-GNR/PAH@Jurkat cells had the highest increase in growth rate ratio (~ 1.6 -fold increase) among all types of encapsulated Jurkat T cells compared to a 24-h culture of encapsulated cells (PSS-GNR/PAH@Jurkat) (Fig. 6). The reason that encapsulated cells could proliferate could be a result of the mild layer-by-layer processing technique. In our case, it appears

that the charge density of PAH, PSS, and PSS-GNRs might have no major effect on cell proliferation. This might be due to the low concentration of PAH, PSS, and PSS-GNRs used for cell encapsulation. Although encapsulated cells could proliferate, it seems that the encapsulating layer could reduce the rate of proliferation when compared to unencapsulated Jurkat T cells. This could have occurred due to the effect of the encapsulating layer that is possibly impacting on the diffusion rate of molecules that pass through encapsulated cells (Cook et al. 2013).

Inflammatory cytokine responses of unencapsulated and encapsulated Jurkat T cells

T cells can produce inflammatory mediators such as IL-6 and TNF- α (Wang et al. 2006). However, high secretions of IL-6 and TNF- α (Wang et al. 2006) can lead to several diseases such as cystic fibrosis (Stecenko et al. 2001), autoimmune disease, and chronic inflammatory proliferative disease (Ishihara and Hirano 2002). Hence, we investigated the secretion of these cytokines from encapsulated and unencapsulated Jurkat cells. IL-2 is a lymphokine that plays an important role in maintaining activated T cell proliferation (Pawelec et al. 1982). Therefore, we evaluated whether different encapsulations applied in our study affected the secretion of IL-2 by Jurkat T cells. Besides IL-6, TNF- α , and IL-2, we also investigated the secretion of IL-1 β , another cytokine involved in inflammation induction (Tang et al. 2012).

The production of cytokines and lymphokines by encapsulated and unencapsulated Jurkat T cells was investigated at 5 and 24 h after cell encapsulation. Unencapsulated Jurkat cells were used as a control sample in our study (Fig. 7). When compared to unencapsulated Jurkat T cells, the levels of TNF- α and IL-1 β at 5 h secreted by PAH@Jurkat, PSS/PAH@Jurkat, and PSS-GNR/PAH@Jurkat cells were similar to unencapsulated Jurkat T cells (Fig. 7a, c). This indicates that the single layer (PAH) or the double layer (PSS/PAH and PSS-GNR/PAH) used for encapsulation of Jurkat T cells had no effect on TNF- α and IL-1 β induction. Similar results were also observed in TNF- α production at 24 h. However, in the case of IL-1 β , a significant IL-1 β reduction was found in PAH@Jurkat cells after treating for 24 h ($P < 0.01$). The reason for this is uncertain. However, it is a possibility that some IL-1 β molecules might not be able to pass through the PAH layer. The zeta potential of PAH@Jurkat cells was less negative than other encapsulating formats, and this could lead to different binding of PAH at the outer layer of cells to other molecules in cell culture media. This binding possibly affected IL-1 β release by blocking the diffusion of IL-1 β . A decrease in IL-2 levels was found in all encapsulated cells after culturing for 5 h. A significant reduction in IL-2 was detected in all encapsulated cells after cells were encapsulated and cultured for 24 h ($P < 0.01$). Werner et al. (2015) also found a reduction in IL-2 by Jurkat T cells encapsulated with polyelectrolytes (Fig. 7d).

In the case of IL-6 (Fig. 7b), at 24 h post-encapsulation, we found that cells encapsulated with PAH, PSS/PAH, and PSS-GNR/PAH secreted IL-6 at a significantly higher level than unencapsulated cells ($P < 0.01$). It was reported that the IL-6 secretion in encapsulated cells could be enhanced by polyelectrolytes used for encapsulation (Mooranian et al. 2016). Based on our results here, an

induction in IL-6 levels was significantly detected in PAH@Jurkat cells at 5 h post-encapsulation. This implies that the PAH layer could first impact IL-6 induction. After cells were encapsulated with the second layer, higher levels of IL-6 were detected. Thus, the second layer of PSS or PSS-GNRs could also be involved in triggering an immune response related to IL-6 expression. Furthermore, the second layer of encapsulation might lead to a decrease in porosity that could cause an accumulation of metabolic by-products related to IL-6 production (Mooranian et al. 2016; Schmidt et al. 2008).

Biological activity in co-culture between macrophage cells and Jurkat T cells encapsulated with polyelectrolytes and PSS-coated GNRs

It is well known that macrophage cells play an important role in the immune system. Because of the lack of information on biological activities between encapsulated cells and macrophages, we were therefore interested in the biological activities of these two cells in co-culture. The co-culture system of macrophages and T cells can be used to acquire more information on immune responses between encapsulated T cells and macrophages.

We used non-activated Jurkat T cells, with and without encapsulation, to investigate whether the encapsulation of cells could influence inflammatory cytokine enhancement in the co-culture system between human macrophages and human T cells. Here, we only focused on the investigation of the double layer encapsulation (PSS/PAH@Jurkat & PSS-GNR/PAH@Jurkat cells) because we aim to use PSS-GNR/PAH@Jurkat cells in a future study for therapeutic applications. The ratio of THP-1 macrophage per Jurkat T cell used in the co-culture system was 1:1. The results showed that there were no significant differences in IL-2 and IL-1 β expression in THP-1 macrophage and Jurkat T cell co-cultures when both encapsulated and unencapsulated Jurkat T cells were used (Fig. 8c, d). This implies that there was no cell-contact-mediated activation of THP-1 macrophages by encapsulated and unencapsulated Jurkat T cells. As stated previously, TNF- α and IL-6 cytokines are inflammatory cytokines and they may be involved in some disease pathogenesis (Rossol et al. 2005). Therefore, it was also worthwhile to test whether encapsulated cells could lead to induction of IL-6 and TNF- α expression.

Our results showed that there was a significant induction of TNF- α in THP-1 macrophages/unencapsulated Jurkat T cells after co-culturing for 24 h when compared with THP-1 macrophages cultured alone. However, there was no increase in TNF- α level when THP-1 macrophages were cultured with encapsulated Jurkat T cells (PSS/PAH@Jurkat or PSS-GNR/PAH@Jurkat) (Fig. 8b). These results

Fig. 7 Different pro-inflammatory responses of TNF- α (a), IL-6 (b), IL-1 β (c), and IL-2 (d) from each type of encapsulated and unencapsulated cells at 5 and 24 h post-encapsulation.

*Significant difference in pro-inflammatory responses at $P < 0.01$ compared with unencapsulated cells at 5 h and $\neq$ at 24 h. Statistical analysis was performed by Tukey–Kramer test ($n \geq 4$)

indicate that the encapsulation of Jurkat T cells could help prevent the induction of TNF- α production through cell-contact-mediated activation of THP-1 macrophages by Jurkat T cells. The layer of PSS/PAH or PSS-GNR/PAH could block the ligand interactions involved in TNF- α production by THP-1 macrophages resulting in no activation of the THP-1 macrophages to enhance TNF- α production. The TNF- α levels in co-cultures of THP-1 macrophages with PSS/PAH@Jurkat or PSS-GNR/PAH@Jurkat cells were similar. This implies that GNRs had no impact on inducing TNF- α . These two types of cell encapsulation resulted in different zeta potential values

(-21.1 ± 0.4 for PSS/PAH@Jurkat and -11.3 ± 0.4 for PSS-GNR/PAH@Jurkat cells), but it seems that the different degree of zeta potential values did not impact on the induction of TNF- α in our system.

In the case of IL-6 expression, we found that there was no significant difference in IL-6 production of THP-1 macrophage/Jurkat T cell (both unencapsulated and encapsulated cells) co-cultures. These results also revealed that there was no cell-contact-mediated activation of THP-1 macrophages through ligand interactions by encapsulated and unencapsulated Jurkat T cells (Fig. 8a). However, it is worth noting that though, there was an induction of IL-6 in

Fig. 8 The expression of inflammatory cytokines IL-6 (a), TNF- α (b), IL-1 β (c), and IL-2 (d) in THP-1 macrophages co-cultured with unencapsulated and encapsulated Jurkat T cells. Two different types of encapsulated Jurkat T cells (PSS/PAH@Jurkat and PSS-GNR/PAH@Jurkat) were used in this co-culture system. *Significant difference in TNF- α level at $P < 0.01$ compared with THP-1 macrophages alone. Statistical analysis was performed by Tukey–Kramer test ($n \geq 7$)

encapsulated Jurkat T cells. Nonetheless, the induction amount of IL-6 was much lower than Jurkat cells treated with a positive control chemical (phorbol 12-myristate 13-acetate; PMA) (Supplementary Material, Fig. S1) and

there was no significant production of IL-6 in THP-1 macrophage/Jurkat T cell co-culture. This implies that the induction amount of IL-6 in encapsulated Jurkat T cells might be at a level that does not lead to an adverse effect.

Conclusions

We demonstrate a single-cell encapsulation of Jurkat T cells and biological activities of cells after encapsulation. Jurkat T cells were successfully encapsulated with PAH, PSS/PAH, or PSS-GNR/PAH. Encapsulated cells mostly maintained viability and their metabolic activity. This could be due to the mild interaction between PAH, PSS/PAH, or PSS-GNR/PAH and the cell membrane. The cell proliferation assay confirmed that encapsulated cells were capable of cell division, and PSS-GNR/PAH@Jurkat cells had the highest cell proliferation rate. In THP-1 macrophage/Jurkat cell co-cultures, the encapsulation of Jurkat T cells could shield some cell surface functions that impacts cell-to-cell signaling between THP-1 macrophages and Jurkat T cells, resulting in no induction of TNF- α or other cytokines observed in the co-cultures. Unlike the macro- or microcapsule cell encapsulation technique, cells were singly encapsulated with a thin layer of polyelectrolytes and polyelectrolyte-coated GNRs, which had no significant effect on cell size. Therefore, this technique should avoid the problem of blood flow blockage when cells are applied in a blood circulation system. As well, with the property of GNRs mentioned earlier, to combine GNRs on a layer of encapsulated cells should help increase the efficiency for diagnostic or therapeutic purposes. Overall, the assessment of biological activities found in this study suggests that the combination of polyelectrolytes and GNRs for single-cell encapsulation was reasonably biocompatible with Jurkat T cells and macrophages. The results from this study support continuing and further developing studies based on using this encapsulation technique, combined with GNRs, in various biomedical applications without adverse inflammatory effects.

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