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Molecular insights for the biological interactions between polyethylene glycol and cells

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ABSTRACT

As the gold standard polymer for drug delivery system, polyethylene glycol (PEG) has excellent biocompatibility. It's reported that the low nonspecific interactions between PEG and body contribute to its biocompatibility. However, here we discover dynamic biological interactions exist between PEG and cells on the molecular level. PEG (2 kD) can induce metabolism modulations and survival autophagy by creating an intracellular hypoxic environment, which act as cellular survival strategies in response to the hypoxia. In the cellular adaption process during hypoxia, PEG-treated cells decrease energy consumption by reducing cell growth rate, increase energy supply by amino acid catabolism in a short period, and survival autophagy over a relatively long period, to keep energy homeostasis and survival. Our research provides molecular insights for understanding the mechanism underlying the excellent biocompatibility of PEG, which will be of fundamental importance for further related studies on other polymers and development of polymeric materials with improved characteristics.

1. Introduction

Over the past decades, the extensive applications of polymeric materials in biomedical field [1–10], such as drug delivery, gene transfection, invasive sensors, tissue engineering and implantable medical devices, have greatly promoted the progress of human medical technology. Currently, polymers have become the focus in the research of biomaterials [11] since they can be modified readily for diverse compositions, properties, and forms to satisfy the clinical requirements [12]. The primary requirement for a biomaterial candidate is the biocompatibility [13]. On the basis of preclinical studies and clinical experiences, polyethylene glycol (PEG) has been considered as a good representative of biomedical polymers due to its advantageous properties, such as high water-solubility, stealth effect, prolonged blood circulation and excellent biocompatibility [1–5,14,15]. However, with the overwhelming applications of PEG, the shortcomings of PEG have also gradually emerged [15–19]. It's reported that PEG can trigger complement activation and cause subsequent hypersensitivity reactions [15,17]. Furthermore, anti-PEG antibodies have occurred in some clinical cases and led to the accelerated blood clearance for PEG or PEGylated products with repeated injection (so called ABC phenomenon) [15,18,19]. In addition, chronic use of PEG with high molecular can lead to accumulation in tissues due to its non-biodegradability, and whether it has adverse effect on the tissues is uncertain [15]. Thus, development of alternative biocompatible polymers is urgently needed. Thus far, the molecular mechanisms underlying the biocompatibility of biomedical polymers remain unclear [10,14,15,20–22], understanding of which will be of fundamental importance for developing novel safer biomedical polymeric materials. Numerous studies on the biocompatibility of PEG focus on the pathological effects and pharmacokinetics of PEG, which mostly

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traced back to the middle of last century (from the 1950s to 1970s), and fail to define the molecular mechanism underlying its biocompatibility [15]. With the rapid development of biology knowledge and techniques, there is an immediate need to update the researches by fundamental systemic evaluation on the interactions between PEG with biological system particularly at the cellular or molecular levels to clarify the molecular mechanism inherent to its excellent performance [15]. Indeed, the investigations that systematically study the interactions between polymers and cells are just beginning and urgently needed for the design and development of therapeutic polymeric materials [20].

In this study, we choose PEG (molecular weight 2 kD) as a representative biocompatible polymer to illuminate its detailed biological interactions with cellular system.

2. Materials and methods

2.1. Materials

PEG (2 kD, linear) was purchased from Sigma (BioUltra, Catalog#84797). The contents of metal impurities (20 metal ions) were extremely low shown in Supplementary Table 2.

2.2. Cell lines and culture

BRL-3A (buffalo rat liver cells), HUVEC (human umbilical vascular endothelium cells), SK-HEP-1 (human hepatocarcinoma cells) and 786-O (human renal adenocarcinoma cells) were authenticated since they were purchased from ATCC. All cell lines were tested negative for mycoplasma contamination. All cells were cultured in basal medium supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich) and 1% penicillin–streptomycin (Sigma-Aldrich), at 37 °C, 5% CO_2 in a humidified atmosphere. The basal medium for BRL-3A and SK-HEP-1 cells was DMEM (high glucose, containing 1% L-glutamine, Thermo Scientific), while that for 786-O cells was RPMI-1640 (Thermo Scientific). HUVEC cells were cultured in completed ECM (ScienCell). All cells were passaged at 80% confluence.

2.3. MTT assay

The effect of different dose and co-culture time of PEG (MW 2 kD) on the growth rate of BRL-3A cells in vitro was evaluated by MTT assay. BRL-3A cells were seeded into 96-well plates at a density of 8×10^3 cells in 180 μL DMEM per well. Each group had five replicate wells. After 24 h, for the determination of the dose effect of PEG on cells, 20 μL PEG solution at serial concentrations was added into each well in PEG treated groups (n = 5) to achieve serial final concentrations (0.001 mM, 0.01 mM, 0.1 mM, 1 mM, 5 mM, 10 mM, 20 mM) in DMEM, while 20 μL phosphate-buffered saline (PBS) was added in each well in the control groups (n = 5). The cells in all groups were further cultured for 24 h. For evaluation of the effect of different co-culture time of PEG (2 kD, 20 mM) on cells, BRL-3A cells were seeded into 96-well plates at a density of 1×10^3 cells in 180 μL DMEM per well. 20 μL PEG solution was added into each well in PEG treated groups (n = 5) at serial time points respectively to achieve a final concentration of 20 mM in DMEM, while 20 μL PBS was added in each well in the control groups (n = 5). The total culture time for groups varying in serial co-culture time with PEG (6 h, 12 h, 24 h, 48 h, 72 h, 96 h) was the same. 20 μL of 5 mg/mL MTT (Sigma) assay stock solution was added in each well and incubated for 4 h at 37 °C. Then removed the supernatant, added 200 μL dimethylsulfoxide (DMSO) in each well and vortexed the plates for 10 min to dissolve the insoluble formazan thoroughly. The optical density (OD) was read at 490 nm by microplate reader (BioTek, SynergyH4).

The role of autophagy on PEG (2 kD, 20 mM) treated cells was determined by the difference of OD values from MTT assay in four groups including control groups, PEG alone treated groups, 3-MA alone treated groups, PEG plus 3-MA treated groups. Each group had five parallel repeats. BRL-3A cells were seeded into 96-well plates at a density of 8×10^3 cells per well. PEG was added at a final concentration of 20 mM in each well in PEG alone treated groups and PEG plus 3-MA treated groups, while PBS was added in each well in control groups. 3-MA was added at a final concentration of 5 mM in each well in 3-MA alone treated and PEG plus 3-MA treated groups at 2 h before adding PEG. All groups were cultured for 24 h for MTT assay described above.

2.4. Apoptosis analyses

Cells were seeded into 6-well plates at a density of 2.5×10^4 cells in 1.8 mL DMEM per well and allowed to adhere overnight. 200 μL PEG (2 kD, 200 mM) was added in wells treated by PEG with serial co-culture time (12 h, 24 h, 48 h, 72 h, 96 h) at a final concentration of 20 mM, while 200 μL PBS was added in the untreated wells. The total culture time for groups varying in serial co-culture time with PEG was the same. Finally, all cells (attached or supernatant) were harvested and washed in cold PBS. Staining of propodium iodide (PI) and annexin V-FITC for cell apoptosis analysis was performed according to Alexa fluor® 488 annexin V/dead cell apoptosis kit (Invitrogen, Catalog nos. V13241 and V13245). Samples were detected on a flow cytometer (BD&LSR Fortessa).

2.5. ROS and mitochondrial membrane potential (MMP) analysis with flow cytometer

Cells were seeded into 6-well plates at a density of 2×10^5 cells in 1.8 mL DMEM per well and allowed to adhere overnight. 200 μL PEG (20 kD, 200 mM) was added in PEG-treated wells at a final concentration of 20 mM and co-cultured for 24 h, while 200 μL PBS was added in the untreated wells. All cells (attached or supernatant) were harvested. ROS analysis was conducted according to commercial CellROX® Oxidative Stress Reagents (Life technologies, Catalog nos.C10444). The cells were stained by CellROX® green reagent at a final concentration of 500 nM and incubated for 30 min at 37 °C. Mitochondrial membrane potential was evaluated by 5,5'-,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimi dazolylcarbocyanide iodine (JC-1) Mitochondrial Potential Sensors (Invitrogen). The cells were stained by JC-1 at a final concentration of 2 μM and incubated for 20 min at 37 °C. Both ROS signal and JC-1 signal were analyzed immediately on a flow cytometer (BD&LSR Fortessa).

2.6. ROS and JC-1 imaging with fluorescence microscope

Placed a clean cover glass in each well of the 6-well plates. BRL-3A cells were seeded into each well at a density of 1×10^5 cells in 1.8 mL DMEM and allowed to adhere overnight. Cells were treated as described above in flow cytometer analysis section. Cells CellROX® Reagent was added in the wells at a final concentration of 5 μM and the cells were incubated for 30 min at 37 °C. Then quickly removed the DMEM, washed the adherent cells twice by PBS and fixed the cells with 4% ice-cold paraformaldehyde for 30 min. The fixed cells were washed with PBS for 3 × 5 min and stained with Hoechst 33342 at a final concentration of 2 μg/mL for 10 min to locate the nucleus. Washed the cells 3 × 5 min with PBS. Took out the cover glass, rinsed it quickly with ultra-water, and covered it on the slide glass with fluorescence antifade mounting medium. The signal of ROS was analyzed on a fluorescent microscope (Leica) at an excitation wavelength of 488 nm within 24 h.