Biomaterials xxx (2018) 1–16

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Near-infrared light-controlled regulation of intracellular calcium to modulate macrophage polarization

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ARTICLE INFO

Article history:
Received 17 December 2017
Received in revised form
6 February 2018
Accepted 4 March 2018
Available online xxx

Keywords:
Near-infrared light control
Photocleavable cap
Upconversion nanoparticle
Calcium regulation
Macrophage polarization

ABSTRACT

Macrophages are multifunctional immune cells with diverse physiological functions such as fighting against infection, influencing progression of pathologies, maintaining homeostasis, and regenerating tissues. Macrophages can be induced to adopt distinct polarized phenotypes, such as classically activated pro-inflammatory (M1) phenotypes or alternatively activated anti-inflammatory and pro-healing (M2), to execute diverse and dynamic immune functions. However, unbalanced polarizations of macrophage can lead to various pathologies, such as atherosclerosis, obesity, tumor, and asthma. Thus, the capability to remotely control macrophage phenotypes is important to the success of treating many pathological conditions involving macrophages. In this study, we developed an upconversion nanoparticle (UCNP)-based photoresponsive nanocarrier for near-infrared (NIR) light-mediated control of intracellular calcium levels to regulate macrophage polarization. UCNP was coated with mesoporous silica (UCNP@mSiO_2), into which loaded calcium regulators that can either supply or deplete calcium ions. UCNP@mSiO_2 was chemically modified through serial coupling of photocleavable linker and Arg-Gly-Asp (RGD) peptide-bearing molecular cap *via* cyclodextrin-adamantine host-guest complexation. The RGD-bearing cap functioned as the photolabile gating structure to control the release of calcium regulators and facilitated the cellular uptake of UCNP@mSiO_2 nanocarrier. The upconverted UV light emission from the UCNP@mSiO_2 under NIR light excitation triggered the cleavage of cap and intracellular release of calcium regulators, thereby allowing temporal regulation on the intracellular calcium levels. Application of NIR light through skin tissue promoted M1 or M2 polarization of macrophages, by elevating or depleting intracellular calcium levels, respectively. To the best of our knowledge, this is the first demonstration of NIR light-mediated remote control on macrophage polarization. This photoresponsive nanocarrier offers the potential to remotely manipulate *in vivo* immune functions, such as inflammation or tissue regeneration, *via* NIR light-controlled macrophage polarization.

1. Introduction

Macrophages are multifunctional immune cells that regulate tissue development and regeneration, homeostasis maintenance, defense against infection, and the progression of diseases [1–3]. When macrophages are activated in response to external stimuli, they are polarized into distinct phenotypes to perform

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https://doi.org/10.1016/j.biomaterials.2018.03.007
0142-9612/

immunoregulatory functions [4]. Macrophages can be polarized into classically activated pro-inflammatory (M1) phenotypes that assist fighting against infection or alternatively activated anti-inflammatory and pro-healing (M2) phenotypes that promote tissue regeneration. Macrophage polarizations have been shown to be regulated by various signals, such as physical and mechanical cues [5], transcriptional signals [6], and chemokines [7]. Unbalanced macrophage polarizations are known to be involved in various pathologies, such as atherosclerosis, obesity, tumor, and asthma [8]. Therefore, controlling macrophage polarizations hold great promise in treating these diseases. Meanwhile, increasing research attention has been dedicated to developing biomaterials to mediate various immunomodulatory functions, such as anti-inflammation [9], immunization [10,11], immune activation [12,13], immune cell targeting [14,15], hematopoietic functions [16], and macrophage polarization-regulated tissue regeneration [17–19].

Owing to the vital roles of macrophages in regulating host immune functions dependent upon their polarization phenotypes, various biomaterials have been designed to regulate macrophage polarization [20–22]. For example, biomaterials have been developed with a variety of chemical functional groups [19], fiber dimensions [23], mechanical properties [24], micropatterned spacing [25], and nanotopography [26], to statically modulate macrophage polarization. In particular, basic calcium phosphate crystals that release calcium ions were synthesized to induce pro-inflammatory function of macrophages, such as the production of pro-inflammatory cytokine IL-1β [27]. There are a number of previous reports that have suggested the role of calcium in regulating macrophage polarization [28–31]. For instance, extracellular calcium levels regulate phosphorylation of protein kinase C β (PKCβ) to modulate macrophage polarization [29]. Furthermore, blocking extracellular calcium influx through the calcium channels inhibits pro-inflammatory polarization of macrophages [28,31]. These findings suggest that regulating intracellular calcium levels of macrophages through controlled intracellular delivery of calcium regulators by biomaterials may modulate their polarization phenotypes.

The chemical design of biomaterials that allow the remote manipulation of polarization phenotypes of macrophages is an attractive strategy to dynamically regulate polarization-specific functions, but has rarely been demonstrated. We recently utilized magnetic remote control of ligand to modulate the adhesion and polarization of macrophages on the surface of biomaterials engineered with mobile magnetic nanoparticles [32]. In addition, ultraviolet (UV) light was employed to temporally modulate ligand presentation on the surface of photosensitive biomaterials for regulating macrophage adhesion [33]. However, UV light is prone to cause photodamage to cells and shows limited tissue penetration depth, thereby limiting its biomedical applications. Alternatively, near-infrared (NIR) light has enhanced tissue penetration and shows minimal cytotoxicity. Recently, lanthanide-doped upconversion nanoparticles (UCNPs) were developed for imaging and photoactivation by NIR light through upconversion of NIR light to UV light [34,35]. Chemical modifications of UCNPs enabled their versatile applications, such as *in vivo* targeting and imaging [11,36,37] as well as diagnostic imaging or monitoring of atherosclerosis plaques [38], rheumatoid arthritis [39], blood pool [40], and intracellular glutathione [41]. UCNPs have been also prepared for photoactivation for use in *in vivo* photodynamic therapy [42,43], ion channel activation [44], photoisomerization [45], and photocleavage [46]. Furthermore, chemical modifications of nanoparticles for spherically shaped assembly [47] as well as conjugation of dual peptide [48] or targeting ligand including RGD peptide [49,50], could be applied to the UCNPs to promote their intracellular uptake. We recently demonstrated that NIR light-controlled intracellular release of UCNP-tethered inductive molecules enhanced stem cell differentiation [51]. However, there have been no precedent studies of utilizing UCNPs to regulate macrophage polarization by NIR light. We believe that the versatility of UCNPs makes them the ideal vehicle for NIR light-controlled intracellular delivery of calcium regulators to modulate macrophage polarization.

In this study, we developed a UCNP-based nanocarrier to mediate NIR light-controlled change in intracellular calcium concentration to regulate macrophage polarization as illustrated in Scheme 1. UCNPs were coated with mesoporous silica shell (UCNP@mSiO_2) as the nanocarrier of calcium regulators. Calcium regulators included (1-(4,5-dimethoxy-2-nitrophenyl)-1,2-diaminoethane-N,N,N′,N′-tetraacetic acid), DMNP-EDTA-Ca^{2+} complex for calcium supply or (1,2-bis(2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester)), BAPTA-AM for calcium chelation. UCNP@mSiO_2 was further conjugated with the RGD peptide-bearing molecular cap *via* a photolabile linker as the photosensitive gating structure to control the release of calcium regulators (supplier or chelator). Our findings showed that NIR light illumination enabled on-demand intracellular release of calcium regulators *via* cleavage of the photosensitive cap and time-regulated control of intracellular calcium levels. This NIR light-induced elevation or depletion of intracellular calcium ions further promoted M1 or M2 polarization of macrophages, respectively, even when NIR light was applied through an intervening layer of animal skin tissue. Dynamic control of macrophage polarization by NIR light can allow remote manipulation of host immune system through regulation of its pro-inflammatory M1 or pro-healing M2 macrophage phenotypes to potentially treat inflammatory diseases or expedite tissue healing and regeneration.

2. Materials and methods

2.1. Synthesis of $NaYF_4$: Yb, Tm upconversion nanoparticles (UCNPs)

UCNPs were synthesized in accordance with a previous protocol [52]. In brief, YCl_3 (0.8 mmol), $YbCl_3$ (0.18 mmol) and $TmCl_3$ (0.02 mmol) were mixed with 6 mL of oleic acid and 15 mL of octadecene (ODE). The solution was heated to 150 °C to form a homogeneous solution and then slowly cooled down to room temperature. 10 mL of methanol solution containing NaOH (2.5 mmol) and NH_4F (4 mmol) was slowly added into the mixture that was then subject to stirring for 30 min. Subsequently, the solution was slowly heated to remove methanol, degassed at 100 °C for 10 min, heated to 300 °C, and maintained 300 °C for 2 h under argon protection. After the solution was cooled down to room temperature under stirring, nanocrystals were precipitated from the solution with ethanol, washed with methanol/ethanol (1:1 v/v) for three times, and then re-dispersed in cyclohexane.

The size and morphology of the UCNPs were characterized using a Transmission Electron Microscope (TEM; Hitachi H7700), and their photoluminescence spectrum with near-infrared light (NIR) laser (980 nm) was recorded with F-4600 spectrophotometer (Hitachi).

2.2. Synthesis and characterization of mesoporous silica-coated UCNPs (UCNP@mSiO_2)

The as-prepared UCNPs (3 mg) were mixed with cyclohexane (10 mL), Triton X-100 (2 mL), 1-hexanol (2 mL), ammonia solution (30 wt%, 200 μL), and DI water (400 μL), and then stirred for 30 min. Tetraethyl orthosilicate (TEOS, 15 μL) and APTES ((3-aminopropyl) triethoxysilane, 3 μL) were added into the mixture. The reaction

Please cite this article in press as: H. Kang, et al., Near-infrared light-controlled regulation of intracellular calcium to modulate macrophage polarization, Biomaterials (2018), https://doi.org/10.1016/j.biomaterials.2018.03.007