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Antioxidant functionalized polymer capsules to prevent oxidative stress

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ABSTRACT

Polymeric capsules exhibit significant potential for therapeutic applications as microreactors, where the bio-chemical reactions of interest are efficiently performed in a spatial and time defined manner due to the encapsulation of an active biomolecule (e.g., enzyme) and control over the transfer of reagents and products through the capsular membrane. In this work, catalase loaded polymer capsules functionalized with an external layer of tannic acid (TA) are fabricated via a layer-by-layer approach using calcium carbonate as a sacrificial template. The capsules functionalised with TA exhibit a higher scavenging capacity for hydrogen peroxide and hydroxyl radicals, suggesting that the external layer of TA shows intrinsic antioxidant properties, and represents a valid strategy to increase the overall antioxidant potential of the developed capsules. Additionally, the hydrogen peroxide scavenging capacity of the capsules is enhanced in the presence of the encapsulated catalase. The capsules prevent oxidative stress in an *in vitro* inflammation model of degenerative disc disease. Moreover, the expression of matrix metalloproteinase-3 (MMP-3), and disintegrin and metalloproteinase with thrombospondin motif-5 (ADAMTS-5), which represents the major proteolytic enzymes in intervertebral disc, are attenuated in the presence of the polymer capsules. This platform technology exhibits potential to reduce oxidative stress, a key modulator in the pathology of a broad range of inflammatory diseases.

Statement of Significance

Oxidative stress damages important cell structures leading to cellular apoptosis and senescence, for numerous disease pathologies including cancer, neurodegeneration or osteoarthritis. Thus, the development of biomaterials-based systems to control oxidative stress has gained an increasing interest. Herein, polymer capsules loaded with catalase and functionalized with an external layer of tannic acid are fabricated, which can efficiently scavenge important reactive oxygen species (i.e., hydroxyl radicals and hydrogen peroxide) and modulate extracellular matrix activity in an *in vitro* inflammation model of nucleus pulposus. The present work represents accordingly, an important advance in the development and application of polymer capsules with antioxidant properties for the treatment of oxidative stress, which is applicable for multiple inflammatory disease targets.

1. Introduction

Polymer capsules have traditionally been employed in the biomedical field as drug/gene/protein delivery vehicles [1–3]. Based on natural or synthetic polymers, these microstructures allow for the controlled spatiotemporal release of the encapsulated entities through polymeric systems that respond to single or multiple biologically relevant stimuli such as pH, [4] temperature [5] and cell biomarkers [6]. Apart from the aforementioned application, advances in biosciences and polymer synthesis have allowed the development of novel applications of polymer capsules in the biomedical field, including their use as microreactors, artificial organelles or cell mimics [7–10].

As microreactors, polymer capsules accommodate active biomolecules (e.g., enzymes) inside their inner cavity that act *in situ* due to a continuous transfer of reagents and products through a shell/membrane with selective permeability [11]. Polymer vesicles

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(i.e., polymersomes), which are fabricated in the absence of a sacrificial template via self-assembly of amphiphilic block copolymers, have been widely employed as nanoreactors which facilitate single or cascade enzymatic reactions [12–15]. In these systems, the permeability of the membrane is finely tuned through the use of copolymers that self-assemble in compartments with a porous membrane, [16] the incorporation of biopores/protein channels in the membrane, [17–20] or the use of copolymers generating compartments whose membranes undergo conformational changes in response to specific stimuli [21,22].

Apart from polymersomes, controlled enzymatic reactions can also be performed within polymer capsules fabricated using a sacrificial template which act as a microreactor [23–25]. These polymer capsules are usually fabricated via a layer-by-layer (LbL) approach which involves the deposition of polymer layers through electrostatic, covalent or hydrogen bonding interactions on a sacrificial core template which is then removed. Additionally, these systems can be further modified via the incorporation of nanoparticles or surface modification processes to yield polymer capsules with advanced functionalities [26,27]. For the encapsulation of enzymes and the subsequent use as microreactors, calcium carbonate ($CaCO_3$) represents the most promising sacrificial template because of its inherent highly porous structure, ease of fabrication, high encapsulation efficiencies, which can be dissolved under mild conditions [28]. For this reason, several enzymatic reactions performed within polymer capsules, fabricated employing $CaCO_3$ as a sacrificial template, have been reported in the literature [29–31]. Despite the promising results observed in terms of encapsulation efficiencies, and enzyme activity, the translation to therapeutic applications has not yet been achieved and only a few studies have reported the use of these capsules in *in vitro* models [32].

We developed polymer capsules that acted as antioxidant microreactors which scavenge reactive oxygen species (ROS). These systems were tested to prevent oxidative stress in an interleukin-1β (IL-1β) induced inflammation model of nucleus pulposus (NP). ROS such as hydrogen peroxide (H_2O_2), hydroxyl (.OH) and superoxide anion ($.O_2^-$) radicals are important signalling molecules that play a pivotal role in several cellular events (including gene expression, transcription factor activation, DNA damage, cellular proliferation and apoptosis) [33,34]. Under normal physiological conditions, the levels of ROS are efficiently regulated by several molecules and enzymes including glutathione, superoxide dismutase and catalase which exhibit antioxidant properties [35]. However, overproduction of ROS can overwhelm the antioxidant capacity of cells resulting in oxidative stress. The overproduction of ROS has been implicated in numerous disease pathologies (including cancer, [36,37] neurodegeneration [38] and diabetes [39]). In degenerative disc disease, several *in vitro* and *in vivo* studies conducted on NP and annulus fibrosus (AF) cells have reported the activation of important signalling pathways including p38 mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinases (ERKs), Jun amino-terminal kinases (JNKs), Akt, and nuclear factor-κB (NF-κB) [40] mediated by excessive ROS that induces senescence and apoptosis [41]. This promotes a catabolic and pro-inflammatory cell phenotype [42,43] [as demonstrated by an up-regulated expression of proteolytic enzymes (matrix metalloproteinases (MMPs), and disintegrins and metalloproteinase with thrombospondin motifs (ADAMTSs)) and pro-inflammatory cytokines (IL-1β, interleukin-8 (IL-8), interleukin-6 (IL-6)]. This causes an imbalance in matrix homeostasis which results in degenerative disc disease which is one of the main causes of low back pain. Considering the socio-economic impact, 60–80% of the population of developed countries are affected by low back pain at some stage in their lives, [44,45] the development of biomaterial-based systems to restore matrix homeostasis has gained significant interest in recent years [46].

We hypothesize that polymer capsules with antioxidant properties will prevent oxidative stress and attenuate the expression of major proteolytic enzymes in an IL-1β induced inflammation model of NP. We fabricated polymer capsules loaded with catalase and functionalized with an external layer of TA via a LbL approach using $CaCO_3$ as a sacrificial template. Following comprehensive physico-chemical and functional characterization of the polymer capsules, their therapeutic efficacy was assessed on NP cells stimulated with IL-1β by analysing intracellular oxidative stress and the expression of matrix metalloproteinase-3 (MMP-3) and disintegrins and metalloproteinase with thrombospondin motif -5 (ADAMTS-5) (Fig. 1).

2. Materials and methods

2.1. Materials

$CaCl_2$, Na_2CO_3, NaCl, ethylenediaminetetraacetic acid (EDTA), poly (allylamine hydrochloride) (PAH) ($M_w \sim 58{,}000$ Da), dextran sulphate (DEX) from *Leconostoc* spp. (DEX) ($M_w > 500{,}000$ Da), fluorescein 5(6)-isothiocyanate (FITC), FITC-dextran sulphate sodium salt ($M_w \sim 500{,}000$ Da), tannic acid (TA), bovine serum albumin (BSA), 50 wt% hydrogen peroxide solution, $FeSO_4{\cdot}7H_2O$, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), TRI Reagent®, Hank's Balanced Salt Solution (HBSS), high glucose Dulbecco's modified Eagle's medium (HGDMEM), penicillin-streptomycin solution (P/S), foetal bovine serum (FBS), protease from *Streptomyces griseus*, collagenase from *Clostridium histolyticum*, fluorimetric hydrogen peroxide assay kit, Bradford reagent and protease inhibitor cocktail were purchased from Sigma-Aldrich (Ireland). AlamarBlue® cell viability reagent, live/dead® viability kit, rhodamine phalloidin, Hoechst staining solution, CellROX® green reagent, propidium iodide, BCA protein assay kit and SuperSignal West Pico Chemiluminescent substrate were purchased from ThermoFisher Scientific (Ireland). Human recombinant interleukin-1β cytokine was purchased from PeproTech (USA) whereas phosphatase inhibitor (PhosSTOP) was purchased from Roche (USA).

2.2. Fabrication and characterization of polymer capsules

2.2.1. Fabrication of polymer capsules

Polymer capsules were fabricated via the LbL approach using $CaCO_3$ microparticles as a sacrificial template and PAH, DEX and TA as polyelectrolytes. For the fabrication of the sacrificial template, 0.66 mL of a 1 M Na_2CO_3 solution and 0.66 mL of a 2 mg/mL catalase solution were poured into an equal volume of 1 M $CaCl_2$ solution. In a particular case, FITC-labelled catalase was employed to demonstrate the successful encapsulation of the enzyme in the final polymer capsules. After vigorous stirring of these solutions for 30 s, the obtained dispersion was centrifuged and the particles were washed several times with a 0.005 M NaCl solution. Then, the particles were suspended in a 2 mg/mL PAH solution of pH 6.5. After fifteen minutes of incubation, the particles were centrifuged and washed several times with a 0.005 M NaCl solution. Then, particles were suspended in a 2 mg/mL DEX solution at pH 6.5. This process was repeated until three layers of PAH and two layers of DEX were alternatively deposited. Finally, the particles were suspended in a 3 mg/mL TA solution at pH 6.5 and incubated for fifteen minutes. After the corresponding washing steps, the particles were submerged in a 0.1 M EDTA solution. After incubating for five minutes, the particles were recovered by centrifugation. This process was repeated four times to guarantee the complete removal of the sacrificial template. The resulting polymer capsules were washed several times with PBS prior to conducting physico-chemical and morphological characterization.