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Nanoinitiator for enzymatic anaerobic polymerization and graft enhancement of gelatin–PAAM hydrogel†

Qi Zhang, Yanan Lv, Mingyu Liu, Xia Wang,* Yongli Mi* and Qigang Wang *

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As an emerging method for mildly molding polymer hydrogel bioscaffolds, the enzymatically polymerized system is mainly based on the screening of various oxidoreductases to produce radicals, but the design of multifunctional nanoinitiators to facilitate hydrogel performance remains challenging. Here, we utilize *N*-hydroxyimide-modified silica nanoparticles as nanoinitiators to simultaneously trigger glucose oxidase anaerobic polymerization and nanoparticle-grafting enhancement of the gelatin–polyacrylamide (PAAM) hydrogel. The enzyme–nanoinitiator system produced nitrogen radicals, which were further converted into carbon radicals *via* GOx-catalyzed glucose reduction, as confirmed by electron paramagnetic resonance (EPR) analysis. Our stretchable hydrogel has a 12-fold increased fracture energy relative to traditional hydrogel due to grafting enhancement by the nanoinitiator. The temperature-dependent physical crosslinking of gelatin endowed our hydrogel facile printing ability. Cytotoxicity assay and 3D cell culture demonstrated the low toxicity of our hydrogel. As the first example of the use of nanoinitiators for enzymatic polymerization, this work provides a biocompatible platform to prepare or print hydrogel bioscaffolds with the required mechanical strength.

Introduction

Hydrogels with hydrophilic macromolecular networks are promising synthetic equivalents for biological applications such as drug delivery,[1,2] cell culture[3–6] and tissue engineering.[7–11] Regarding hydrogel designs or 3D printing, the current research mainly focuses on selecting natural or synthetic polymer components and optimizing the network crosslinks.[12–14] However, the initiation techniques are also a critical factor in obtaining hydrogel bioscaffolds with adjustable mechanical strength. Conventional radical-polymerized methodologies *via* photo or thermal initiators limit the biological applications due to the harsh operating conditions and toxic initiator residues.[15–17] As early as the 1950s, Parravano *et al.* reported the first enzymatic polymerization of methyl methacrylate by using xanthine oxidase to produce free radicals from substrates.[18] Recently, tremendous works have been performed to synthesize many kinds of macromolecules using oxidoreductase-initiated radical polymerization.[19,20] The earliest work involving the preparation of polymer hydrogel *via* enzyme-initiated radical polymerization was developed by Bowman and co-workers using glucose oxidase (GOx) and ferrous ion as the reagents.[21] Our group has also developed several oxidoreductases, such as horseradish peroxidase (HRP),[22] laccase,[23] and cascade enzymes (HRP-GOx)[24] to catalyse electron-transfer reactions and thus generate radicals capable of initiating polymerization. Surprisingly, we have recently discovered that our anaerobic GOx/*N*-hydroxyimide-initiated system could produce free radicals only through glucose reduction without oxidizing agents, such as O_2 and H_2O_2. Normally, GOx catalyses the conversion of glucose into glucolactone, with H_2O_2 as the reductive product of O_2. In an anaerobic environment, *N*-hydroxyimide compounds might be reduced by GOx/glucose systems to serve as the initiator of radical polymerization.

It is well known that nanocomposite (NC) hydrogels with inorganic nanoclay,[25] layered double hydroxide,[26] graphene oxide[27,28] and silica nanoparticles[29,30] could significantly improve the compressive and tensile properties due to physical or chemical interactions between inorganic nanoparticles and polymer chains to form sacrificial bonds. Among NC hydrogels, silica-based nanocomposite hydrogels are thought to be suitable biological platforms due to their low toxicity, physiological inertness and easy modifiability. Here, *N*-hydroxyimide-modified SiO_2 nanoparticles (*N*-hydroxyimide@SiO_2) are selected as the nanoinitiators for enzymatic polymerization. Beyond the effective initiation of radical polymerization, this novel enzyme–nanoinitiator system is expected to enhance hydrogel mechanical strength and

School of Chemical Science and Engineering, Tongji University, Shanghai 200092, China. E-mail: 15174@tongji.edu.cn, keymix@tongji.edu.cn, wangqg66@tongji.edu.cn

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RESEARCH ARTICLE

Neutrophils-Inspired Supramolecular Nanogel for Magnetocaloric-Enzymatic Tandem Therapy

Qi Zhang,[‡] Jiaojiao Wu,[‡] Jingjing Wang,[‡] Xia Wang, Chu Wu, Mengwei Chen, Qing Wu, Maciej S. Lesniak, Yongli Mi, Yu Cheng* and Qigang Wang*

Abstract: Neutrophils can responsively release reactive oxygen species (ROS) to actively combat infections by exogenous stimulus and cascade enzyme catalyzed bio-oxidation. Inspired by this, there is a paucity of enzyme-laden nanodrugs for bio-oxidation assistant antitumor research. We designed supramolecular nanogel as artficial neutrophil by enzymatic interfacial self-assembly of peptides (Fmoc-Tyr(H_2PO_3)-OH) with magnetic nanoparticles (MNPs) and electrostatic loading of chloroperoxidase (CPO). The MNPs within nanogel can elevate H_2O_2 level in cancer cells under programmed alternating magnetic field (AMF) smiliar to the neutrophil activator, and the loaded CPO within protective peptides nanolayer sustainedly converts the H_2O_2 to singlet oxygen ($^1O_2$) for neutrophils-inspired tumor therapy. As a proof of concept study, both the H_2O_2 and $^1O_2$ in cancer cells increase stepwisely under the programmed alternating magnetic field. Therefore, an active enzyme dynamic therapy by magantic-stimulated oxygen stress and sustained enzyme bio-oxidation is proved with the studies on both cells and animals.

Introduction

Conventional cancer treatments including surgery, chemotherapy and radiotherapy suffer from uncontrollable toxic side effects to normal cells as well as drug and radio-resistance, which increase the incidence of tumour regrowth and lead to a poor prognosis.[1] The regulation of reactive oxygen species (ROS) by biological oxidation at the tumuor area is an emerging strategy for modulating the redox status of cells for cancer treatment. Reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals(•OH) and hydrogen peroxide (H_2O_2), is the by-product of organism oxygen metabolism and acts as a double-edged sword in intracellular signalling and regulation of cell fate.[2] In contrast to the normal cells, cancer cells with elevated ROS levels depend heavily on the antioxidant defence system.[3] Therefore, a proper ROS-elevating strategy to precisely break the vulnerable redox balance of cancer cells is the key precondition for effective cancer treatment. Exogenous ROS can be produced from the physical environmental stress (such as UV or heat exposure), while the major sources of endogenous ROS normally derives from the biochemical enzymatic oxidation. Nowadays, physical approaches including photodynamic therapy (PDT)[4] and sonodynamic therapy (SDT)[5] have been reported to damage cancer cells via the phsical light or ultrasound excitation that can transfer the external energy to produce exogenous $^1O_2$ for cancer therapy. Moreover, biocatalysis, which is capable of elevating the intracellular ROS, has been reported as an alternative bioinspired approach for anticancer treatment by yielding toxic hydroxyl radicals using bio-fenton reaction or producing the chromogenic substrate for photoacoustic therapy.[6] The enzymatic bio-oxidation has been taken as a sustained pathological-responsive biochemical theraputic way for anticancer treatment as single oxygen-generator by imitating process of the innate immune system combating infections and bacterials in our group. Recently we have proposed one new kind of stretagy which used superoxide dismutase (SOD) to elevate the intracellular H_2O_2 and combined with CPO enzyme to convert it to $^1O_2$ to kill cancer cells, named as enzyme dynamic therapy (EDT).[7] However, the methodology combining the advantages of physical and biochemical approaches for anticancer has never been reported.

Actually, in our innate immune systems, marvelous neutrophils can perfectly combine the precisely activation by exogenous inflammatory cytokines with the sustained biochemical anti-infection and cell fate regulation by the secretion of oxidase and myeloperoxidase. During these processes, NADPH oxidase can produce endogenous H_2O_2 or superoxide radicals, which was tandemly transformed to $^1O_2$ or hypochlorous acid by myeloperoxidase.[8] Such activable and regulatable biocatalysis provides us a promising inspiration for the physical-biochemical tandem design for regulatable ROS-elevated cancer therapy, for which the physical stimulus can be exerted as the short-term activator and the enzyme can be provided as the constant ROS output by biocatalysis.

Herein, combining the physical-activable property of PDT &SDT and the efficient enzymatic bio-oxidation of EDT, we proposed a new class of enzyme-laden magnetic nanogels, MNP-CPO@Nanogels, as the activable and programmed regulated neutrophil-mimics for cancer therapy by upregulation of the intracellular $^1O_2$, named as magnetocaloric-enzymatic tandem therapy (METT). To our minds, the bioinspired design of neutrophil-mimics should initially involve the exogenous production of H_2O_2 and subsequent biocatalytic generation of

[a] Q. Zhang, Prof. X. Wang, C. Wu, Dr. Q. Wu, Prof. Y. Mi, Prof. Q. Wang
School of Chemical Science and Engineering
Tongji University
1239 Siping Road, Shanghai (China)
E-mail: wangqg66@tongji.edu.cn
[b] J. Wu, J. Wang, M. Chen, Prof. Y. Cheng
Institute for Regenerative Medicine, Institute for Translational Nanomedicine, Shanghai East Hospital, Tongji University School of Medicine. 1800 Yuntai Road, Shanghai (China)
E-mail: yucheng@tongji.edu.cn
[c] Prof. M. S. Lesniak
Feinberg School of Medicine
Northwestern University
676 North Saint Clair Street, Suite 2210, Chicago (USA)
[‡] These authors contributed equally to this work.