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Biological thiols-triggered hydrogen sulfide releasing microfibers for tissue engineering applications

Sheng Feng [a], Yu Zhao [b], Ming Xian [b,*], Qian Wang [a,*]

[a] Department of Chemistry and Biochemistry and Nanocenter, University of South Carolina, Columbia, SC 29208, USA
[b] Department of Chemistry, Washington State University, Pullman, WA 99164, USA

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ABSTRACT

By electrospinning of polycaprolactone (PCL) solutions containing *N*-(benzoylthio)benzamide (NSHD1), a H_2S donor, fibrous scaffolds with hydrogen sulfide (H_2S) releasing capability (H_2S-fibers) are fabricated. The resultant microfibers are capable of releasing H_2S upon immersion in aqueous solution containing biological thiols under physiological conditions. The H_2S release peaks of H_2S-fibers appeared at 2–4 h, while the peak of donor alone showed at 45 min. H_2S release half-lives of H_2S-fibers were 10–20 times longer than that of donor alone. Furthermore, H_2S-fibers can protect cells from H_2O_2 induced oxidative damage by significantly decreasing the production of intracellular reactive oxygen species (ROS). Finally, we investigated the H_2S-fibers application as a wound dressing *in vitro*. Given that H_2S has a broad range of physiological functions, H_2S-fibers hold great potential for various biomedical applications.

Statement of significance

Hydrogen sulfide, as a gaseous messenger, plays a crucial role in many physiological and pathological conditions. Recent studies about functions of H_2S suggests H_2S-based therapy could be promising therapeutic strategy for many diseases, such as cardiovascular disease, arthritis, and inflammatory bowel disease. Although many H_2S donors have been developed and applied for biomedical studies, most of H_2S donors have the shortage that the H_2S release is either too fast or uncontrollable, which poorly mimic the biological generation of H_2S. By simply combining electrospinning technique with our designed biological thiols activated H_2S donor, NSHD1, we fabricated H_2S releasing microfibers (H_2S-fibers). This H_2S-fibers significantly prolonged the releasing time compared to H_2S donor alone. By adjusting the electrospinning parameters, tunable releasing profiles can be achieved. Moreover, the H_2S fibers can protect cardiac myoblasts H9c2 and fibroblast NIH 3T3 from oxidative damage and support their proliferation as cellular scaffolds. To our knowledge, this is the first report of electrospun fibers with H_2S releasing capacity. We anticipate this H_2S-releasing scaffold will have great potential for biomedical applications.

1. Introduction

Hydrogen sulfide (H_2S) has long been considered as a malodorous toxic gas. Abe and Kimura first reported the possible role of endogenous H_2S in the neuromodulation [1], indicating the biological relevance of H_2S as a gasotransmitter. Together with nitric oxide (NO) and carbon monoxide (CO), H_2S forms part of a group of active gaseous molecules that modulate cellular functions through intracellular signaling cascades [2]. Most, if not all, of endogenous formation of H_2S is attributed to four enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and the tandem enzymes cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3-MST) [3–5]. Tightly controlled endogenous H_2S has involved in diverse physiological and pathophysiological processes, including learning and memory, neurodegeneration, regulation of inflammation and blood pressure, metabolism, and anti-apoptosis [6]. Possessing all positive effects of NO without generating a toxic metabolite such as $ONOO^-$ [7], H_2S holds great therapeutic potential for various diseases and has drawn increased attention of biomedical scientists.

* Corresponding authors.
E-mail addresses: mxian@wsu.edu (M. Xian), wang263@mailbox.sc.edu (Q. Wang).

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Limited available H_2S releasing agents (i.e. H_2S donors), such as sulfide salts, natural polysulfide compounds such as diallyl trisulfide, synthetic H_2S donors such as Lawesson's reagent derivative, GYY4137, have been developed and applied for the studies of the physiological and pathological functions of H_2S [8]. Recently, a polymeric H_2S donor synthesized by conjugating 5-(4-hydroxyphenyl)-3H-1,2-dithiole-3-thione (ADT-OH) to polymers showed effects of potentiating lipopolysaccharide-induced inflammation and altered cellular trafficking [9]. However, the shortage of aforementioned H_2S donors is that the H_2S release is either too fast or uncontrollable, which poorly mimic the biological generation of H_2S. For example, sulfide salts Na_2S and NaHS, as the most widely used H_2S donor, have a fast and spontaneous H_2S release in aqueous solutions. H_2S gas concentration reaches a maximum within 20 s and falls exponentially thereafter [10]. Other commonly used H_2S donors, such as GYY4137 [11], polysulfide such as diallyl disulfide (DADS) and diallyl trisulfide (DATS) [12], ADT-OH [13], and thioamides [14] have peaking times ranging from several minutes up to 15 min. To overcome this drawback, we have developed a series of *N*-(benzoylthio)benzamides derivatives (NSHD), which are controllable and have a relative slow-releasing profile [15]. The H_2S release of these donors can be triggered by biological thiols such as cysteine and glutathione (GSH), which prevail in the biological system. Therefore, NSHDs are promising candidates for H_2S-based therapeutic strategy.

Electrospinning is a simple, cost-effective, and versatile technique in which natural or synthetic polymers are fabricated into fibers with diameters ranging from tens nanometers to micrometers. The unique advantages, such as high surface to volume ratio, adjustable porosity, and the flexibility to form various sizes, make the electrospun fibers an ideal system for a broad range of biomedical applications, such as wound dressing, blood vessel tissue engineering, neural repair, bone or cartilage tissue engineering, and controlled drug release, as described in several reviews [16–18]. Previously, researchers have functionalized electrospun fibers with biological relevant macromolecules, such as low molecular weight heparin [19], growth factor bone morphogenetic protein 2 (BMP2) [20], neural growth factor [21], and specific genes [22]. Notably, several groups have generated electrospun fibers with the capability to release gaseous messenger nitric oxide [23–27]. Though emerging role of H_2S in physiological and pathophysiological processes indicates H_2S holds great therapeutic potential [28], to the best of our knowledge, no H_2S-releasing electrospun fibers (H_2S-fibers) has been generated and evaluated so far.

Fibrous scaffolds generated by electrospinning have been used to generate substitutes and grafts for various tissue regeneration and achieved significant progress [17]. The electrospun fibers served as cell scaffolds for the therapeutic cells to adhere, proliferate, and differentiation, in the damaged tissues or organs [16]. However, in the process of transplantation of organs, tissue substitutes, and therapeutic cells, ischemia *in vitro* and post-transplantation is the most well-known cause of malfunction of transplanted grafts [29,30]. Since the production of endogenous H_2S and the exogenous administration of H_2S elicit a wide range of protective actions, especially in cardiovascular systems, including vasodilation, anti-inflammatory, antioxidant, and down regulation of cellular metabolism under stress [28,31], H_2S-fibers would enhance the regenerative capacity of tissue engineering grafts by protecting surrounding cells from ischemia.

We hypothesized that electrospinning biodegradable polymers doped with a H_2S donor (NSHD1) will generate H_2S-fibers with a controllable slow-releasing profile, which could be used as cell scaffolds to protect cell from ischemia reperfusion injury. To test this hypothesis, we fabricated H_2S-fibers by electrospinning biodegradable polycaprolactone solution containing NSHD1. The resultant H_2S-fibers not only supported the growth of cardiac myoblasts H9c2 and fibroblast NIH 3T3, but also protected cells from hydrogen peroxide (H_2O_2) induced oxidative damage by releasing H_2S. Given the broad biological functions of H_2S, we anticipate this H_2S-releasing scaffold will have great potential for biomedical applications.

2. Materials and methods

2.1. Electrospinning of H_2S-releasing fibers

Poly(ε-caprolactone) (PCL) (Average Mn ca. 70–90 kDa, Sigma) were dissolved in 1,1,1,3,3,3-hexafluro-2-propanol (HFIP) (Sigma Aldrich) to obtain 1–12% w/w solutions. For samples containing dopant, the polymer was dissolved in HFIP first, followed by the addition of NSHD1. Each polymer solution was drawn into fibers by a home-made electrospinning system described before [32]. Briefly, the polymer solution was transferred to a 1.0 mL plastic syringe (BD) with a 21 G blunt needle (BD precision glide). A syringe driver was used to control the solution flow rate at 15 μL min^{-1}. A high voltage supply (HVR Orlando, FL) was used to build up a voltage of 6–7 kV electric filed between the needle and the grounded collector. The distance between the collector and needle was fixed at 10 cm. The temperature of the electrospinning environment was 25 °C, and the humidity was below 1%. The random fibers were obtained by using a stationary collector. And the aligned fibers were obtained by using a rotating drum (12 cm in diameter) as the collector. The rotating rate was 1000 rpm.

2.2. Fiber analysis

The morphology of PCL fibrous scaffolds and elemental analysis via EDX were examined by a scanning electron microscopy (SEM, Zeiss Ultra Plus FESEM). Fibrous scaffolds samples were dried with nitrogen, and coated with gold for 40 s with Desk II cold sputter coater (Denton Vacuum, Morristown, NJ). To examine the uniformity of the fibrous diameters, several randomly selected areas were imaged. The same condition was used for EDX. Fibrous diameters were measured using ImageJ (National Institutes of Health). Fourier transform infrared spectrometry (FTIR) was conducted on a Shimadzu 8400 FTIR spectrometer in the range of 500–4000 cm^{-1}.

2.3. Hydrogen sulfide release

Reactions for the measurement of H_2S release kinetics were run in triplicate. In each test, 50 mg fibers samples were immersed in 50 mL PBS (pH 7.4) containing 1 mM cysteine. Reaction aliquots (1.0 mL) were taken to UV–Vis cuvettes containing zinc acetate (100 μL, 1% w/v in H_2O), $FeCl_3$ (200 μL, 30 mM in 1.2 M HCl), and *N*,*N*-dimethyl-1,4-phenylenediamine sulfate (200 μL, 20 mM in 7.2 M HCl) at predetermined time points. The Absorbance at 670 nm was measured 20 min thereafter. H_2S concentrations were calculated according to the Na_2S standard curve. To determine the release half-life, 1% w/v zinc acetate was added to the 50 mL PBS containing 50 mg fibers sample. Then reaction aliquots were taken to UV–Vis cuvettes containing $FeCl_3$ (200 μL, 30 mM in 1.2 M HCl), and *N*,*N*-dimethyl-1,4-phenylenediamine sulfate (200 μL, 20 mM in 7.2 M HCl) at predetermined time points. First-order half-life of H_2S release was determined by plotting time vs. ln(1/(1−% released), with $t_{1/2} = \ln(2)/\text{slope}$.

2.4. Cell lines and cell cultures

The rat cardiomyocyte cell line H9c2 and the immortalized mouse fibroblast cell line NIH 3T3 were purchased from American