

Breathing Micelles for Combinatorial Treatment of Rheumatoid Arthritis

Siyue Tao^{1,2}, Tao You³, Jinmin Hu³, Fengdong Zhao^{1,2}

1. Department of Orthopaedic Surgery, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine

2. Key Laboratory of Musculoskeletal System Degeneration and Regeneration Translational Research of Zhejiang Province

Address: No. 3, Qingchun Road East, Hangzhou, 310016, P.R.China.

3. The First Affiliated Hospital of USTC, Division of Life Science and Medicine, University of Science and Technology of China
Hefei 230001, Anhui, P.R.China.

E-mail: 12018284@zju.edu.cn

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INTRODUCTION: Rheumatoid arthritis progressively impairs joint function and ultimately leads to disability, significantly reducing the quality of life and posing a serious public health problem. Higher organisms, including animals and plants, generate energy through respiration to maintain normal physiological functions. In the typical process of aerobic respiration, oxygen (O₂) is inhaled and carbon dioxide (CO₂) is exhaled for gas exchange. In addition to O₂ and CO₂, various gases such as carbon monoxide (CO) and nitric oxide (NO) are produced by organisms. Excessive production of NO is a hallmark of RA, and selective consumption of NO using NO scavengers has shown potential for treating RA. Furthermore, studies have indicated that CO has potential anti-inflammatory effects. Although NO and CO have traditionally been considered atmospheric pollutants with toxicity, research has revealed their important regulatory roles in numerous physiological/pathological processes. For instance, the occurrence and progression of inflammation-related diseases, such as arthritis and inflammatory bowel disease, are closely associated with high NO concentrations, while endogenous CO effectively inhibits inflammatory responses. Therefore, we simulated gas exchange in biomimetic respiration, combining a "breath-in" pro-inflammatory NO-releasing unit (NOSM) with a "breath-out" anti-inflammatory CO-releasing unit (CORM) to generate a combined anti-inflammatory respiratory micelle. With this approach, we aim to provide a therapeutic strategy for rheumatoid arthritis.

METHODS: In this study, we designed a Breathing Micelles (BM) platform capable of inhaling nitric oxide (NO) and exhaling therapeutic carbon monoxide (CO). The respiratory micelles nanoparticles were functionalized with ortho-phenylenediamine (oPDA), which reacts with NO, and 3-hydroxyflavone (3-HF) derivatives, which release CO, both within a benzotriazole (BTA) moiety, enabling NO consumption and CO release upon visible light irradiation. The BMs were characterized using fluid dynamics, UV/Vis spectroscopy, nuclear magnetic resonance spectroscopy, and gel permeation chromatography. The in vitro activities of the respiratory micelles were evaluated through assessments of biocompatibility, hemolysis, intracellular NO scavenging/CO release, and anti-inflammatory effects. Finally, the in vivo anti-inflammatory performance of the respiratory micelles was assessed in a rat adjuvant-induced arthritis (AIA) model.

RESULTS SECTION: The average hydrodynamic diameter of the micelles was 32 nm, and their morphology remained stable after continuous NO treatment and light irradiation. UV-Vis spectroscopy and NMR results confirmed the formation of BTA derivatives in the micelles upon NO addition, and visible light-mediated CO release resulted in decreased absorbance at 270 and 334 nm. Comparison with NOSM and CORM monomers indicated that NO absorption and CO release were mutually exclusive. In vitro cell experiments demonstrated the excellent safety of the BMs in both prokaryotic and eukaryotic organisms, and they were readily internalized by RAW264.7 cells. Griess assay, NO/CO fluorescent probes, and ELISA confirmed that BM effectively scavenged excessive NO induced by LPS in the intracellular environment and reduced the secretion of IL-6 and TNF- α through CO release. In the rat adjuvant-induced arthritis (AIA) model, BM exhibited superior therapeutic effects compared to NOSM and CORM micelles, as evidenced by improved hind paw morphology, micro-CT imaging, tissue histopathology scores, and lower levels of serum and tissue inflammatory factors. Furthermore, BM showed better anti-inflammatory and anti-osteoclastogenic effects compared to the classical RA therapeutic drug dexamethasone.

DISCUSSION: We have successfully developed a novel biomimetic respiratory micelle (BM) that can inhale and exhale different biologically relevant gases. BM effectively scavenges excessive NO and releases therapeutic CO, demonstrating combined anti-inflammatory capabilities both in vitro and in vivo. In a rat arthritis model, BM exhibits superior therapeutic effects compared to any individual component of BM as well as traditional RA treatment drugs.

SIGNIFICANCE/CLINICAL RELEVANCE: This work has developed a novel biomedically promising biotherapeutic micelle.

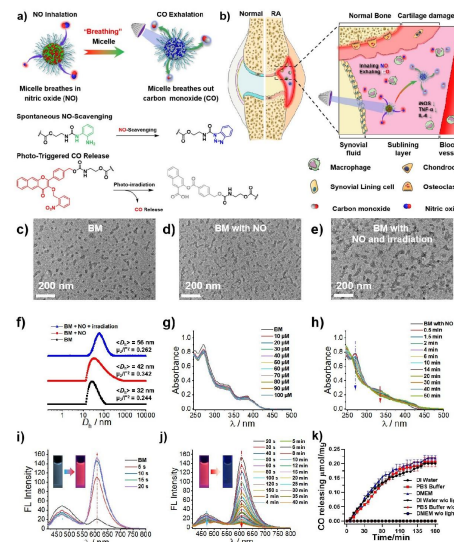


Figure 1. Synthesis and in vitro characterization of BM. (a) The working principle of the "breathing" micelles (BM) that inhale NO via the transformation of o-phenylenediamine (oPDA) into benzotriazole (BTA) moieties and exhalate CO through the photorelease of the 3-HF derivatives with the formation of 3-hydroxyflavone (3-HF) moieties. (b) BM synthesis scheme. (c) TEM images of BM. (d) DLS size distribution of BM. (e) Z-average size distribution of BM. (f) UV-Vis spectra of BM. (g) Fluorescence spectra of BM. (h) Fluorescence spectra of BM. (i) Fluorescence spectra of BM. (j) Fluorescence spectra of BM. (k) Fluorescence spectra of BM.

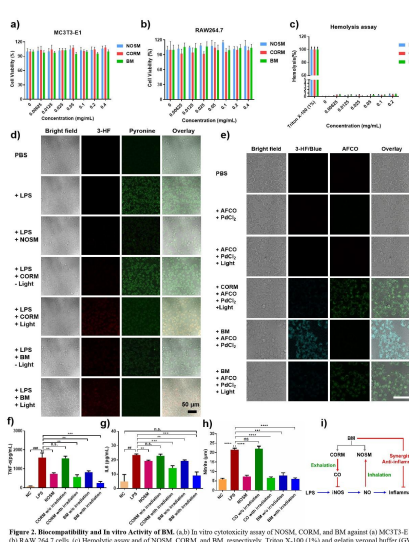


Figure 2. Biocompatibility and in vitro activity of BM. (a) Hemolysis assay. (b) Hemolysis assay. (c) Hemolysis assay. (d) Hemolysis assay. (e) Hemolysis assay. (f) Hemolysis assay. (g) Hemolysis assay. (h) Hemolysis assay. (i) Hemolysis assay. (j) Hemolysis assay. (k) Hemolysis assay.

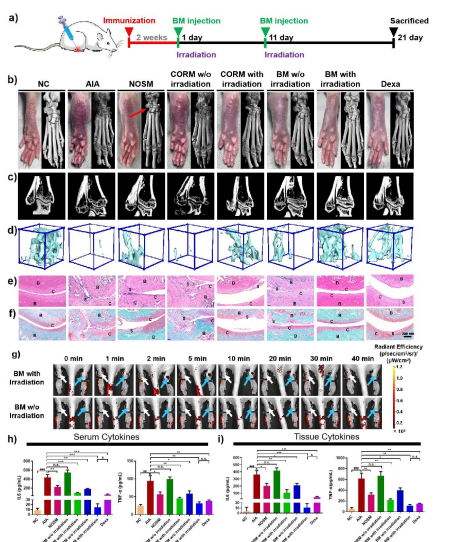


Figure 3. In vivo anti-RA activity of BM. (a) Experimental timeline. (b) Representative images of paws. (c) Representative images of paws. (d) Representative images of paws. (e) Representative images of paws. (f) Representative images of paws. (g) Representative images of paws. (h) Representative images of paws. (i) Representative images of paws. (j) Representative images of paws. (k) Representative images of paws.