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Real-time observation of aortic vessel dilation through delivery of sodium nitroprusside via slow release mesoporous nanoparticles

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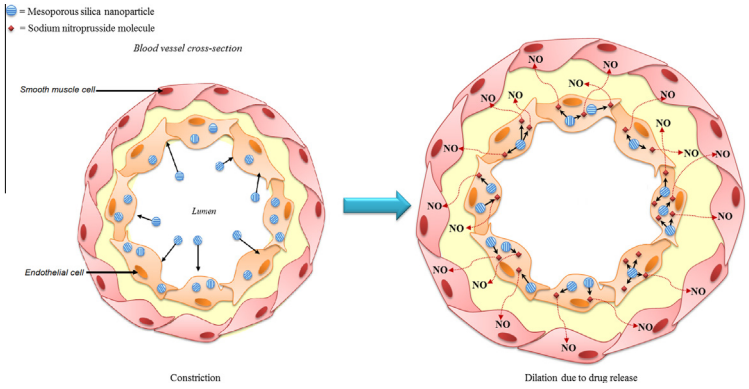
GRAPHICAL ABSTRACT



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ABSTRACT

Spherical mesoporous nanoparticles (MNPs) with a diameter of ~100 nm were synthesised via a sol-gel method in the presences of organic template (with and without fluorescein dye encapsulation). The template molecules were removed by acidic extraction to form a regular pore lattice structure. The nanoparticle size and morphology were analysed using transmission electron microscopy and dynamic light scattering analysis. The MNPs were further characterised by zeta potential, nitrogen adsorption measurements and infra-red spectroscopy. The interior pores had an average diameter of ~3 nm and were loaded with an endothelial-independent vasodilator, sodium nitroprusside (SNP). The optimal drug loading and drug release was determined in high potassium physiological salt solution using dialysis and atomic absorption spectroscopy. We demonstrate that the initial instantaneous release is due to the surface desorption of the drug followed by diffusion from the pores. Furthermore, these drug loaded MNPs (with and without fluorescein dye encapsulation) were added to viable aortic vessels and release in real-time was observed, *ex vivo*. MNPs and loaded with and without SNP were incubated with the vessel (at 1.96×10^{12} NP mL^{-1}) over a 3 h time period. The real-time exposure to unloaded MNPs resulted in a small attenuation in constriction that occurred after approximately 1 h. In contrast, MNPs loaded with SNP led to a rapid relaxation of aortic vessels that was sustained over the 3 h period ($p < 0.001$).

1. Introduction

Progress in nanoparticle fabrication techniques has enabled the tailoring of nanoparticle properties for the development of

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innovatively designed biological drug delivery vehicles [1–3]. Orally and intravenously administered drugs circulate throughout the body and may have a number of systemic side effects, often leading to poor efficiency of the drug [4]. Consequently, there is a drive for the development of new drug delivery systems. In particular, mesoporous nanoparticles (MNPs) can be fabricated using colloidal methods with small diameters that are able to enter cells. Regular pore structure is produced during the surfactant template synthesis method [5]. The resulting MNPs have high surface to volume ratio's with pore channels that can be loaded with guest molecules. The exterior surface is available for functionalisation to enable targeted therapy of intracellular structures [6]. Furthermore, these particles have been used for diagnostic imaging and cellular localisation by confocal microscopy and flow cytometry by incorporation of fluorescent dye molecules within the MNPs silica framework [7]. Once the nanoparticles have been drug loaded, the release is governed by constrained pore diffusion enabling these particles to be utilised for slow drug release applications [8,9]. Previous studies have used MNPs as hosts (such as MCM-41and SBA-15) and loaded with guest molecules (such as drugs, enzymes, DNA and luminescent dyes) to produce effective systems in which the host-guest interaction plays a vital role in their application [10].

The ordered pore network of MNPs allows fine control of the drug loading and release kinetics, while the large surface area allows adsorption of the required dosage of drug [5]. Release kinetics has been demonstrated from MNPs using luminescent molecules and ibuprofen [9,11–14]. One study showed that 80% of ibuprofen was released after only 50 min from MNPs with pore size of 3.4 nm and particle diameter of 50 μm [15]. Another study demonstrated that it took 31 h for the release of 60% of ibuprofen loaded into MNPs (diameter 950 nm and pore size of 2.5 nm). There has been an abundance of studies showing that MNPs are internalised into the cytoplasm of mammalian cells with low cytotoxicity (LD_{50} values greater than 1 mg mL^{-1}) demonstrating their biocompatibility [16].

The aortic vessel is the largest artery in vertebrates and plays an important role in the transport of blood and the maintenance of blood pressure (Fig. 1). Arteries are comprised of three layers, an endothelial layer facing the lumen, a smooth muscle layer, and an outer adventitial layer [17]. Vessel viability and the integrity of the endothelial and smooth muscle cell layers can be verified by examining vascular responses to vasodilator and vasoconstrictor agonists [18]. A major vasodilator released naturally by endothelial cells is nitric oxide (NO) and is important in the maintenance of vessel diameter. Reduced bioavailability of NO has been demonstrated in a number of vascular related conditions, including diabetes and hypertension [19]. The development of nanoparticles that can deliver sustained and controlled release of NO could have enormous impact on human health. One approach has been the development of silica hydrogel nanoparticle matrices, whereby the entrapped NO molecules are released when exposed to moisture [20]. Sodium nitroprusside (SNP) is an endothelial independent vasodilator that causes relaxation of the smooth muscle cells by delivery of NO, which is a labile ligand, contained within the SNP molecular complex [21–23]. In this study, we report for the first time the *ex vivo* real-time vasodilator action of SNP on arterial vessels delivered via MNPs. Controlled release of SNP from MNPs is a promising avenue for the development of therapeutic intervention in cardiovascular disease.

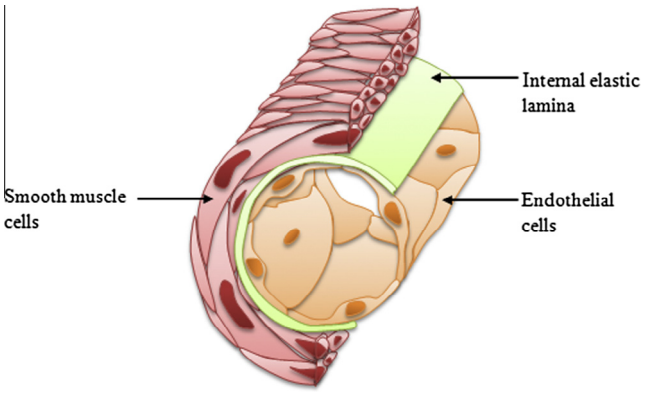

Fig. 1. Graphical illustration of a cross-section of the aortic vessel.

2. Materials and methods

2.1. Materials

Fluorescein isothiocyanate (FITC), (3-aminopropyl)trimethoxysi lane (APTMS), tetraethyl orthosilicate (TEOS), hydrochloric acid (HCl, 37%) and sodium nitroprusside (SNP) were purchased from Sigma-Aldrich. Hexadecyltrimethyammonium bromide (CTAB), anhydrous dimethylformamide (DMF), sodium hydroxide (NaOH) and methanol (MeOH) were purchased from Fisher-Scientific. Salt solutions were prepared using sodium chloride (NaCl), potassium chloride (KCl), magnesium sulphate heptahydrate ($MgSO_4{\cdot}7H_2O$), potassium phosphate monobasic (KH_2PO_4), calcium chloride ($CaCl_2{\cdot}2H_2O$), ethylenediaminetetraacetic acid dipotassium salt dihydrate ($K_2EDTA{\cdot}2H_2O$) purchased from Fisher-Scientific.

Physiological Salt Solution (PSS) was prepared using the following chemical composition [mM]: 119 NaCl, 4.7 KCl, 1.2 $MgSO_4$.7 H_2O, 25 $NaHCO_3$, 1.17 KH_2PO_4, 0.03 $K_2EDTA{\cdot}2H_2O$, 5.5 glucose and 1.6 $CaCl_2{\cdot}2H_2O$; pH 7.4. Potassium Physiological Salt Solution (KPSS) was prepared using the following chemical composition [mM]: 78.2 NaCl, 60 KCl, 1.2 $MgSO_4$.7 H_2O, 25 $NaHCO_3$, 1.17 KH_2PO_4, 0.03 $K_2EDTA{\cdot}2H_2O$, 5.5 glucose and 1.6 $CaCl_2{\cdot}2H_2O$; pH 7.4 as previously described by Farooq et al. [24].

2.2. Synthesis of non-dye encapsulated mesoporous nanoparticles

Mesoporous silica nanoparticles (MNPs) were synthesised using a modified template directed self-assembly method published by Slowing et al. without the incorporation of FITC dye molecules [25]. Briefly, anhydrous DMF (5 mL) was mixed with APTMS (50 μL). CTAB (1 g) was dissolved in H_2O (480 mL) and 2 M NaOH solution (3.5 mL) was added and stirred at 80 °C. TEOS (4 mL) was added followed by the addition of the APTMS mixture (500 μL). The resulting mixture was stirred vigorously at 80 °C for 2 h producing an opaque white suspension. The MNPs were collected and washed with MeOH by centrifugation (14,000 rpm/15 min) several times. The surfactant template (CTAB) was extracted from the as-synthesised product by refluxing the product in MeOH (200 mL) and concentrated HCl (2 mL) for 24 h. The MNPs were washed using the same procedure as before. The acid extraction step was repeated twice.

2.3. Synthesis of dye encapsulated mesoporous nanoparticles

Fluorescein isothiocyanate encapsulated mesoporous nanoparticles (FITC MNPs) were synthesised via the same method as the MNPs except that FITC (2.5 mg) was added to the mixture of anhydrous DMF and APTMS and then stirred for 3 h [25]. The resulting product was a yellow suspension.