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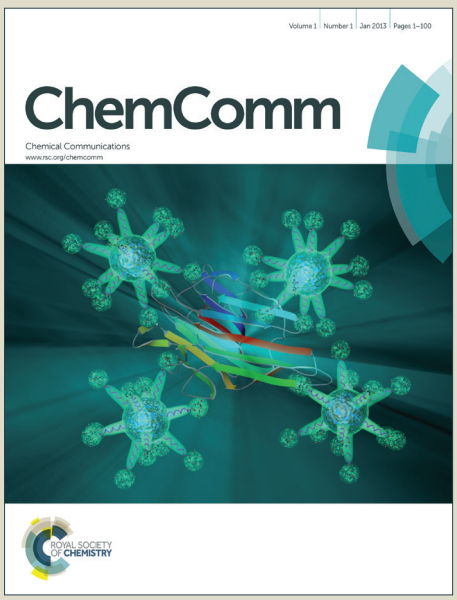

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Programmable Drug Release Using Bioresponsive Mesoporous Silica Nanoparticles for Site-specific Oral Drug Delivery

Amirali Popat [a,b,c], Siddharth Jambhrunkar[a], Jun Zhang[a], Jie Yang[a], Honwei Zhang[a], Anand Meka[a], Chengzhong Yu[a]*

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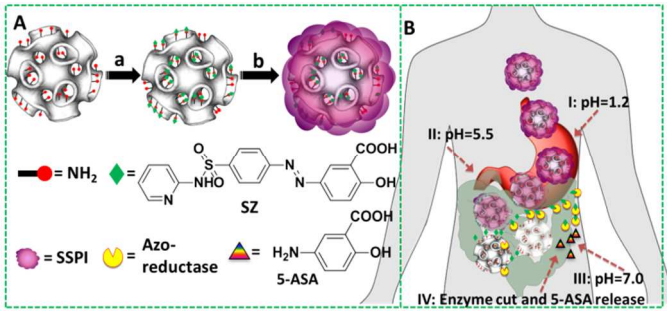
Novel mesoporous silica nanoparticles (MSNs) responsive to multiple biological stimuli (pH and Enzyme) were prepared through conjugation with structure modified soy protein isolate. The particles show an extremely high pro-drug (Sulfasalazine) loading with programmable drug release in simulated gastric fluid.

Intelligent nanomaterials delivering cargo at the targeted site with zero premature release is an enchanting area of research in the field of material science. In the past decade, mesoporous silica nanoparticles (MSNs) have been in the limelight as one class of the most promising nanocarriers owing to their biocompatibility, enormous loading capacity, and adaptable surface chemistry just to name a few.[1, 2] Nevertheless, the drug release profile from conventional MSNs suffers from premature and uncontrollable release urging the need for finely tuning the drug release to compete with its polymeric and/or lipidic counterpart. To ameliorate the performance, many MSNs based smart drug delivery system responding to the internal stimuli such as pH[3, 4, 5, 6, 7], enzyme[6, 7, 8, 9], glucose[10], and external triggers such as temperature[11], light[12, 13] have been proposed with improved drug release characteristics. In the quest of further calibrating the drug release, recently MSNs with dual stimuli responsiveness have emerged as the new area of nanomedicine. Numerous systems which respond to pH/light, pH/pH, pH/time, and glucose/pH have been proposed in order to augment the drug release performance.[14, 15]

Nevertheless, such dual stimuli-response systems typically show improved drug release performance in cell delivery using generic drug/dye molecules [2, 4, 6, 13, 16], but rarely target a diseased organ. Despite several advancements in drug delivery, oral delivery is still the most preferred route and dominates overall drug delivery market. Large variations in pH (1.2-7.4) and digestive enzymes make delivery through the oral route much more complicated. As a result, many pH or enzyme responsive drug delivery systems have been proposed specially using MSNs.[5, 7, 9, 14, 17] A most common strategy used to control the drug release is to bind the drug molecules using either electrostatic or ionic interaction on the surface of MSNs.[17, 18] The release of cargo is mainly triggered by change in acidity and ionic strength of the release medium which can lead to uncontrolled and premature drug release.[17, 19, 20] Additionally, weak electrostatic and ionic interaction leads to poor control over drug release in biologically relevant conditions.[17] Alternatively, the drug molecules are attached via chemical bonding and the release is triggered by physical or biological stimuli.[7, 19] However, the drug loading capacity of such a system is low, making them unsuitable for pre-clinical studies.[7, 17] In another approach, MSNs have been coated with pH responsive polymers or hydrophilic proteins with relatively small molecular weights.[5, 20] However, such systems show burst release at pH > 5, leading to uncontrolled drug release due to rapid dissolution of coating before reaching to small intestine (pH of 7). Apart from the big pH change in gastrointestinal tract (GIT), there are several other factors such as digestive enzymes (pepsin and pancreatine), gut flora etc., which plays an important role in drug absorption and release. So far, these critical factors are rarely considered in the design of such smart nanomaterials.



Scheme1. Schematic representation of A) synthesis of MSN-NH2-SZ@SSPI. a: SZ is loaded into amino functionalized MCM-48 (MSN-NH2) to form MSN-NH2-SZ, b: coating of SSPI using amide chemistry leads to MSN-NH2-SZ@SSPI. B) Oral delivery and site-dependent programmable release of SZ into 5-ASA in GIT from MSN-NH2-SZ@SSPI.

Hence, a more realistic system is needed in order to achieve a programmable drug release in alignment with the diverse biological stimuli in GIT. Herein we report the first example of a multi-stimuli responsive oral delivery system based on MSNs and designed for the targeted delivery in GIT. As shown in Scheme 1A, MCM-48 type MSNs with a cubic bicontinuous pore structure, high pore volume and large surface area are chosen as drug carriers after functionalisation with primary amino groups (MSN-NH_2). Sulfasalazine (SZ) is chosen as an anionic pro-drug and loaded into positively charged MSNs to form MSN-NH_2-SZ through ionic interaction. The composite is further coated by a rationally designed succinylated soy protein isolate (SSPI). Soy protein isolate (SPI) is a low cost neutraceutical with a high molecular weight, [21] thus hydrophobic and insoluble in water. SSPI is chosen because 1) the carboxylic groups after surface modification provide binding site for MSN-NH_2; 2) succinylation increases the hydrolysis of proteins in the presence of enzymes present in small intestine; and 3) SSPI coating is stable at pH 5. The resultant MSN-NH_2-SZ@SSPI nanoparticles show both pH and enzyme responsiveness depending upon the location of nanoparticles in GIT (Scheme 1B). The choice of amino functionalised MSNs provides high drug loading with zero release in pH 1.2 while SSPI coating restricts the release of SZ in stomach (pH of 1.2) and duodenum (a medium high pH of ~5), thus two major problems in SZ formulation, i.e., low encapsulation efficiency and precise control over release of SZ can be addressed[7, 17]. MSN-NH_2-SZ@SSPI is programmed to release the pro-drug in small intestine (pH 7.4) in the presence of pancreatine enzyme via protein hydrolysis to open the gate, providing slow and sustained pro-drug (SZ) release, which cannot be achieved in previous reports.[5, 20] Finally, in the presence of azo-reductase produced by colon microflora, SZ is broken down into its active metabolite 5-aminosalycilic acid (5-ASA), an effective drug for the treatment of colon disease.

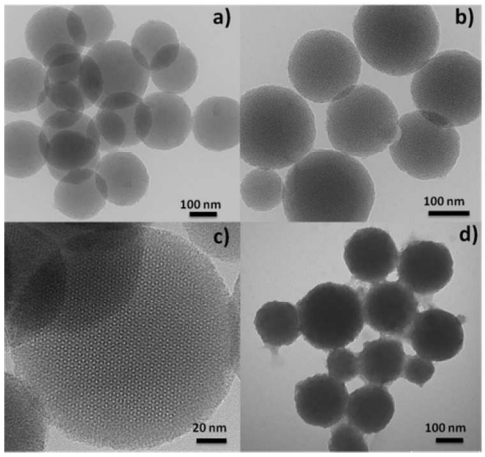


Figure 1. TEM images of a) MCM-48, b) MSN-NH_2, c) HRTEM of MSN-NH_2 clearly indicating the uniform mesopores, d) MSN-NH_2@SSPI

MCM-48 nanoparticles with small particle size and an *Ia3d* pore structure were synthesised by a modified sol-gel method (Supporting Information).[22] Transmission electron microscopy (TEM) image shows that typical MCM-48 types MSNs have a uniform particle size of 150-180 nm (Fig 1a). The amino functionalisation process has little impact on particle morphology and size of the particles (Fig 1b). The high resolution TEM image of MSN-NH_2 recorded from the [111] direction confirms the highly ordered bicontinuous cubic structure (Fig 1c). This highly ordered structure was also confirmed by low angle X-ray diffraction (XRD) patterns of all the samples which commensurate the structure to be *Ia3d* symmetry (Fig S1). N_2 sorption isotherm of pure MCM-48 revealed a type IV isotherm with a sharp capillary condensation step at P/P_o of ~ 0.3 characteristics of small sized mesopores. MCM-48 showed surface area of 1505 m^2g^{-1} and uniform 2 nm pores. After functionalisation MSN-NH_2 exhibits a typical isotherm with decrease in surface area (829 m^2g^{-1}) and pore volume (0.51 cm^3g^{-1}) affirming successful functionalization (Fig S2, Table S1). The decrease in surface area (260 m^2g^{-1}) and mesopore volume (0.22 cm^3g^{-1}) was even more pronounced after SZ loading suggesting the drug is adsorbed inside the mesopores. This is evident from XRD pattern of MSN-NH_2-SZ displaying decrease in intensity of the 211 peak and disappearance of the 220 peak. Grafting of SSPI was monitored by various techniques. Firstly, TEM image of MSN-NH@SSPI showed clear wrapping of SSPI on the outer surface of MSN-NH_2 (Fig 1d). It is suggested that SSPI is coated onto the outer surface of MSN-NH_2 owing to its extremely large molecular size (~180,000 Da), making it impossible to go inside 2-3 nm pores. Finally the drug loaded and protein coated particles (MSN-NH_2-SZ@SSPI) showed almost no porosity confirming the coating of protein on the surface of MSN. Detailed physicochemical parameters of all samples are summarised in Table S1.

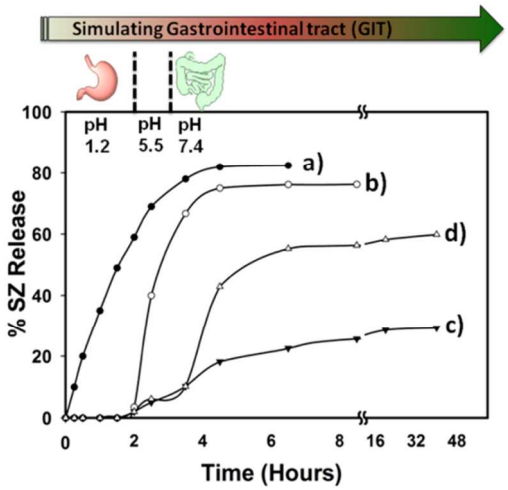


Figure 2. The release profiles of SZ from a) MCM-48-SZ, b) MSN-NH_2-SZ, c) MSN-NH_2-SZ@SSPI without enzymes and d) MSN-NH_2-SZ@SSPI in the presence of enzymes (pepsin in pH 1.2, and pancreatine in pH 7.4) present in simulated GIT fluids.

The pro-drug SZ was loaded into MSN-NH_2 to obtain MSN-NH_2-SZ (See details in SI). Thermogravimetric analysis (TGA) showed almost ~25% (W/W) SZ can be loaded into MSN-NH_2 (Fig S3, Table S2). As a prodrug, SZ is given in extremely high doses, hence it is important to encapsulate high amount of SZ in nanoparticles. To the best of our knowledge this is the first nanoparticle based system with a very high loading of SZ. This relatively high loading is due to the choice of nanocarriers (MSN-NH_2) with large number of amino groups and high surface area. Furthermore, the choice of MCM-48 type MSNs with 3D cubic pores is innervated by their higher adsorption and release

performance compared to its 2D counterparts.[23] Compared with the literature[17], MSN-NH2 shows high zeta potential at all pH, which could attract more molecules of anionic SZ, hence high loading capacity (Fig S4). Finally, to achieve programmed release, modified soy protein (SSPI) with abundant carboxylic (COOH) groups was conjugated with amino (NH_2) groups of MSN-NH_2-SZ using the carbidomide chemistry to form MSN-NH_2-SZ@SSPI (For details see supporting information, Materials and Methods).

Successful grafting of SSPI was also observed by change in zeta potential with respect to pH of the medium (Fig S4). Clearly, the isoelectric point (PI) of SPI is decreased from pH 5 to 3.2 validating successful succinylation of the SPI (Fig S4). Unlike MSN-NH_2 which showed positive zeta potential irrespective of pH, MSN-NH_2@SSPI displayed negative zeta potential at pH >5 verifying the successful wrapping of SSPI onto MSN-NH_2 (Fig S4). Fourier transform infrared spectroscopy (FTIR) further supported successful drug loading and protein grafting (Fig S5).

^{13}C-NMR was performed in order to investigate and understand the bonding between SZ, MSN-NH_2, and SSPI (Fig S6). MSN-NH_2 showed characteristic chemical shifts for propyl carbon (δ= 8.5, 22 and 43 ppm). A weak band near δ=50 is due to residual aminopropyltriethoxysilane (APTES) and solvent impurity.[7] MSN-NH2-SZ showed an additional peak in up field region (δ<50 ppm) characteristic of C-N of SZ. Resonances around δ=100-150 corresponds to phenyl rings present in SZ. Interestingly, peaks for carbonyl (C=O) at about 170 ppm does not appear in MSN-NH_2-SZ. This is believed to be due to high mobility of drug in mesopore making them invisible in CP NMR due to low dipolar coupling.[24] This interesting observation also demonstrates that lot of SZ molecules are within the nanopores. In addition to the above peaks MSN-NH_2@SSPI and MSN-NH_2-SZ@SSPI show strong band in the downfield region δ~170 ppm due to amide bonds between particles and protein.[5] Due to overlapping of various resonances between SZ and SSPI it is very difficult to assign peak number to each carbon atom.

To demonstrate effectiveness of our novel programmed system, we simulated the GIT in-vitro by taking into account pH, enzymes normally present in GIT and gastric emptying time after oral drug delivery. Although numerous studies showed pH responsive drug release from MSNs, most of them have excluded the presence of proteolytic enzymes in their in-vitro release test. Herein we present the first of its kind in-vitro release from MSNs with pepsin and pancreatin to simulate stomach and small intestine respectively. The release profiles of SZ at pH 1.2 (stomach, 2 h), pH 5.5, (duodenum, 1 h) and pH 7.4 (small intestine, >8 h) in absence and presence of enzymes are shown in Fig 2. As a control we adsorbed the SZ into bare MCM-48 particles (negative zeta potential) denoted as MCM-48-SZ and compared its release behaviour in similar conditions.

As shown in Fig 2a MCM-48-SZ showed burst release in pH 1.2 releasing ~60% of SZ in 2 h and reaching plateau in 6 h at the intestinal pH. It is to be noted that release of SZ from MCM-48-SZ showed no change irrespective of the pH highlighting the need for better controlled release. This is due to weak interaction between negatively charged MCM-48 and anionic SZ i.e drug release is controlled by mere diffusion. On the contrary, MSN-NH_2-SZ shows zero or no release at pH 1.2 for 2 h (Fig 2b) (average gastric emptying time in stomach). This is due to protonation of primary amino groups making the electrostatic interaction stronger between MSN-NH_3+ and SZ- at such a low pH.[17] At pH 5.5 in 1 h (average residence time in duodenum) however, about 40% SZ is released followed by about 80% release in 12 h at pH 7.4 (Fig 2b). As the pH of the medium increases the interaction between MSN-NH_3^+ and SZ^- becomes weak as justified by zeta potential change in MSN-NH_2 with respect to pH of the medium (Fig S4) making the release faster.

SZ is a pro-drug which gets converted into its active metabolite 5-ASA and sulfapyridine (SP) in the presence of azo-reductase producing bacteria present in lower part of small intestine/colon (Reaction S1). Hence, it is very important not only to protect the SZ release in stomach but also release it in lower part of small intestine in a sustainable manner. As depicted in Fig 2c, MSN-NH_2-SZ@SSPI showed no noticeable SZ release in pH 1.2 or pH 5.5 announcing the superiority of such coated system over uncoated system.

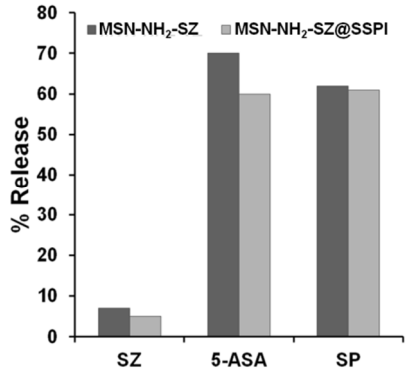


Figure 3. Release of SZ, 5-ASA, SP from MSN-NH_2-SZ and MSN-NH2-SZ@SSPI in presence of bacterial azo-reductase after 24 hours.

SSPI is partially soluble (pH >5.5) protein and degrades in presence of pancreatic enzyme present in small intestine (pH 7.4) removing the gate for SZ to release. Interestingly, at pH 7.4 MSN-NH_2-SZ@SSPI showed very slow drug release due to the presence of large and partially soluble SSPI on the surface of nanoparticles (Fig 2c). The release of SZ in presence of both pepsin at pH 1.2 and pancreatine at pH 7.4 is given in Fig 2d. In pH 1.2 the presence of pepsin had no effect on degradation of SSPI hence no SZ release was observed in first 2 h. Due to poor solubility of SSPI in pH 5.5 only ~10% of SZ was leaked from MSN-NH_2-SZ@SSPI unlike MSN-NH_2-SZ where ~40% of SZ was released. Furthermore, in pH 7.4 due to degradation of SSPI in the presence of pancreatine triggered the release of SZ with a burst followed by sustained release from MSNs (Fig 2d). The obtained results confirmed our hypothesis, i.e., combination of amino groups and novel SSPI coating protects the SZ in pH 1.2 and 5.5 both in presence and absence of enzyme while in pancreatine rich release medium, SZ is released in sustained manner over the period of 48 h.

Finally, to monitor the release of 5-ASA and SP from MSN-NH_2-SZ and MSN-NH_2-SZ-SSPI after 12 h the release medium was changed to simulated bacterial medium.[7] The degradation of released and bound SZ to 5-ASA and SP was monitored using HPLC (see details in Supporting Information). SZ was stable in pH 7.4 in the absence of bacterial medium while more than 95% of SZ was degraded to 5-ASA and SP in the presence azo-reductase producing

bacteria present in the release medium (Fig 3). Additionally, equal percentage of 5-ASA and SP were detected by HPLC confirming degradation is efficient to release both drugs inside colon. Preliminary kinetics studies are done to delineate the release mechanism of SZ from different nanocarriers (Table S2). Finally, we studied the colloidal stability of our particles in PBS using dynamic light scattering (DLS) method (for details please refer Fig S7).

Conclusions

In summary, we have successfully synthesised MSNs based multi-stimuli responsive delivery system with a programmable drug release behaviour responding to the variational biological stimuli in gastrointestinal tract. Our novel system features a high pro-drug loading (~25 % of SZ, 4 times higher than literature values.[7, 17]), and site-specific and controlled SZ release in the presence of pancreatine in small intestine. The pro-drug is further broken down by the in-situ enzyme azo-reductase to release 5-ASA and SP in colon. This unique and precisely programmed oral drug delivery system will be useful in targeting small intestine or colon which is absorption site of most orally delivered drugs.

Notes and references

[a] Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD 4072, Australia Fax: +61 7 33463973; Tel: +61 7 3346 3283; *E-mail: c.yu@uq.edu.au

[b] The School of Pharmacy, The University of Queensland, Brisbane, QLD 4072,

[c] Mucosal Diseases Group, Mater Research Institute – The University of Queensland, Translational Research Institute, 37 Kent St, Woolloongabba, QLD 4102.

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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