Effects of Fragrance-Loaded Mesoporous Silica Nanocolumns on Central Nervous System

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Fragrances have rapid effect on our central nervous system, such as making the spirit of relaxation, relieving tensions and refreshing ourselves. However, the release of fragrances is not stable. Here, we added photo-driven mesoporous silica nanocolumns loaded with sandela 803 (MS-C@S803) into wallpaper to obtain fragrant wallpaper (MS-C-W@S803). We then analyzed the effects of MS-C-W@S803 and pure sandela 803 treated wallpaper (W@S803) on the CNS of mice and explored the internal mechanism of these effects. Besides, we evaluated the short-term (7 days) and long-term (30 days) effects of the fragrance treated wallpaper. In behaviouristics level, we detected the anxiolytic effects via elevated plus maze and open field test. In tissue level, we analysed the neural activity in hippocampus, hypothalamus and olfactory bulb regions via measuring the electrophysiological signal. In cell level, we tested the nerve regeneration in hippocampus, substantia nigra and corpus striatum via immunofluorescence staining with the anti-BrdU antibody. In molecule level, we measured the expression of dopamine, acetylcholine, γ-aminobutyric acid (GABA) and N-methyl-D-aspartic acid (NMDA) via liquid chromatography-mass spectrometry. Finally, we find that MS-C-W@S803 had anxiolytic effects on the CNS of mice, and the effects were more significant as time progresses.

KEYWORDS: Sandela 803, Light-Activated Release, Central Nervous System, Neural Activity, Nerve Regeneration.

INTRODUCTION

Fragrances have widespread uses in cosmetics, perfumes and toilet soaps.1–3 The light scent emitted by them can relieve tension, makes the spirit of relaxation, refresh ourselves, help tranquilize the mind and improve sleep.4–6 Interestingly, we feel more cheerful and more relaxed immediately after smelling fragrances. That is to say, the effects of fragrances on our central nervous system are rapid and powerful. However, a majority of fragrances are easy to oxidation,7–8 hydrolysis9 and especially volatilization.10–11 So the pure fragrances are released rapidly, which severely affects their storage, performances and useful life. In particularly, we often feel pungent and uncomfortable when we smell magnificent aroma. Therefore, it is necessary to find ways to release the scents slowly.

With the developments of nanotechnology, there have been many kinds of nanomaterials with the ability of slow release.12–14 The essences of changing release rate are the balances of interaction and de-interaction. These interactions mainly include covalent interactions,15–16 coordination interactions,17–18 hydrophilic–hydrophobic interactions,19 electrostatic interactions,20–21 interfacial interactions,22–23 and so on. Above all, interfacial interactions based on mesoporous silica nanoparticles are lasting and reversible. However, preparation of nanomaterials with the only ability of slow release is far from enough. Firstly, the fragrance loaded nanoparticles are best to be added into a kind of living goods such as wallpaper. Besides, the aroma do not need to be released anytime. For example,
the aroma molecules are best not to be released when the fragrance products are stored or we sleep in the dark. Therefore, nanoparticles with the ability of photo-driven releasing fragrance need to be designed and prepared.

In this study, as shown in Figure 1, mesoporous silica nanocolumns modified with azobenzene derivatives (MS-C) were prepared. Sandela 803, a kind of representative synthetic santalum album essential oil, was then encapsulated into the MS-C via both interfacial interactions and hydrophobic interactions to prepare fragrance loaded nanomaterials (MS-C@S803). The N=N bonds in azobenzene derivatives can cis-trans isomerize after stimulate by light.24 So in the light, the dynamic azobenzene derivatives were seemed like impeller to impel the sandela 803 molecules from the mesoporous.25 In the dark, by contrast, the static azobenzene derivatives served as gatekeepers to prevent the guest molecules from escaping. So the MS-C@S803 had the ability of light-driven releasing sandela 803. The MS-C@S803 was then added into wallpaper to prepare fragrant wallpaper (MS-C-W@S803). The MS-C-W@S803 was further glued on the wall of mouse cage to mimic the wall of our house. Finally, the effects of pure sandela 803 treated wallpaper and MS-C-W@S803 on central nervous system of mice were studied. The effects was evaluated in four level: behavioural level, tissular level, cellular level and molecular level. Firstly, the elevated plus maze and open field test were performed to detected the effects on emotion of mice in behavioural level. Secondly, neuropotentials were measured to study the effects on neural activity of specific regions of the brain in tissular level. Thirdly, BrdU staining was performed to detect the effects on nerve regeneration in cellular level. Finally, the expression of neurotransmitters were measured to detect the effects on nerve conduction in molecular level. According to the analysis of these results, the effects of pure sandela 803 and photo-driven

![Diagram](image-url)
and slowly releasing sandela 803 on central nervous systems of mice were primary discussed.

**EXPERIMENTAL REAGENTS AND INSTRUMENTS**

Sandela 803 was obtained from Shanghai research institute of fragrance and flavor industry. 3-(Triethoxysilyl) propyl isocyanate (TESPIC), acetylcholine (Ach) chloride, \( \gamma \)-aminobutyric acid (GABA) and dopamine (DOPA) hydrochloride was obtained from Alfa Aesar. 4-aminoazobenzene (AZO) and formic acid was obtained from Tokyo Chemical Industry (TCI). Superdry tetrahydrofuran (THF) was purchased from J&K Scientific Ltd. Hexadecyltrimethylammonium bromide (CTAB), Tetraethyl orthosilicate (TEOS), ammonium hydroxide (28% solution in water), N-methyl-D-aspartic acid (NMDA) and extra dry toluene (PhMe) were obtained from Acros Organics.

**Preparation of Photo-Driven Wallpaper Loaded with Sandela 803 Based on Mesoporous Silica Nanocolumn (MS-C-W@S803)**

Firstly, TESPIC-AZO was synthesized via addition reaction. TESPIC (1731.52 mg) and AZO (1380.68 mg) were dissolved into superdry THF (50 mL) and refluxed at 80 °C for 24 h. Hexane (300 mL) was then added to facilitate the crystallization of product at \( \sim \)20 °C. The orange needle-like crystals were separated by filtration. Secondly, CTAB (1000 mg) and 28% ammonium hydroxide (25 mL) were dispersed into deionized water (336 mL) and heated to 80 °C. When the solution was clear, TEOS (6.55 mL) dissolved into ethanol (5 mL) was immediately added in a drop-wise manner into the surfactant sol with stirring for 2.5 h. The precipitate was collected by filtration and calcined at 550 °C for 5 hours to remove the CTAB surfactants. Thirdly, the precipitate (250 mg) and TESPIC-AZO (778.1 mg) were dissolved into ethanol (12.5 mL) and then added into the CTAB surfactant. Fourthly, the particles (MS-C) were collected via filtration. Lastly, MS-C (250 mg) was dispersed into deionized water (125 mL). Sandela 803 (200 mg) was dissolved into ethanol (12.5 mL) and then added into the solution under vigorous stirring in the light for 24 h. The organic solvents and unloaded sandela 803 were then removed by dialyzing in a Cellu SepH1-membrane (MWCO 7000) against deionized water in the dark to obtain MS-C@S803. Finally, wallpaper (50 cm\(^2\)) was immersed in MS-C@S803 aqueous solutions (1 mg/mL) under stirring. After 12 h, the wallpaper was then dried at 40 °C for 1 h in an oven to obtain MS-C-W@S803.

**Animals**

Female C57 mice (3 weeks and 6 weeks, respectively) were purchased from the Academy of Military Medical Sciences of China. All procedures involving experimental animals were performed in accordance with protocols approved by the Committee for Animal Research of Peking University, China. MS-C-W@S803 was glued on the three side wall of mouse cage. The 3-week-old mice were cultured for 7 days and the 6-week-old mice were cultured for 30 days.

**Open Field Test**

The open field test referred to the method of Heijtz and his colleagues.\(^{26}\) In brief, mice were placed individually in open field box (48 cm \( \times \) 48 cm; Acti-Mot detection system; TSE) and the spontaneous motor activity was recorded. The following parameters were automatically recorded: distance travelled in all the box and in the center of the box.

**Elevated Plus Maze Test**

The elevated plus maze test referred to the methods reported by Mansouri et al.\(^{27}\) Briefly, the elevated plus maze consisted of two open arms and two closed arms, 10 \( \times \) 50 (length \( \times \) width) with an open roof arranged such that the two arms of each type were opposite to each other. The maze was elevated 50 cm above the ground. Mice were placed individually in the center of the maze and the spontaneous motor activity was recorded. The following parameters were automatically recorded: distance travelled in all the maze and in the open arms of the maze.

**Electrophysiology Test**

Mice were anesthetized by isoflurane and the head of them placed on the stereotaxic apparatus and the skulls were exposed. The hippocampus, hypothalamus and olfactory bulb regions were located via The Mouse Brain: In Stereotaxic Coordinates. The Electrophysiology signals in above-mentioned brain areas were detected by a physiological recorder (Techman Soft, China).

**Immunohistology**

Brains were rapidly excised and fixed in 4% (w/v) paraformaldehyde after the mice were sacrificed. The fixed

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**Table I. The conditions of MS.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ach</th>
<th>DOPA</th>
<th>GABA</th>
<th>NMDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRM</td>
<td>146.10</td>
<td>153.98</td>
<td>104.10</td>
<td>148.07</td>
</tr>
<tr>
<td>Ionization mode</td>
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<td>ESI(+)</td>
<td>ESI(+)</td>
<td>ESI(+)</td>
</tr>
<tr>
<td>Ion spray voltage</td>
<td>87.10</td>
<td>137.0</td>
<td>87.1</td>
<td>88.0</td>
</tr>
<tr>
<td>Temperature (°C)</td>
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<td>5000</td>
<td>5000</td>
<td>5000</td>
</tr>
<tr>
<td>Ion source gas 1</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Ion source gas 2</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Curtain gas</td>
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<td>15</td>
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<td>15</td>
</tr>
<tr>
<td>DP</td>
<td>31</td>
<td>41</td>
<td>26</td>
<td>61</td>
</tr>
<tr>
<td>CE</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>CXP</td>
<td>4</td>
<td>12</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>
samples were then embedded in paraffin blocks to prepare tissues sections at a thickness of 5 μm. After deparaffinization, the brain sections were stained with BrdU antibody and DAPI. The images were visualized by an optical microscope. The magnification used in the experiment was 20×.

**Expression of Neurotransmitters**

The expression of neurotransmitters was measured via liquid chromatography-mass spectrometry (LC-MS). Briefly, brain tissue sample was weighed, then 4 volumes of physiological saline was added and homogenized to obtain a brain homogenate sample. 50 μL of brain homogenate sample was taken, then 305 μL of methanol was added, fully whirled for 3 min and centrifuged at 4000 r/min for 10 min, 100 μL of supernatant was taken to test by LC-MS. The condition of MS was shown in the Table I. For LC, the chromatographic column was Polar RP column (3 mm × 50 mm ID., 2.6 μm, phenomenex) and the Column temperature was 40 °C. The run time was 4 minutes. The mobile phase condition was shown in Table II. The peaks of DOPA, Ach, GABA and NMDA was at 0.56, 0.56, 0.53 and 0.57 minutes, respectively.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow velocities (mL/min)</th>
<th>Methanol (0.1% FA) (%)</th>
<th>Water (0.1% FA) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.35</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>0.8</td>
<td>0.35</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>0.9</td>
<td>0.35</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>1.4</td>
<td>0.35</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>1.41</td>
<td>0.35</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>0.35</td>
<td>2</td>
<td>98</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

All data were expressed as mean ± SD unless otherwise indicated. Statistical significance was analyzed using one-way ANOVA. Statistical differences in behavioral data were determined using two-way repeated measure ANOVA.

**RESULTS**

**Characterisation of MS-C@S803**

The photo-responsive silane coupling agent TESPIC-AZO was synthesized via an addition reaction (Fig. 2(A)). The molecular weight of the obtained product was detected by matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOFMS). As shown in Figure 2(B), there was a peak of 445.13, which was the peak of TESPIC-AZO. So TESPIC-AZO was synthesized successfully.

![Figure 2.](image-url)  
(A) The reaction formula of TESPIC-AZO. (B) The MALDI-TOFMS of TESPIC-AZO.
The MS-C was prepared via a sol–gel method and the modification of TESPIC-AZO. The chemical structures of pure mesoporous silica, TESPIC-AZO and MS-C was detected via fourier transform infrared spectroscopy.

As shown in Figure 3, for TESPIC-AZO, the obvious absorption peak of the –CH$_3$ at 2975 and 2870 cm$^{-1}$. And the peak of 2930 cm$^{-1}$ was attributed to –CH$_2$. Besides, the absorption band of 1560 cm$^{-1}$ was assigned to the stretching vibration of benzene ring. There was no obvious absorption peak in the spectrum of the pure mesoporous silica nanocolumns, which indicated that CTAB template can be completely removed by calcination. By contrast, MS-C displayed the peaks of TESPIC-AZO. The peak 1560 cm$^{-1}$ was attributed to the stretching vibration of benzene ring. And the obvious absorption peak of the –CH$_3$ at 2975 and 2870 cm$^{-1}$. In addition, there was no absorption peak of the –CH$_3$ at 2975 and 2870 cm$^{-1}$, which indicated that there was no free TESPIC-AZO in
MS-C. From the above, these results demonstrated that all
the TESPIC-AZO was attached to the pure mesoporous
silica nanocolumns.

The morphology of the MS-C was observed via
scanning electron microscope (SEM). As shown in
Figure 4(A), the MS-C was columnar and the diameter
and height was about 170 and 250 nm, respectively. After
loaded with sandela 803, the morphology and size was not
changed (Fig. 4(B)).

The Effects of MS-C-W@S803 on the
Behaviouristics of Mice

Firstly, open field test was performed to evaluate the
effect of MS-C-W@S803 on the behaviouristics of mice.

We placed the different groups of mice in open field box
individually. The spontaneous motor information including
total distance travelled and distance in the center of the
open field (zone 5) was detected for 5 minutes. As shown
in Figure 5(A), after cultured in MS-C-W@S803 treated
mouse cages for 7 days, both the total distance and the
distance in zone 5 was increased obviously. In addition,
the increased effect was more obvious after cultured for 30
days. The quantitative results were shown in Figures 5(B)–
(H). The movement distance in zone 5 of the mice in MS-
C-W@S803 group was up to 1521.98 mm after 7 days.
The distance of mice in W@S803 and pure W group,
by contrast, was only 448.71 and 175.69 mm, respec-
tively. The movement distance ratio of zone 5 of mice

![Figure 6](image-url)

**Figure 6.** Representative tracks of movement patterns of different samples in elevated plus maze. The green curves rep-
resented the movement of mice. (B–H) The quantitative results of Figure A. The mean ± SD is shown (n = 3). *P < 0.05, **P < 0.01,
***P < 0.005.
in MS-C-W@S803 group was up to 6.62%. The ratio in W@S803 and pure W group, by contrast, was only 2.85% and 1.27%, respectively. In addition, after 30 days, the distinction was clearer. The movement distance and the ratio in zone 5 of MS-C-W@S803 reached 2366.78 mm and 7.84%, respectively.

Secondly, elevated plus maze test was also performed to evaluate the effect of MS-C-W@S803 on the behaviouristics of mice. As shown in Figure 6, both the total movement distance and movement in open arms of MS-C-W@S803 group were increase obviously compared with W@S803 and pure W group both for 7 days and 30 days. The quantitative results were shown in Figures 6(B)–(H). The movement distance in open arms of MS-C-W@S803 was 2951.47 mm after 7 days. The movement distance of W@S803 and pure W, by contrast, were only 1384.52 and 419.85, respectively. The movement distance ratio of open arms in MS-C-W@S803 group was up to 25.49%. The W@S803 and pure W group were only 15.80% and 9.21%. Both the movement distance and ratio in open arms were enhanced after 30 days. They reached 3856.01 mm and 29.79%, respectively.

**Figure 7.** The electrophysiology signals cultured with different samples for 7 days in (A) hippocampus, (C) hypothalamus, (E) olfactory bulb and the quantitative results in (B) hippocampus, (D) hypothalamus, (F) olfactory bulb. The mean±SD is shown (n = 3). *P < 0.05, **P < 0.01, ***P < 0.005.

The Effects of MS-C-W@S803 on the Electrophysiology of Mice

The potential of hippocampus, hypothalamus and olfactory bulb regions were detected by a physiological recorder. As shown in Figure 7(A), for hippocampus region, the amplitudes were increased when the mice was cultured in the MS-C-W@S803 treated mouse cages for 7 days. The potential difference was up to 2664.09 μV compared with the 1483.05 and 1321.41 μV of W@S803 and pure W groups, respectively. As shown in Figures 7(B) and (C), the amplitudes in both hypothalamus and olfactory bulb regions were also increased when cultured with MS-C-W@S803. The potential difference in hypothalamus region was 2261.35 μV after cultured with MS-C-W@S803 while the values were 1492.31 and 1342.78 μV, respectively after cultured with W@S803 and pure W. For olfactory bulb region, the amplitude value of MS-C-W@S803 was 2984.62 μV. By contrast, the values of W@S803 and pure W were only 1333.62 and 595.86 μV, respectively.

After cultured for 30 days, the potential was also measured. As shown in Figures 8 and 9, after 30 days,
the amplitudes of among hippocampus, hypothalamus and olfactory bulb regions were increased. The potential differences were reached 6481.94, 2989.01 and 3909.30 μV, respectively.

The Effects of MS-C-W@S803 on the Nerve Regeneration

The effects of MS-C-W@S803 on the nerve regeneration were evaluated by immunofluorescent staining with anti-BruU antibody. As shown in Figure 10, the nerve regenerations in corpus striatum, substantia nigra and olfactory bulb were detected. There were more nerve regenerations in MS-C-W@S803 group than that in W@S803 and pure W groups. Besides, there were more nerve regenerations cultured for 30 days than that cultured for 7 days. Finally, there were more nerve regenerations in olfactory bulb region than that in corpus striatum, substantia nigra regions.
DISCUSSION

Fragrances can relieve tension, make the spirit of relaxation, refresh ourselves, help tranquilize the mind and improve sleep. And the effects of fragrance were rapid and most of them were on the CNS. But the fragrances are released rapidly due to the strong volatility. If the concentrations of fragrances in air are high, they will make us uncomfortable. So we prepared fragrance encapsulated nanoparticles to overcome this obstacle and then analyse the effects of nano-fragrance and pure fragrance on CNS.

The photo-driven mesoporous silica nanocolumns were prepared to encapsulate sandela 803 and release this fragrant molecule when stimulated by light. The MALDI-TOFMS result demonstrated that we synthesized photosensitive silane coupling agent TESPIC-AZO successfully. In the following, the TESPIC-AZO was used to be modified on the mesoporous silica nanocolumns to prepare light-activated mesoporous silica nanocolumns (MS-C). MS-C was then encapsulated sandela 803 to obtain MS-C@S803. It was then added into wallpaper to prepare fragrant wallpaper with the ability of photo-driven releasing sandela 803 (MS-C-W@S803). Finally, we glue the wallpaper on the three side wall of mouse cages and mice were cultured in these cages for 7 days and 30 days.

The effects of MS-C-W@S803 and pure sandela 803 treated wallpaper (W@S803) on the behaviouristics of mice were evaluated via open field test and elevated plus maze. The open field test has been widely used to analyse the anxiety-like behaviour of animals. An increase in the time spent and movement distance in the center of the open field is regarded as a powerful marker for the anxiolytic effect. The results in Figure 5 show that the motor activity of the mice that cultured with MS-C-W@S803 was increased obviously. The movement distance of the mice in MS-C-W@S803 group was much longer than that in W@S803 and pure W groups. In addition, both the movement distance in the center of the open field and its ratio were significantly increased. So mice cultured with MS-C-W@S803 could improve their mood and fight anxiety. We also performed elevated plus maze to analyse the effects on the behaviouristics. The elevated plus maze test has been used effectively to assess the neurobehavioral profile of animals under the influences of anxiolytic agents. An increase of movement distance in open arms was the marker for the anxiolytic effect. The result in Figure 6, both the movement distance of MS-C-W@S803 group in the open arms and its ratio was increased obviously compared with the distance of the other two groups. Besides, the anxiolytic effect was more significant as time progresses.

We then analyse the effects in tissue level. The electrophysiology signal reflects the neural activity. The fragrance influences CNS via sense. And the amplitudes of MS-C-W@S803 group in olfactory bulb were much larger than the amplitudes of the other two groups. So the olfactory event-related neural activity was enhanced. Besides,
both the memory related neural activity, internal secretion and mood related neural activity of MS-C-W@S803 group were also enhanced because the potential difference of them in hippocampus and hypothalamus region was larger than others. In addition, the effects on increasing neural activity were also more significant as time progresses.

In the following, the effects in cellular level were detected. Olfactory bulb participate in olfaction. Most of dopaminergic neurons were distributed in substantia nigra. Corpus striatum was associated with muscular movement. The nerve regeneration abilities of mice cultured with MS-C-W@S803 were enhanced remarkably among in above-mentioned regions. Besides, the effects were also more significant as time progresses.

Finally, the effects in molecular level were detected. DOPA could create exhilaration and focused attention. Ach participates in learning memory and keep our conscious. GABA could participate in the effects of antianxiety. NMDA takes part in internal secretion and mood related neural activity. The expressions of all the above-mentioned neurotransmitters in MS-C-W@S803 group were increased.

CONCLUSIONS

In this study, the effects of MS-C@S803 treated wallpaper (MS-C-W@S803) and pure sandela 803 treated wallpaper (W@S803) on CNS were analysed and compared. In behavioural level, MS-C-W@S803 processed a better anxiolytic effect than W@S803. In the following, the mechanism of the anxiolytic effect was investigated in tissue, cell and molecule levels. The neural activity in all of olfactory bulb, hippocampus and hypothalamus regions were enhanced. Besides, the abilities of nerve regeneration in all of olfactory bulb, substantia nigra and corpus striatum regions of mice cultured with MS-C-W@S803 were enhanced compared with the mice cultured with W@S803. In addition, the expression of DOPA, Ach, GABA and NMDA in the brain of MS-C-W@S803 was increased. Interestingly, the anxiolytic effects were more significant as time progresses.

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REFERENCES


