Hierarchical rippled and crumpled PLA microstructure generated through double emulsion: the interesting roles of Pickering nanoparticles

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EXPERIMENTAL SECTION

Reagents and Materials. Commercial available biodegradable material, polylactic acid (PLA, 2003D), was purchased from Nature Works. Polyvinyl alcohol (PVA) (Kuraray, PVA-217, DP=1700, 88.5% hydrolysed), was kindly provided by Kuraray Co., Ltd. (Japan). Dichloromethane (DCM), ammonium bicarbonate (NH₄HCO₃, analytical grade), ferric chloride (FeCl₃·3H₂O), ethylene glycol, sodium acetate anhydrous, sodium citrate, and tetraethyl orthosilicate (TEOS, 98%) purchased from Aladdin (Shanghai, China). Deionized water (DI, 18.2 MΩ cm⁻¹) was obtained through a Milli-Q water-purification system. Pure SiO₂ was kindly donated by Professor. Yongsheng Yan. All other chemicals and solvents were of reagent grade or better, and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of Fe₃O₄@SiO₂ nanoparticles. Firstly, the citrate-modified magnetic Fe₃O₄ nanoparticles were prepared through a solvothermal reaction previously reported with minor modification.[1] Briefly, FeCl₃·6H₂O (4.05 g) and sodium acetate anhydrous (7.2 g) were dissolved in ethylene glycol (40 mL) with magnetic stirring at 160 °C for 1.0 h. The homogeneous solution was transferred into a teflon-lined stainless steel autoclave, and the autoclave was then sealed to heat at 200 °C for 12 h. After reaction, the autoclave was cooled to room temperature, and the supernatant of mixture was discarded. The obtained Fe₃O₄ particle suspension was washed successively with ethanol and H₂O for three times, and dried under vacuum for 6.0 h at 60 °C.

Secondly, the core/shell Fe₃O₄@SiO₂ particles were generated by a modified Stöber method.[2] Briefly, 10 mg of Fe₃O₄ nanoparticles were dispersed in a mixture of 9.0 mL of deionized water, 40 mL of ethanol, and 1.0 mL of ammonia solution, and the mixture was homogenized by sonication for 10 min. Under continuous stirring, 0.2 mL TEOS was added to the mixture, and the reaction was then performed at 40 °C for 12 h. The product was collected by external magnet, washed several times with ethanol and water, and finally dispersed in water for further use.

Fabrication of hierarchical rippled and crumpled PLA microstructure. PLA microstructure with hierarchical curled, rippled, and crumple textures were fabricated utilizing double emulsion by simply addition of Fe₃O₄@SiO₂ nanoparticles.[3,4] Typically, 1.25 mL of NH₄HCO₃ aqueous solution (5%, w/v) containing Fe₃O₄@SiO₂ particles was dispersed in another PLA/DCM solution (6.25%, w/v) under a high-speed homogenization (23,000 rpm, 90 s, IKA T25) to prepare the primary water-in-oil (W₁/O) reverse emulsion. Then the W₁/O emulsion (1.0 mL) was poured into 5.0 mL of PVA aqueous solution (0.1%, w/v), and followed by the emulsification for another 1.0 min, resulting in the formation of the double emulsion (W₁/O/W₂). Subsequently, the resultant W₁/O/W₂ double emulsion was poured into 20 mL of 0.1% (w/v) PVA solution, and DCM was allowed to evaporate at 30 °C under shaking (500 rpm) for half an hour to yield PLA microstructures. Finally, the PLA microstructures ith hierarchical curled, rippled, and crumple textures were collected by an external magnet, washed with deionized water to completely remove surfactants and dried under vacuum. (For comparison, pure SiO₂ nanoparticles were specifically utilized as the Pickering nanoparticles as well, and the fabrication method followed the aforementioned protocol.)

Characterization. The Fourier transform infrared (FT-IR) spectra of the samples were tested on the Nicolet NEXUS-470 FTIR apparatus (U.S.A.), which was recorded using KBr pellets for solid samples. The morphology
of the products was analyzed via the field emission scanning electron microscopy (SEM, JSM-7100F), which was
carried out with an electron microscope equipped with a field emission electron gun and an energy dispersive
spectrometer (EDS). The morphology of the Fe₃O₄@SiO₂ nanoparticles was characterized by transmission
electron microscopy (TEM, JEM-2100). The magnetic properties were measured using a VSM (VSM 7410, Lake
Shore, USA). TGA of the samples were performed (about 10 mg) using a Diamond TG/DTA instruments (Perkin-
Elmer, U.S.A.) under a nitrogen atmosphere up to 800 °C with a heating rate of 5.0 °C min⁻¹. The measurement
of the liquid surface tensions was directly obtained from dynamic contact angle meter and tensiometer (DCAT 21,
Data Physics Instruments GmbH). The Brunauer–Emmett–Teller (BET) surface area and pore volume were
measured by N₂ adsorption using an adsorption apparatus (Micromeritics, ASAP 2020, USA). Raman spectra were
collected in the range of 500 cm⁻¹ to 3500 cm⁻¹ using the 560 nm laser excitation line.

Detailed discussions:

**TGA analysis, magnetic property and BET specific surface area.** The TGA analysis of the prepared PLA
microstructure with different amount of Fe₃O₄@SiO₂ nanoparticles involved was utilized to determine the mass
fraction of the Fe₃O₄@SiO₂ nanoparticles, and the results are given in Table S1. Generally, the calculated mass
fractions of the residual Fe₃O₄@SiO₂ nanoparticles for different patches in Fig. S5A were 4.53%, 12.92%, 20%,
25.02 %, and 33.10%, respectively, which were slightly lower than the theoretical values. This result indicated that
a small portion of Fe₃O₄@SiO₂ nanoparticles was not involved during the fabrication process, which is in
consistence with black accumulations presented in Fig. S3. Additionally, the room temperature magnetic
properties of PLA microstructure with different amounts of Fe₃O₄@SiO₂ nanoparticles were measured by VSM.
As shown in Fig. S5B, all the products exhibited typical ferromagnetic curves, and their values of saturation
magnetization were 0.37 emu g⁻¹, 0.68 emu g⁻¹, 1.33 emu g⁻¹, 1.59 emu g⁻¹ and 2.03 emu g⁻¹, respectively,
which were closely related to the mass fractions of Fe₃O₄@SiO₂ particles in PLA microstructure. Though their saturation
magnetization values were far lower than original Fe₃O₄@SiO₂ nanoparticles (42.45 emu g⁻¹, Fig. S5C), PLA
microstructure possessed sufficient magnetic responsive ability, as the obtained PLA microstructure have to
withstand such great load originate from the Fe₃O₄@SiO₂ nanoparticles and the drag force attributed to their huge
volumes. Besides, when the conditions that the PLA microstructure possess a huge inner cavity and the
Fe₃O₄@SiO₂ nanoparticles account for a relatively small fraction of the composite but a big mass fraction of the
microstructure are taken into consideration, we conclude that the PLA microstructure bears sufficient magnetic-
stimulation for easy guidance, which had potential various applications such as oil/water separation.

Additionally, the nitrogen adsorption/desorption isotherm linear blot of the representative PLA
microstructure displayed in Fig. S5D indicated that there were mesopores presented as well, which may be created
owing to gas penetration. In the meanwhile, the BET surface area given as 7.9383 m² g⁻¹ was comparable 197
times larger than the smooth sphere type PLA ones (0.0402 m² g⁻¹), which was resulted from their hierarchical
textures with the combination of macropores, mesopores, and hollow nature together. The unique PLA
microstructure bearing hierarchical rippled and crumples textures in combination with hierarchical pores and
magnetic nature were highly promising in various areas and demonstrated the highly superiority and feasibility of
the proposed protocol.

**EDS, micro-Raman, and XRD discussions:** In order to further elucidate the existence of Fe₃O₄@SiO₂
nanoparticles inside the PLA microstructure, micro-Raman spectra and powder XRD measurements were
conducted together. As shown in Fig. S7, the micro-Raman spectra of PLA microstructure with different scanning
depth exhibited many same absorption peaks in the range of 3500-500 cm⁻¹. The characteristic Raman bands in the
range of 3100-2800 cm⁻¹ were identified as C-H stretching modes of -CH₃ and -CH₂ groups.[5] However, the main
bands can be observed in the region of the Raman spectra below 1800 cm⁻¹. For neat PLA, the band associated
with C=O stretching appeared at 1769 cm⁻¹, while -CH₃, C-H bending and C-O-C stretching bands located in the
region of 1500-800 cm\(^{-1}\).\(^5\) And the strong band at 873 cm\(^{-1}\) were assigned to the characteristic \(-\text{C-COO}^\text{-}\) stretching of the repeated unit for PLA.\(^5\) By comparing the Raman spectra of interior and exterior surface of PLA microstructure, it was clear in Fig. S7B that the peaks of pristine Fe\(_3\)O\(_4\)@SiO\(_2\) nanoparticles appeared at 550 cm\(^{-1}\) and 670 cm\(^{-1}\), which indicated that Fe\(_3\)O\(_4\)@SiO\(_2\) nanoparticles were located at the interior surface of PLA microstructure.\(^6\) Moreover, the diffraction peaks with 20 at 30.4°, 35.6°, 43.3°, 53.5°, 57.3°, and 62.8° from the crystalline phase of the Fe\(_3\)O\(_4\)@SiO\(_2\) nanoparticles were observed in Fig. S7C.\(^7\) After incorporating into PLA shell, particularly, the PLA microstructure exhibited the same characteristic diffraction peaks in analogous to pure core/shell Fe\(_3\)O\(_4\)@SiO\(_2\) nanoparticles. Howbeit, there was an intensive and sharp peak at around 16.8° and the other smaller peak at about 19.2° that were both ascribed to the crystallinity phase of PLA microstructure, which were in consistence with the pseudo orthorhombic \(\alpha\) structure with the chains in a left handed 10/3 helices conformation and the \(\beta\) structure with 3/1 chain conformation deprived from neat PLA, respectively.\(^8\)

**Classic spreading coefficient and the partial wetting theory:** In fact, according to the classic spreading coefficient and the partial wetting theory,\(^9,10\) these resultant equilibrium configuration of the double emulsion system can be rationally determined and predicted based on the interactive interfacial tensions among different phases (\(\gamma_{ij}\), \(\gamma_{ik}\), and \(\gamma_{jk}\)), and the spreading coefficients \(S_i\) for each phase are defined as

\[
S_i = \gamma_{jk} - (\gamma_{ij} + \gamma_{ik})
\]

\[
S_j = \gamma_{ik} - (\gamma_{jk} + \gamma_{ij})
\]

\[
S_k = \gamma_{ij} - (\gamma_{ik} + \gamma_{jk})
\]

Typically, the resulting equilibrium configuration of double emulsions can be divided into three categories, i.e. partial wetting (PW), complete wetting (CW), as well as complete dewetting (CD), as shown in Figure 1E.\(^9\) The internal water phase (W\(_1\)), the intermediate oil phase (O) and the external water phase (W\(_2\)) are referred as Phase 1, Phase 2, and Phase 3, respectively. According to the spreading coefficient and partial wetting theory, \(\gamma_{13}\) is assumed to be almost 0 mN m\(^{-1}\) under the circumstances that Phase 1 and Phase 3 are completely soluble and no interface exists the spreading coefficient.\(^10\) Thus, when \(\gamma_{13} < \gamma_{23}\) (\(S_i=0\)), the possible combination of \(S_i\) are

\[
S_i<0; S_j<0; S_k<0,
\]

\[
S_i>0; S_j<0; S_k<0,
\]

\[
S_i<0; S_j<0; S_k>0,
\]

which result in PW, CW, and CD, respectively. Herein the results of measured interfacial tension among different phases were given in Table 1. When pure water phase (W\(_1\)) was designated as Phase 1, the spreading coefficients were negative, and the partial wetting was determinative. As a result, traditional robust core shell microcapsules could be obtained (Fig. 1D). However, when NH\(_4\)HCO\(_3\) or NH\(_4\)HCO\(_3\) combined with Fe\(_3\)O\(_4\)@SiO\(_2\) nanoparticles were added into Phase 1, the interfacial tension of \(\gamma_{12}\) decreased, and then the spreading coefficients of the two systems were \(S_i>0; S_j<0; S_k<0\), indicating that the equilibrium configuration of them were complete wetting. In this case, the extrusions generated as shown in Fig. 1B and Fig. 1C were derived from the decomposition of NH\(_4\)HCO\(_3\), rather than the dewetting of inner water droplets. In the case that Fe\(_3\)O\(_4\)@SiO\(_2\) nanoparticles were involved, the final spherical shape of the PLA microstructure could be well maintained.
Fig. S1 TEM images of the synthesized Fe$_3$O$_4$@SiO$_2$ nanoparticles.
Fig. S2 Surface textures of PLA microstructure with fine textures of hierarchical rippled, and crumpled morphologies (A) and plate-like PLA architecture.
Fig. S3 the corresponding optical images of the double emulsions using different amounts of Fe₃O₄@SiO₂ nanoparticles: 0 mg ml⁻¹ (A), 10 mg ml⁻¹ (B), 30 mg ml⁻¹ (C), 50 mg ml⁻¹ (D), 70 mg ml⁻¹ (E), 90 mg ml⁻¹ (F), and corresponding magnified optical images from (a) to (f).
Fig. S4 SEM images of PLA microstructure using different amounts of Fe₃O₄@SiO₂ nanoparticles: 10 mg mL⁻¹ (A), 50 mg mL⁻¹ (B), and 90 mg mL⁻¹ (C), and TEM images of the yielded microstructure with varied amounts of Fe₃O₄@SiO₂ nanoparticles from 50 mg mL⁻¹ (E) and 90 (F) mg mL⁻¹.
Fig. S5 TGA analysis of the prepared PLA microstructure with different amounts of \( \text{Fe}_3\text{O}_4@\text{SiO}_2 \) nanoparticles involved (A), room temperature magnetic properties of PLA microstructure with different ratios of \( \text{Fe}_3\text{O}_4@\text{SiO}_2 \) nanoparticles were measured using VSM (B), VSM spectra of pristine \( \text{Fe}_3\text{O}_4@\text{SiO}_2 \) nanoparticles (C), and the nitrogen adsorption/desorption isotherm linear blot of the representative PLA microstructure indicating the existence of mesopores spontaneously (D).
Fig. S6 EDS results of the exterior and interior surface of the PLA microstructure.
Fig. S7 The micro-Raman spectra of the PLA microstructure under different scanning depths (A), Raman spectra of the interior and exterior surface of the PLA microstructure ranging from 300 cm$^{-1}$ to 1500 cm$^{-1}$ (B), and powder XRD patterns of the PLA microstructure and pristine Fe$_3$O$_4$@SiO$_2$ nanoparticles (C).
Fig. S8 SEM image of porous PLA microspheres with varied concave pores presented under polymer concentration at 1.58% (w/v) (A), 3.125 % (w/v) (B) and 6.25 % (w/v) (C), respectively, PLA microstructures obtained under polymer concentration at 6.25 % (w/v) with Fe$_3$O$_4$@SiO$_2$ nanoparticles added (D).
Fig. S9 Representative SEM images of the final products with the homogenization speed varied ranging from 10,000 rpm to 20,000 rpm: 10,000 rpm (A), 12,000 rpm (B), 14,000 rpm (C), 16,000 rpm (D), 18,000 rpm (E), and 20,000 rpm (F), respectively.
Fig. S10 Illustration of the proposed extrusion, force equilibrium and three catalogues of the resulting double emulsion configurations with predicted final morphological products according to classic spreading coefficient and partial wetting theory (E), $\theta_i$ and $\theta$ are denoted as the contact angle between corresponding interface.
Fig. S11 Corresponding SEM images of PLA microstructures with pure SiO₂ chosen as candidate Pickering nanoparticles (A) and magnified SEM image (B), TEM image of the obtained pure SiO₂ nanoparticles (C) and PLA microstructure fabricated via the addition of SiO₂ nanoparticles (D).
Fig. S12 Water contact angle of the PLA microstructures (A), illustration of oil-adsorption assay (B), fluorescent image of cells seeded plate-like PLA (C) and PLA microstructure (D), and corresponding magnified fluorescent images (c and d), SEM images of cells cultivated with the plate-like PLA (E) and PLA microstructure (F).
Fig. S13 The MTT assay was further conducted to evaluate the cytotoxicity of plate-like PLA and PLA microstructure towards fibroblasts.
Table S1 Comparison of the theoretical and virtual mass fraction of Fe$_3$O$_4$@SiO$_2$ nanoparticles in PLA microstructure with varied Fe$_3$O$_4$@SiO$_2$ nanoparticles added (noted as PLA microstructure (X), X is referred as the amount of Fe$_3$O$_4$@SiO$_2$ nanoparticles added. For example, PLA microstructure (10) means the addition of 10 mg mL$^{-1}$ Fe$_3$O$_4$@SiO$_2$ nanoparticles added).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Theory</th>
<th>Reality</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA microstructure (10)</td>
<td>4.0%</td>
<td>4.53%</td>
</tr>
<tr>
<td>PLA microstructure (30)</td>
<td>12.0%</td>
<td>12.92%</td>
</tr>
<tr>
<td>PLA microstructure (50)</td>
<td>20.0%</td>
<td>20.00%</td>
</tr>
<tr>
<td>PLA microstructure (70)</td>
<td>28.0%</td>
<td>25.02%</td>
</tr>
<tr>
<td>PLA microstructure (90)</td>
<td>36.0%</td>
<td>33.10%</td>
</tr>
</tbody>
</table>

Table S2 Summary of interfacial tensions, and the spreading coefficients of different Phase 1 as well as the theoretical configurations.

<table>
<thead>
<tr>
<th>Constitution of Phase 1, 2 and 3</th>
<th>$\gamma_{13}$</th>
<th>$\gamma_{12}$</th>
<th>$\gamma_{23}$</th>
<th>$S_1$</th>
<th>$S_2$</th>
<th>$S_3$</th>
<th>Theory</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O-PLA-PVA</td>
<td>0</td>
<td>20.50</td>
<td>16.80</td>
<td>-3.7</td>
<td>-37.3</td>
<td>3.7</td>
<td>PW $^{b)}$</td>
</tr>
<tr>
<td>NH$_4$HCO$_3$-PLA-PVA</td>
<td>0</td>
<td>3.56</td>
<td>16.80</td>
<td>13.24</td>
<td>-20.36</td>
<td>-13.24</td>
<td>CW $^{b)}$</td>
</tr>
<tr>
<td>NH$_4$HCO$_3$/Fe$_3$O$_4$@SiO$_2$-PLA-PVA</td>
<td>0</td>
<td>3.50</td>
<td>16.80</td>
<td>13.3</td>
<td>-20.30</td>
<td>-13.30</td>
<td>CW</td>
</tr>
</tbody>
</table>

a) The units of the interfacial tensions and the spreading coefficients are both mN m$^{-1}$.

b) PW and CW are abbreviations of partial wetting and complete wetting, respectively.

References