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Intra-articular injection of indomethacin-methotrexate *in situ* hydrogel for the synergistic treatment of rheumatoid arthritis

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1. Formulation optimization

First, the effects of different ratios of drug to PEI-SS, and different drug concentrations on the preparation of single drug-loaded nanoparticles were investigated (Table S1-S2). According to the results of Fig.S1, IND-NPs with good particle size dispersion could be formed when the ratio of IND to PEI-SS reached at 1:2, and the particle size distribution ranged from 80 nm to 300 nm. According to the results of Fig.S2, MTX-NPs with good particle size dispersion could be formed when the ratio of MTX to PEI-SS reached at 1:3, and the particle size distribution ranged from 100 nm to 350 nm. The results showed that the ratio of drug to carrier material was a key factor affecting the successful preparation of the nanoparticles. When the ratio of the drug to carrier material was above the optimal ratio, the drug was continuously precipitated during dialysis process because the carrier material was not enough to electrostatically load with the excess drug. When the ratio of the drug to carrier material was below the optimal ratio, the nanoparticles prepared by the solvent exchange method had a serious pile-up phenomenon, and the PDI index also became larger. This was because too much carrier material could not play the role of electrostatic compression, resulting in the increase of particle size dispersion. In addition, the results showed that the higher the concentration of drugs and PEI-SS was, the faster the formation rate of nanoparticles was and the larger the particle size was. Moreover, when the concentration of drugs and PEI-SS was too high, the stability of prepared nanoparticles became worse. When 20 mg IND and 40 mg PEI-SS were dissolved in 2 ml DMSO, the prepared IND-NPs had a wide range of particle size span and an asymmetric distribution of particle size, and it precipitated after being placed at room temperature for a while. When 20 mg MTX and 60 mg PEI-SS were dissolved in 2 ml DMSO, the particle size of the prepared MTX-NPs was too large, about 1 μm, and the MTX-NPs precipitated after being placed at room temperature for a while. Therefore, in order to keep the drug loading of IND and MTX as much as possible and ensure the stability of the

nanoparticle solution, 10 mg of IND and 10 mg or 5 mg of MTX were selected for further research on dual drug-loaded nanoparticles (Table S3). In order to ensure that the ratio of IND to PEI-SS was 1:2, and the ratio of MTX to PEI-SS was 1:3, when both the amount of IND and the amount of MTX was 10 mg, the amount of PEI-SS was 50 mg. However, it was found during the experiment that the stability of the prepared nanoparticles was extremely poor, possibly due to the excessive drugs and PEI-SS. When the amount of IND was 10 mg, the amount of MTX was 5 mg, and the amount of PEI-SS was 35 mg, the D-NPs with particle size of about 103 nm could be prepared and showed good stability. In order to further improve the drug loading, we reduced the amount of PEI-SS to 30 mg or 25 mg to investigate whether the D-NPs could be successfully prepared. When the amount of PEI-SS was 30 mg, the prepared D-NPs had a particle size of about 82 nm and good stability. In addition, it could be known from Table S4 that when IND was 10 mg, MTX was 5 mg and PEI-SS was 30 mg, the encapsulation efficiency and drug loading of the prepared D-NPs (Formulation III) were slightly higher than that of D-NPs based on Formulation II (10 mg IND, 5 mg MTX and 35 mg PEI-SS). Moreover, the less amount of PEI-SS was conducive to improve the biotoxicity of D-NPs as well. However, when the amount of PEI-SS was 25 mg, the drugs (10 mg IND and 5 mg MTX) were continuously precipitated during the dialysis process. The reason might be that the amount of PEI-SS was not enough to fully accommodate the drugs. Therefore, the formulation of D-NPs was finally determined to be 10 mg IND, 5 mg MTX and 30 mg PEI-SS, taking into account particle size of nanoparticles, particle size distribution, stability of nanoparticle solution, encapsulation efficiency and drug loading. Accordingly, the formulation of IND-NPs was determined to be IND 10 mg, PEI-SS 20 mg and the formulation of MTX-NPs was determined to be MTX 5 mg, PEI-SS 15 mg.

Table S1. Formulation optimization of PEI-SS-IND nanoparticles (IND-NPs)

Formulation	I	II	III	IV	V	VI
IND (mg)	5	5	5	2.5	10	20
PEI-SS (mg)	10	15	20	5	20	40
IND : PEI-SS	1:2	1:3	1:4	1:2	1:2	1:2
DMSO (ml)	2	2	2	2	2	2

Table S2. Formulation optimization of PEI-SS-MTX nanoparticles (MTX-NPs)

Formulation	I	II	III	IV	V	VI
MTX (mg)	5	5	5	2.5	10	20
PEI-SS (mg)	10	15	20	7.5	30	60
MTX : PEI-SS	1:2	1:3	1:4	1:3	1:3	1:3
DMSO (ml)	2	2	2	2	2	2

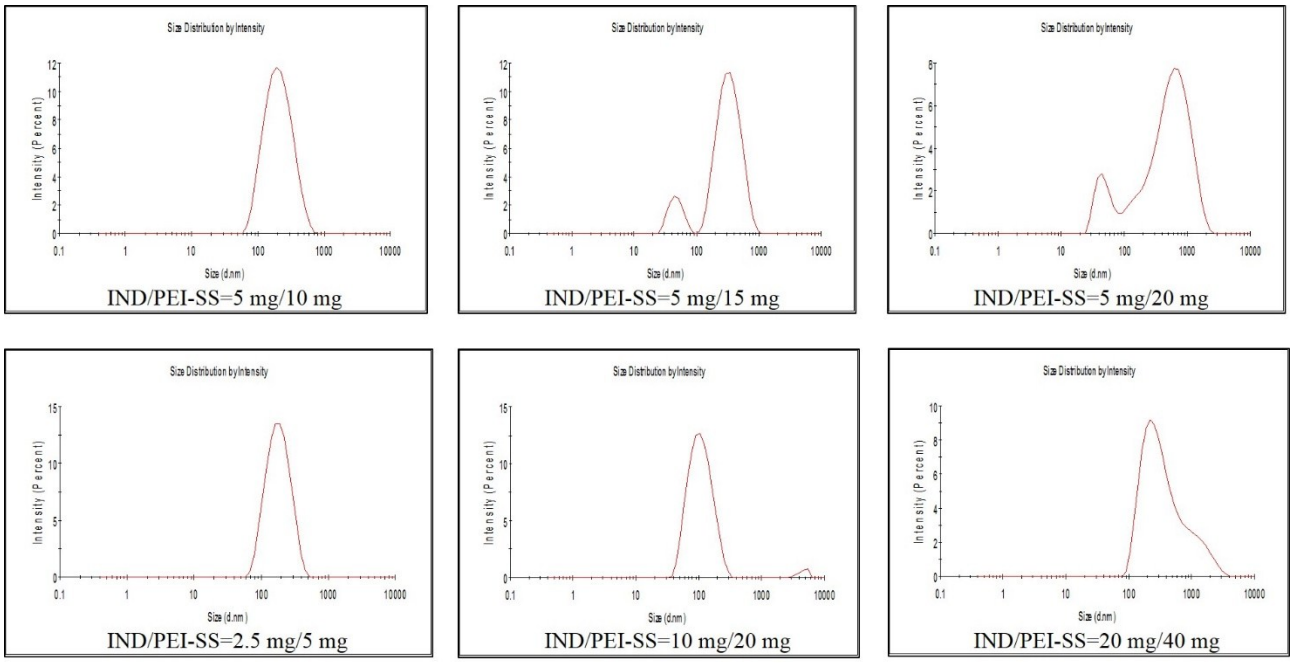

Fig.S1. Particle size distribution of IND-NPs formulations

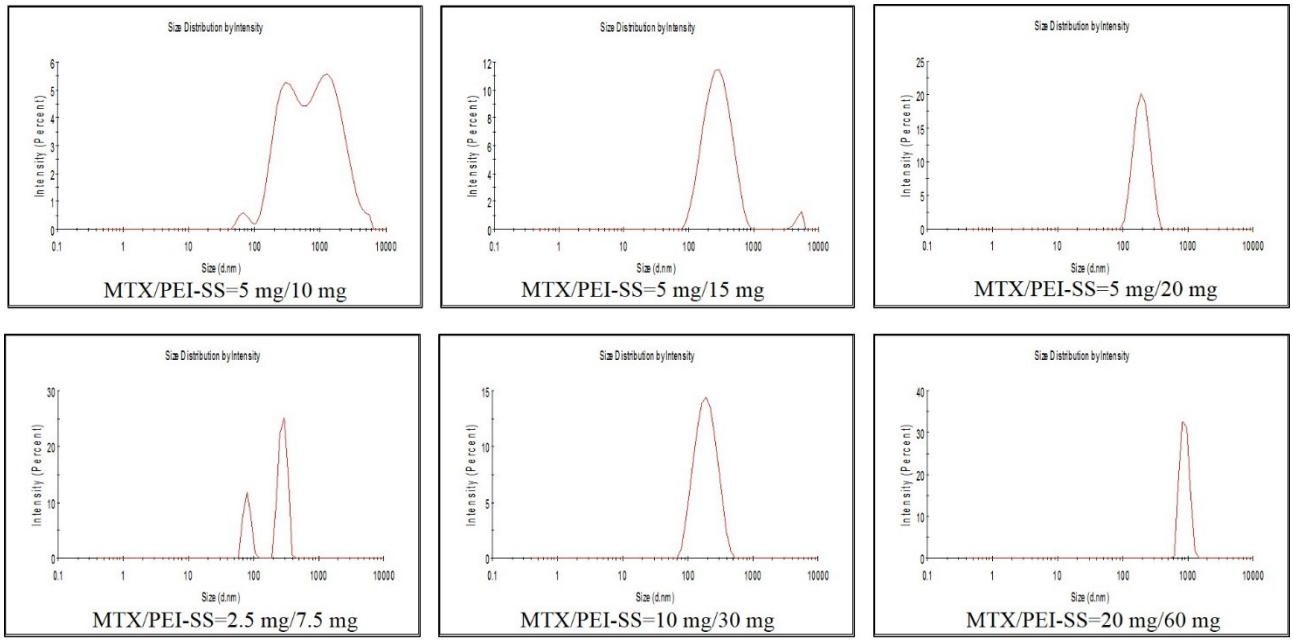

Fig.S2. Particle size distribution of the MTX-NPs formulations

Table S3. Formulation optimization of D-NPs

Formulation	I	II	III	IV
IND (mg)	10	10	10	10
MTX (mg)	10	5	5	5
PEI-SS (mg)	50	35	30	25
Drugs : PEI-SS	1:2.5	1:2.33	1:2	1:1.67
DMSO (ml)	2	2	2	2

Table S4. Characterization of D-NPs in different formulations

Formulation	Size (nm)	PDI	Zeta potential (mV)	Encapsulation efficiency		Drug loading	
II	103.26±0.022	0.254±0.044	59.05±2.81	IND	86.21±1.9%	IND	17.94±1.05%
				MTX	88.54±3.8%	MTX	9.21±0.23%
III	82.71±0.016	0.184±0.016	57.30±1.45	IND	88.64±2.1%	IND	20.47±0.18%
				MTX	88.86±4.4%	MTX	10.26±0.26%

2. TEM scan of release medium

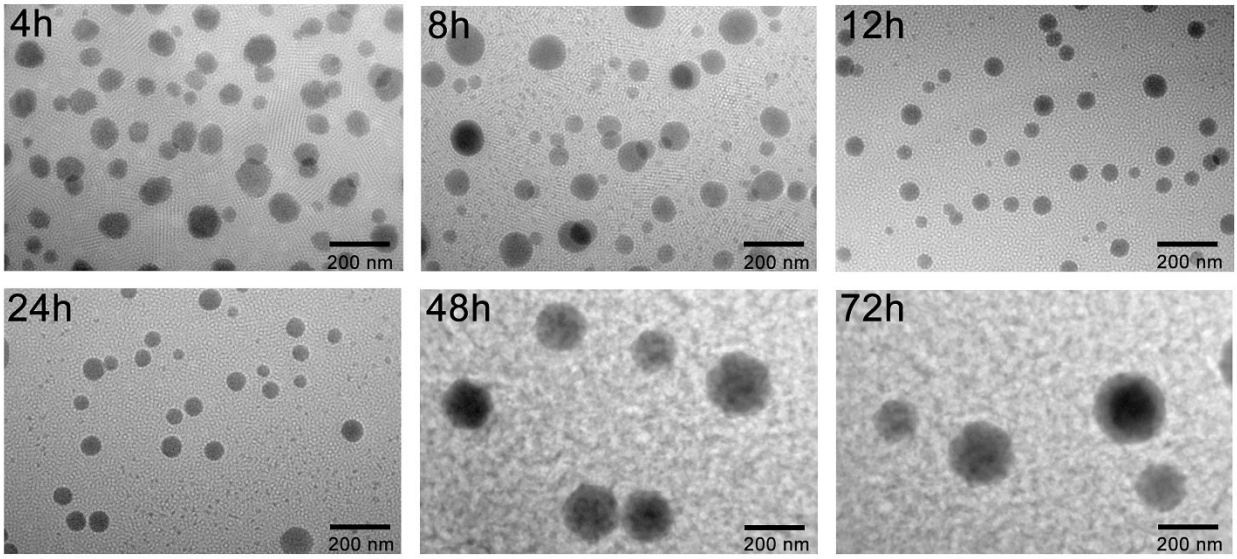

Fig.S3. Transmission electron microscope (TEM) scanning images of the D-NGel release medium at different time points. Scale bar, 200 nm.