**Supplementary Material**

**Loading Psoralen into liposomes to enhance its stimulatory effect on the proliferation and differentiation of mouse calvarias osteoblasts**

Xiaoran Lia, Vasil M. Garamusb, Na Lic, Zhe Zhed, Regine Willumeit-Römerb, Aihua Zoua, ∗

**Table S1** Variables and levels used in 33 factorial designs for PSR-loaded liposome

|  |  |  |  |
| --- | --- | --- | --- |
| levels |  | factors |  |
| A | B | C |
| 1 | 1 | 2:1 | 30:1 |
| 2 | 2 | 3:1 | 40:1 |
| 3 | 3 | 4:1 | 50:1 |

*Note*: Ais the phospholipid concentration (mg·mL−1); Bis the weight ratio of phospholipid to cholesterol (w/w); Cis the weight ratio of phospholipid to drug (w/w).

**Table S2** The orthogonal design and experimental results (n = 3)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Formulas | | | A | | B | | C | | EE(%) | DL(%) | |
| 1 | | | 1 | | 1 | | 1 | | 61.8 ± 1.2 | 3.57 ± 1.3 | |
| 2 | | | 1 | | 2 | | 2 | | 67.0 ± 1.4 | 3.30 ± 1.1 | |
| 3 | | | 1 | | 3 | | 3 | | 72.5 ± 1.3 | 3.25 ± 0.9 | |
| 4 | | | 2 | | 1 | | 3 | | 71.4 ± 1.2 | 3.02 ± 1.2 | |
| 5 | | | 2 | | 2 | | 1 | | 77.9 ± 1.3 | 3.90 ± 0.9 | |
| 6 | | | 2 | | 3 | | 2 | | 71.2 ± 1.1 | 3.42 ± 0.8 | |
| 7 | | | 3 | | 1 | | 2 | | 79.6 ± 0.9 | 3.30 ± 1.3 | |
| 8 | | | 3 | | 2 | | 3 | | 71.5 ± 1.2 | 3.06 ± 1.3 | |
| 9 | | | 3 | | 3 | | 1 | | 85.2 ± 1.6 | 4.23 ± 1.6 | |
| EE  (%) | K1j | 67.1 | | 70.9 | | 75.0 | |  | |
| K2j | 73.5 | | 72.1 | | 72.6 | |  | |
| K3j | 78.7 | | 76.3 | | 71.8 | |  | |
| R | 11.6 | | 5.4 | | 3.2 | |  | |
|  | K1j | 3.37 | | 3.30 | | 3.90 | |  | |  |
| DL  (%) | K2j | 3.45 | | 3.42 | | 3.34 | |  | |  |
|  | K3j | 3.53 | | 3.63 | | 3.11 | |  | |  |
|  | R | 0.16 | | 0.33 | | 0.79 | |  | |  |

*Note*: EE is the entrapment efficiency, DL is the drug loading. Kij is the average value of each test in i level, R is the value of range.

As shown in the Table S2, Taking the entrapment efficiency as the index, the range (R) of phospholipid concentration was the highest, the weight ratio of phospholipid to cholesterol was the second, and the effect of each factor on the entrapment efficiency was phospholipid concentration> the weight ratio of phospholipid to cholesterol> the ratio of phospholipid to drug, namely A>B>C. Taking drug loading as index, the range (R) of phospholipid to drug was the highest, the weight ratio of phospholipid to cholesterol was the second, and the effect of each factor on the drug loading was the weight ratio of phospholipid to drug> the weight ratio of phospholipid to cholesterol> The concentration of phospholipids, namely C>B>A. For the factor of the phospholipid concentration (A) and the weight ratio of phospholipid to cholesterol (B), the average value K of 3st level was the highest. For the weight ratio of phospholipid to drug (C), 1st level was the highest. According to the analytical results, the optimal formula should be A3B3C1, i.e. the concentration of phospholipids was 3mg·mL−1, the weight ratio of phospholipid to cholesterol was 4:1 and the weight ratio of phospholipid to drug was 50:1, which was selected as optimal formula composition for further investigation.

**Table S3** Mean Particle size changes of PSR/liposomes in thirty days

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Formulation | 1 day(nm) | 7 days(nm) | 15 days(nm) | 30 days(nm) |
| 1 | 126.5 ± 1.2 | 128.7 ± 1.4 | 132.0 ± 1.7 | 129.4 ± 1.8 |
| 2 | 107.9 ± 2.3 | 109.2 ± 1.9 | 112.6 ± 2.1 | 111.1 ± 1.7 |
| 3 | 114.0 ± 2.3 | 113.6 ± 2.4 | 115.3 ± 1.5 | 111.5 ± 2.0 |
| 4 | 123.8 ± 2.5 | 124.4 ± 2.3 | 125.5 ± 1.6 | 123.6 ± 2.6 |
| 5 | 113.7 ± 0.9 | 115.3 ± 2.0 | 113.3 ± 2.1 | 112.5 ± 1.3 |
| 6 | 114.1 ± 1.8 | 120.7 ± 1.9 | 121.3 ± 2.3 | 115.5 ± 2.1 |
| 7 | 114.8 ± 2.1 | 121.5 ± 2.1 | 124.2 ± 1.1 | 121.1 ± 1.4 |
| 8 | 114.5 ± 1.2 | 114.6 ± 1.3 | 115.2 ± 1.2 | 115.5 ± 2.0 |
| 9 | 109.8 ± 2.0 | 115.8 ± 1.7 | 117.2 ± 1.4 | 121.8 ± 2.4 |