• 论 著•

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1. 　　　　　　　　　　　，　　　　200433

2. 　　　　　　　　　　　　，　　　　200433

[　] 目的　　　　　　　　　　　　　　　　　　　　　　　　　　　，　　　　　　　　　　　　　　　　　方法

　　　　　　　　　，　　Franz　　　　　　，　　　　　　　　　　，　　　　　　　　　　　　　　　，

　　　　　；　　　　　　　　　　　　　　　　　　　　，　　　　　HPLC

结果　　　　　　　　　　　　　　　　　48 h

　　，　　　　　　　　　　　　　: 　　　　　　125.99 μg/cm², 　　　55.31 μg/cm², 　　　181.30 μg/cm²;

42.15 μg/cm², 　　　32.76 μg/cm², 　　　74.91 μg/cm²　　　　　　　　　　　　　(P< 0.01) ,

　　　　　　　　　　(P< 0.01) 结论　　　　　　　　　　　　　　　　　　　　　，

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[　　] 　；　；　　－　　　　　　；　　；

[　　　　] R 943.43　　　[　　] A　　[　　　　] 0258-879X(2010) 12-1341-05

Study on microneedle technique in enhancement of transdermal nanoparticle delivery

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[Abstract] Objective To investigate the microneedle technique in enhancement of transdermal nanoparticle delivery and the distribution of poly (D,L-lactic-co-glycolic acid) (PLGA) nanoparticles in the skin and the transdermal microconduits. Methods Double fluorescent PLGA nanoparticles were used to show the transdermal transport process. Franz diffusion cell was used for the transdermal study. The nanoparticle suspension was added to the donor chamber. The hairless mouse skin in the microneedle group was treated by microneedle technique and that in the control group was not treated. Penetration of nanoparticles was visualized by confocal laser scanning microscopy (CLSM). Distribution of nanoparticle diameter was quantified by HPLC. Results The CLSM images revealed that the nanoparticles were delivered into the microconduits created by microneedles and entered the epidermis and the dermis. The quantitative results showed that no nanoparticles reached the receptor compartment 48 h after addition of the nanoparticles in both groups. In microneedle group the nanoparticle amount was 125.99 μg/cm² in the epidermis and 55.31 μg/cm² in the dermis, with the total amount in the skin being 181.30 μg/cm²; in the the control group, the nanoparticle amount was 42.15 μg/cm² in the epidermis and 32.76 μg/cm² in the dermis, with the total amount in the skin being 74.91 μg/cm². Microneedle technique significantly increased the amount of nanoparticles entering the skin ($P< 0.01$), and the amount in the epidermis was significantly more than that in the dermis ($P< 0.01$). Conclusion Our results suggest that microneedles can enhance the intradermal PLGA nanoparticle delivery, and the nanoparticles deposit in the skin to achieve sustainable drug release, which is beneficial for topical drug administration.

[Key words] skin; microneedles; poly (D,L-lactic-co-glycolic acid); nanoparticles; cutaneous administration

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[11- 13]

[9, 14]　　　　[15]

1

1.1　仪器和材料

（
　　，　　　）, FD5- 3P　　　　　　　（SIM　　，
），TGL20M　　　　　　　　（
　　，　　　）; Zeta sizer 3000HS
（Malvern　　，　　）, 2010　　　　　　（JE-
OL　　，　　），　　 －　　　　　　（PLGA,
50∶50, M_w = 15 000,　　　　　　　　　，
），　　　　　（PVA, 1788, M_w = 22 000,
　　，　　），　　　　　　（EDC）　N–
　（NHS, Sigma　　，　　），

，

1.2　双荧光纳米粒的制备和表征

1.2.1　双荧光纳米粒的制备[16]　　　　　　　W/O/
W　　　　　　　　　 － 6　　　　，
100 μl　　　　　　　10 mg PLGA　　　1 ml
　100 μg/ml　　　　　　　　，
，　　　　　60 s（200 W），　　　4 ml 3%
(W/V)　PVA　　　　，　　　　60 s（100 W），
　　　　　50 ml 0.1% PVA　　　，
　4 h（600　/ min）　　　　　　　　　，
　PLGA
4 ℃, 17 600× g　　 30 min,

3　,　3%　　　　　　　　　，
　　　　 － 6　　　　　　pH 5.7
，　　　　10 mg/ ml,　　　10 μl
EDC（150 mg/ ml）　10 μl NHS(50 mg/ ml),
　　1 h, 17 600× g　　30 min,　　　，
　　　3　,　　pH 7.4

，

1.2.2　形态 粒径和Zeta电位的测定
　　　　　　　　　，　　　　，

（1 mg/ ml），
　　　　Zeta

1.2.3　药物含量测定　　　　:　　　Dikma
Diamonsil C18(5 μm, 200 mm × 4.6 mm),　　:
SHIMADZU RF – 10AXL　　　　，　　:
：= 95∶5,　　: 1.0 ml/ min,　　: Ex
465 nm, Em 502 nm　　　　　　 －
6,　　　　　　0.1 0.5 1.0 4.0 10 ng/
ml　　　　　　 － 6
20 μl　　　，　　　　3　,
　　　 － 6　　　C　　　A
，

1.2.4　包封率和载药量的测定
　500 μl　　　　　　，　　2.5 ml　，
，　，　　 － 6　　，
　　（%）= （　　　　 － 6
／　　　　　 － 6　　　）×
100%;　　（%）= （　　　 － 6
／　　）× 100%

1.2.5　双荧光标记的验证
　　　　，　，

1.2.6　纳米粒的体外释放
　50.0 ml 10.0 mmol/ L pH 7.4
（　0.02%　　　, 0.02% F68
）　，　　，
，　100　/ min　　，(37 ±0.5)℃
　　　　　　　　　　　　1
ml,　　　　　17 600× g　　30
min,　　　，　　 － 6

1.3　纳米粒经皮给药　　，
（　　8 N,　　2
min)　　　　（　0.5 cm²），

pH 7.4
(0.8 mg/ml), 700 μl

, 32℃ pH
7.4 , 300
/min , ,

,

,

z ,

, z ,

4.77 μm 488 nm 635 nm

– 6

0.5 ml 2.5 ml , ,

HPLC – 6

50℃ , 3 min,

,

, 2 ml , ,

17 600×g 30 min, 500 μl 2.5 ml

, , HPLC – 6

: = (

– 6 /) × 100%,

,

1

Fig 1 TEM photographes of nanoparticles

A: Coumarin– 6– NP; B: Coumarin– 6– NP– PE

2 – 6
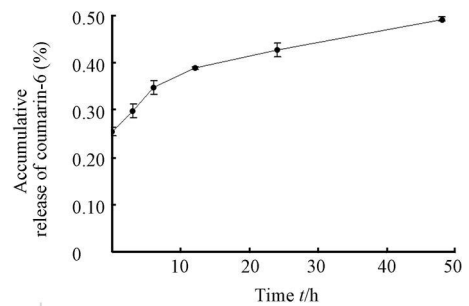
Fig 2 *In vitro* releases of coumarin– 6 from nanoparticles

2

2.1 药物含量测定

A ,
– 6 C , : $C=$
0.000 1 A + 0.039 4, 0.1~ 10.0 ng/ml, R= 0.999
3 (0.1 5 10 ng/ml)
98.34%, RSD 0.80% 3

, RSD
2.31%, RSD 2.49%($n=$ 6)

2.2 纳米粒子的形态与表征

1, ,

, coumarin– 6– NP

(160.1 ±1.97) nm, Zeta (– 30.9 ±0.85)
mV; coumarin– 6– NP– PE (214.1 ±
1.73) nm, Zeta (– 22.4 ±0.75) mV

2.3 包封率和载药量的测定及体外释放实验

, – 6 (61.40±0.22)%,
(0.61±0.00)% 2 , 48 h –
6 0.5%,

– 6,
– 6

2.4 双荧光纳米粒的验证

3A
– 6 , 3B

,

, ,

, PLGA

2.5 纳米粒的经皮递送

4 : – 6

,

; ,

, 4 I J
488 nm 635 nm

, 5 :

,

2.6 纳米粒的分布

2.6.1 接收液中的纳米粒

, 48 h

,

[17]

，

2.6.2 皮肤中的纳米粒   6   ，

，   ；

，

（ $P < 0.01$ ）；

（ $P < 0.01$ ）

[18]   75   /

cm^2，   11   / cm^2，

，

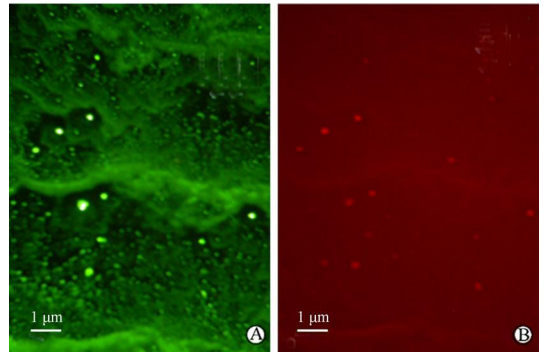，   ，

图 3

**Fig 3   Images of fluorescent nanoparticles
by fluorescence microscopy**

A: Coumarin－6－NP; B: Coumarin－6－NP－PE
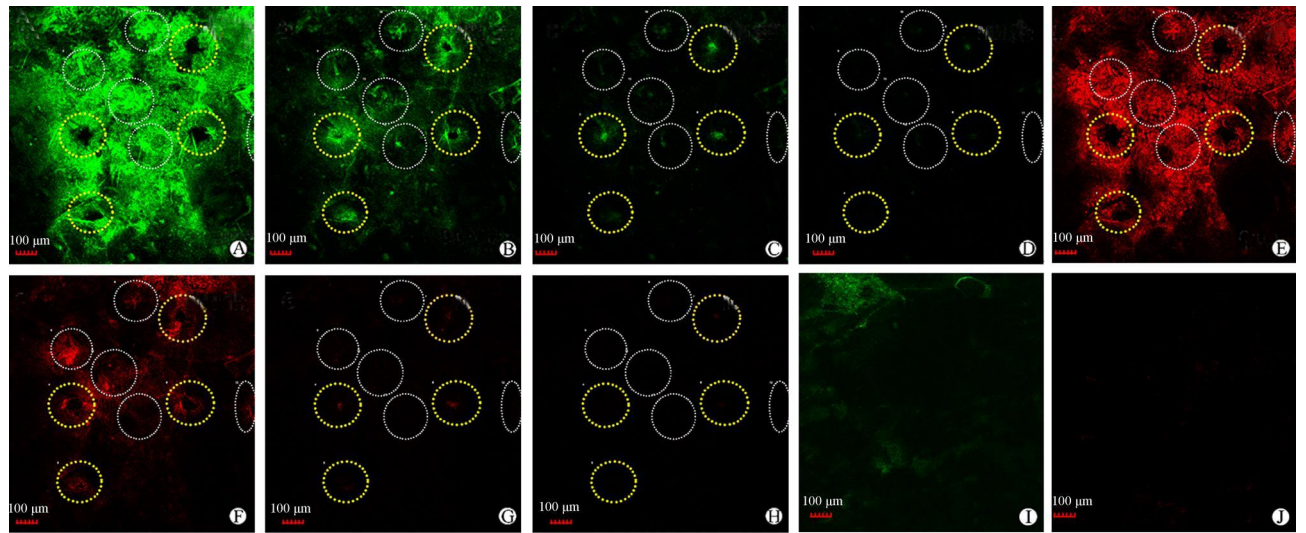

图 4   12 h

**Fig 4   Confocal microscopic images of skin at different depths in microneedle group 12 h
after application of double fluorescence labeled nanoparticles**

The distribution of coumarin 6 at different depths was shown as: (A) 0 μm, (B) 14.31 μm, (C) 28.62 μm, and (D) 42.93 μm; the R－PE distribution at different depths was shown as: (E) 0 μm, (F) 14.31 μm, (G) 28.62 μm, and (H) 42.93 μm. The yellow circles were microconduits produced by microneedles and the white circles were the hair follicles which were in a mess after being pressed by microneedle arrays. (I) and (J) showed autofluorescence of the blank hairless mouse skin excited at 488 and 635 nm, respectively
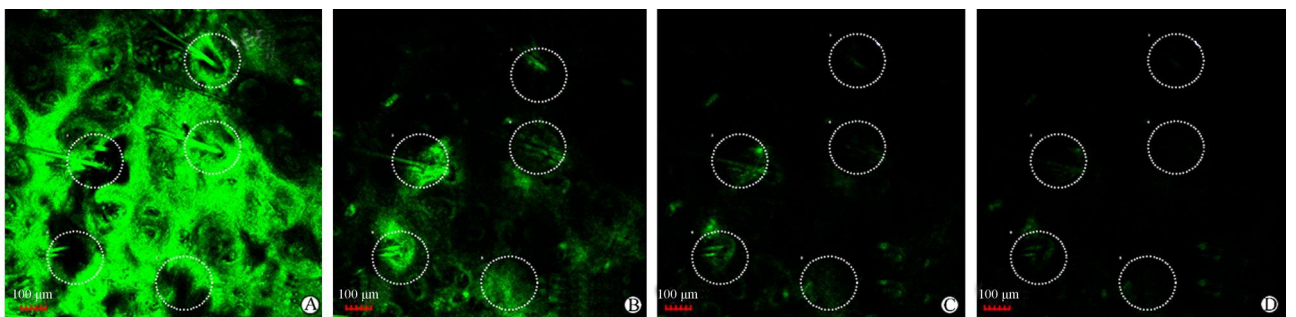

图 5

**Fig 5   Confocal microscopic images of nanoparticle delivery in control group
12 h after application of double fluorescence labeled nanoparticles**

The distribution of coumarin－6 at different depths was shown as: (A) 0 μm, (B) 14.31 μm, (C) 28.62 μm, and (D) 42.93 μm. The white circles were the hair follicles which were in a mess after being pressed by microneedle arrays
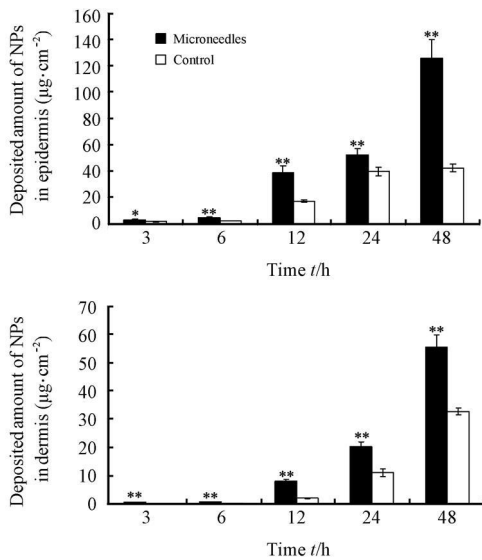
6

**Fig 6　Amount of nanoparticles(NPs)
in epidermis and dermis**

* $P < 0.05$, * * $P < 0.01$ vs control group. $n = 6$, $\bar{x} \pm s$

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