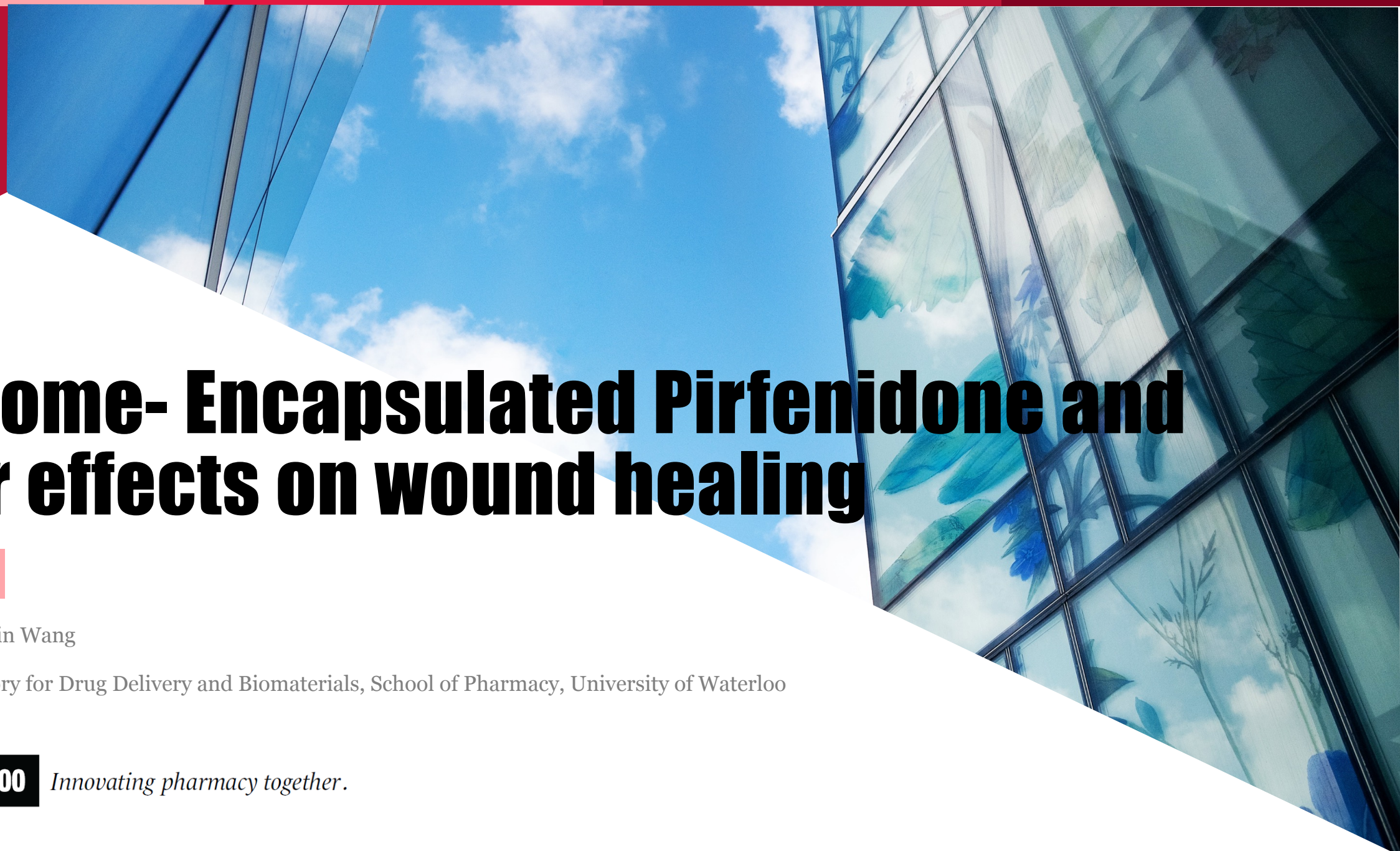

Exosome- Encapsulated Pirfenidone and their effects on wound healing

6/30/22

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YOU+WATERLOO *Innovating pharmacy together.*

BACKGROUND

Background

1) Wound healing and scar management.

2) Why drug delivery system is important for wound care?

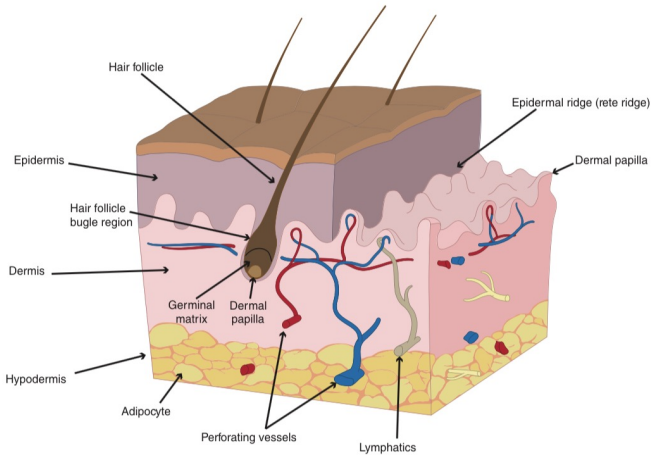
3) What is the advantage of exosomes as drug carriers?

4) How to prevent scar formation during wound healing?

Background

Skin is the largest organ in human body.

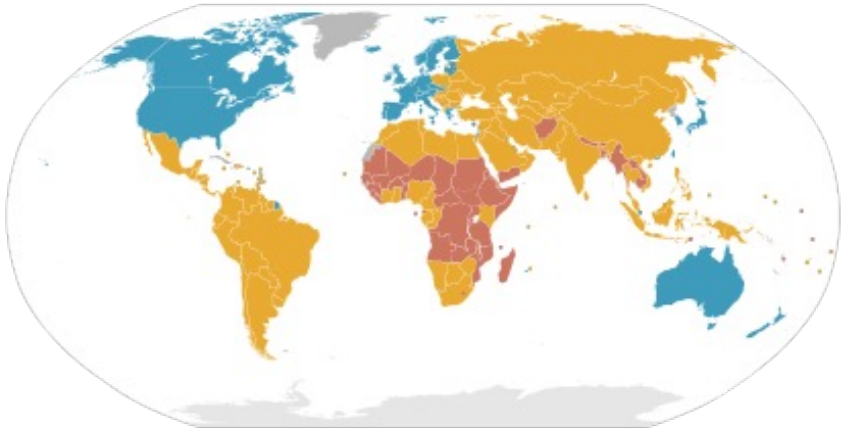
- Protect the body from bacteria and other pathogens
- Prevent internal organs from dehydration.
- Function: sensation, temperature regulation.



Consequences of scar formation

- physiological problem (itching and pain)
- psychosocial trauma (diminished self-esteem, anxiety and depression)

Wound healing & Scar management



Population:
In developed countries: more than 100 million patients develop scars from surgeries each year.

Cost:
Globally, the scar treatment market is estimated to reach around $32 billion by 2027[2].

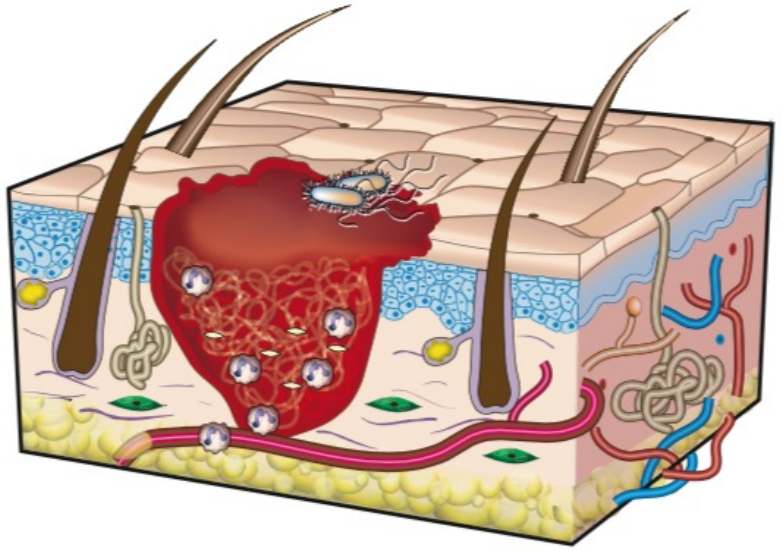
Ideal outcomes of wound healing for patients:

1) Avoid bacterial infections
2) Accelerate wound closure
3) Improve appearance by reducing surface area, thickness and discoloration of scars
4) Minimize the pain.

Drug delivery system & and wound care

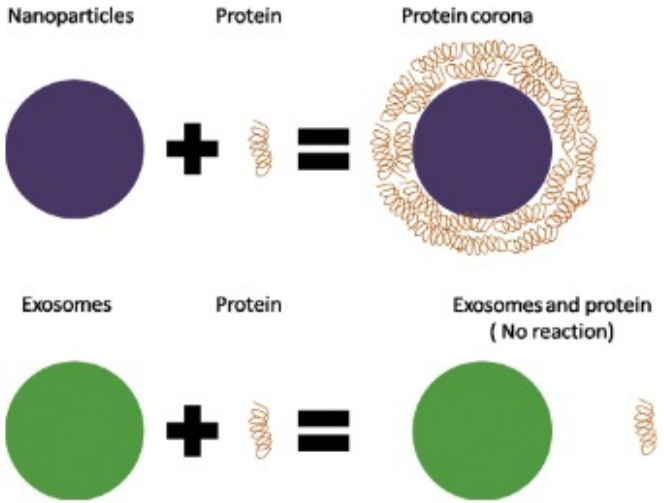
The importance of DDS for wound care

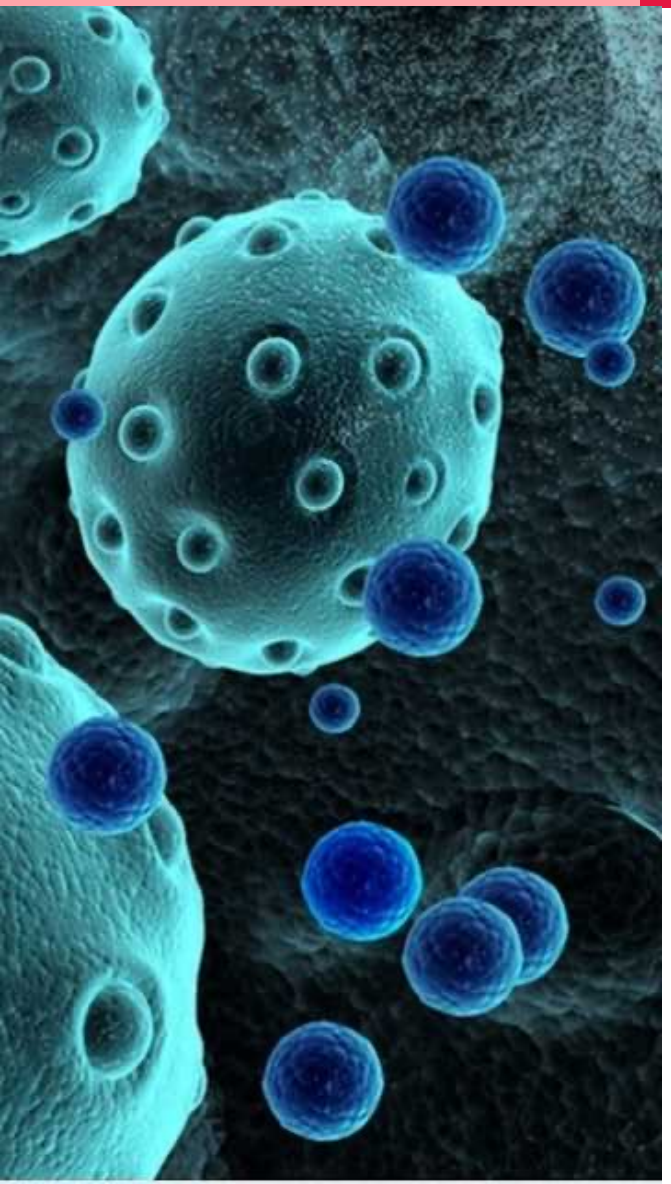
1) Disrupted vasculature (Inefficient systemic drug delivery)



2) Complexity of wound exudate (protein, cytokines, leucocytes, lysozyme, macrophages, and neutrophils)

Formation of protein corona





Exosomes

- **Origin**

Secreted by various cells

- **Size**

Nanosized (30–150 nm) extracellular vesicles

- **Function**

Natural transporters for cell-to-cell communication

- **Structure**

Lipid bilayer membrane + proteins + nuclear components

- **Distribution**

Distribute in blood, breast milk, saliva, urine, and cerebrospinal fluid

Exosomes and wound healing

Theraputic

Cardiovascular disease

Chronic kidney disease
Acute kidney injury

Alzheimer's Disease

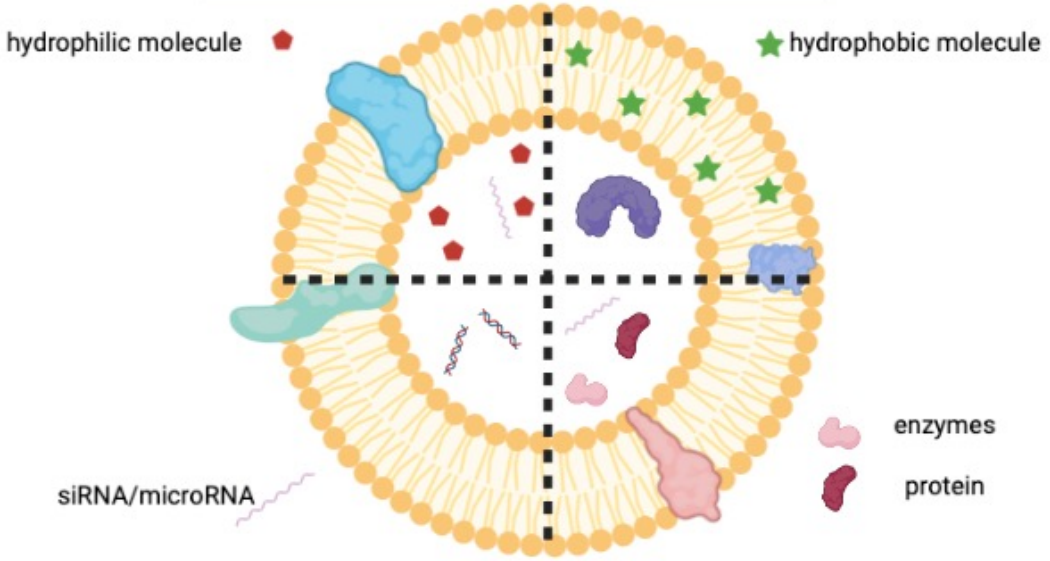
Wound healing

- Adipose mesenchymal stem cells
- Umbilical cord-derived exosomes
- Human amniotic epithelial cells

Osteoarthritis

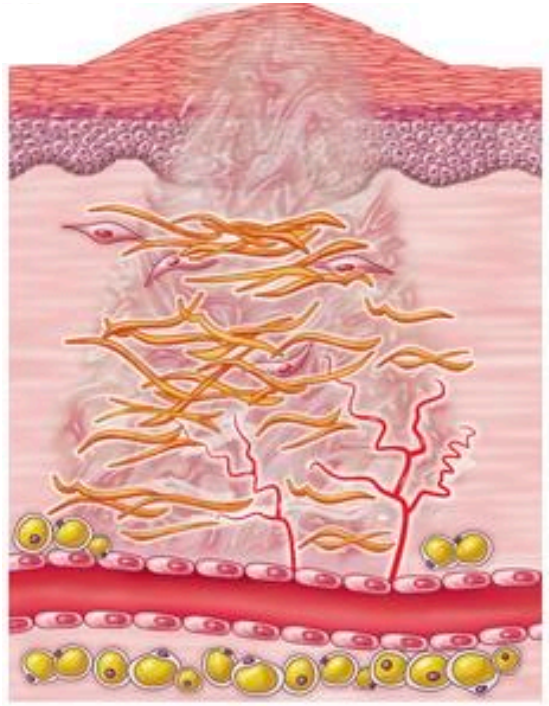
Spinal cord injury

Drug delivery



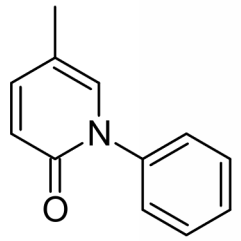
Scar reduction therapy and Pirfenidone

- Fibroblasts' contribution to wound healing and scar formation
- Proliferation
- Migration
- Collagen expression



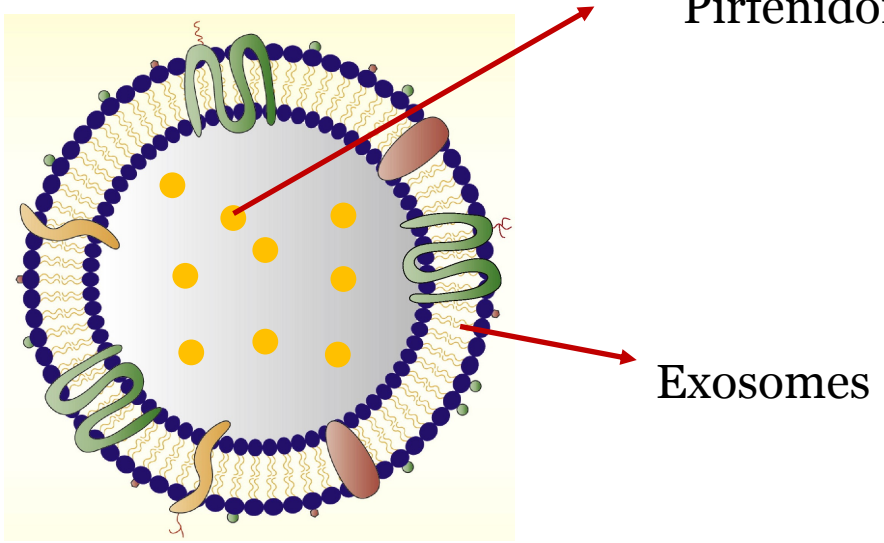
JCI Insight 2018 Sep 20;3(18):e99911.

- Pirfenidone
- A FDA approved compound for treatment of idiopathic pulmonary fibrosis
- Anti-inflammatory and anti- fibrotic effects
- p38 kinases inhibitor in TGF-β1 stimulated human dermal fibroblasts
- TGF-β Smad independent p38 MAPK signaling pathway and mitigated profibrotic gene expression profiles.



HYPOTHESIS

Hypothesis



Drug Delivery System

- Human dermal fibroblast-derived exosomes loaded with Pirfenidone (PFD) as an intracellular drug delivery system.
- Exosomes will encapsulate PFD into dermal fibroblasts and improve fibroblasts' proliferation and migration.
- PFD-exosomes could reduce collagen overexpression.

KEY FINDINGS

Isolation of Exosomes Derived From Human Dermal Fibroblasts

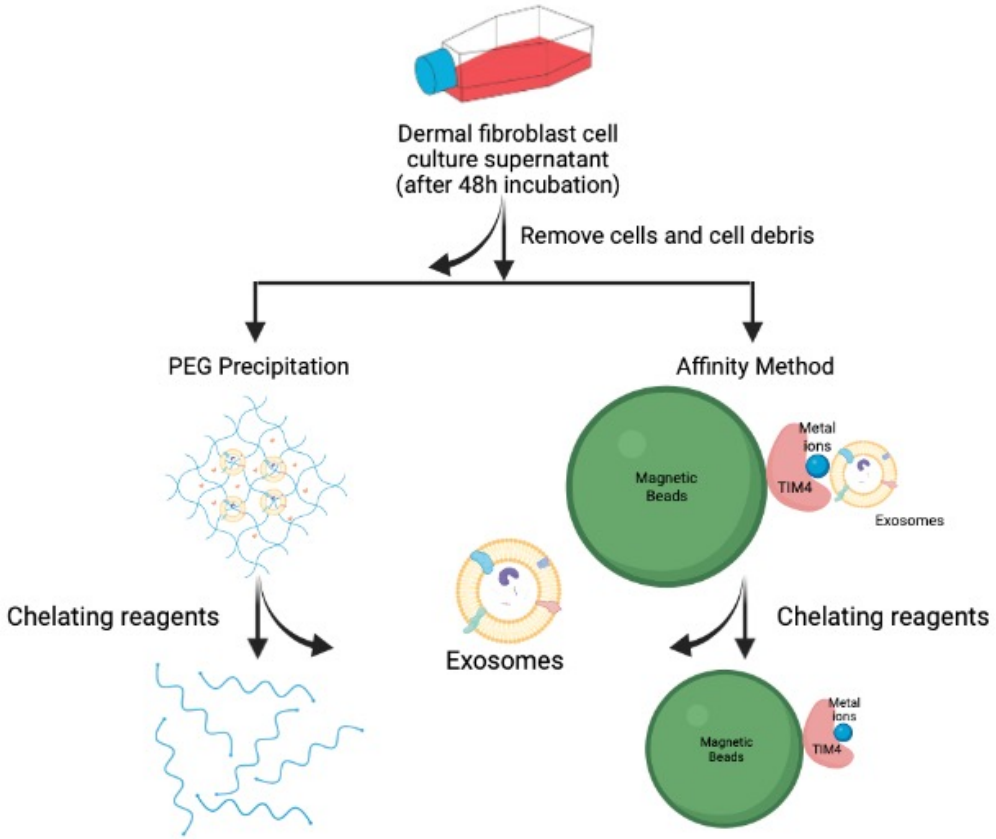


Figure.1 Mechanism of exosomes isolation with PEG precipitation method and affinity method.

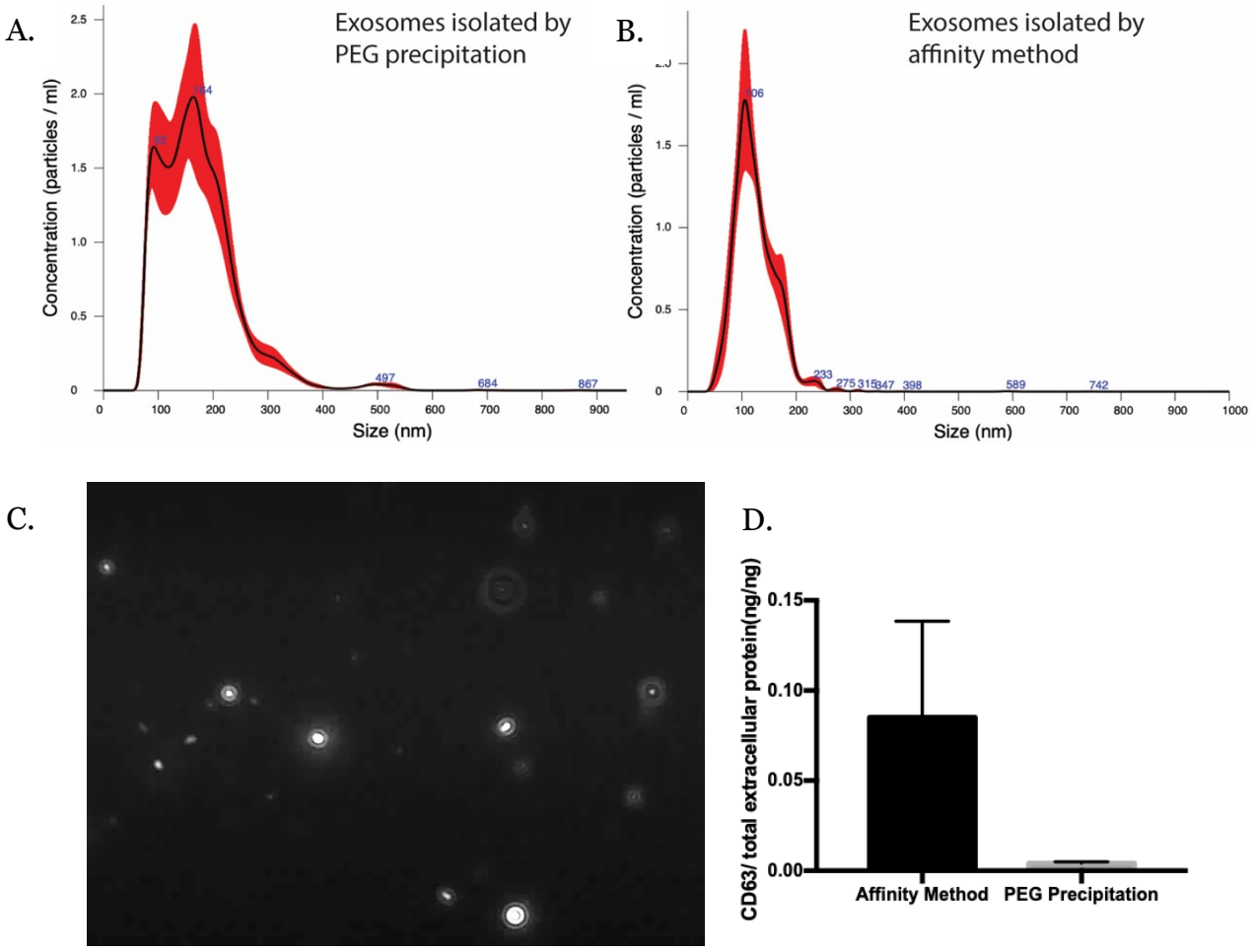


Figure 2. (A) (B) and (C)Size distribution of exosomes was analyzed by nanoparticle tracking analysis. (D). Purity of hDFs-exosomes isolated from 20 mL cell culture supernatant by different method

Characterization of exosomes

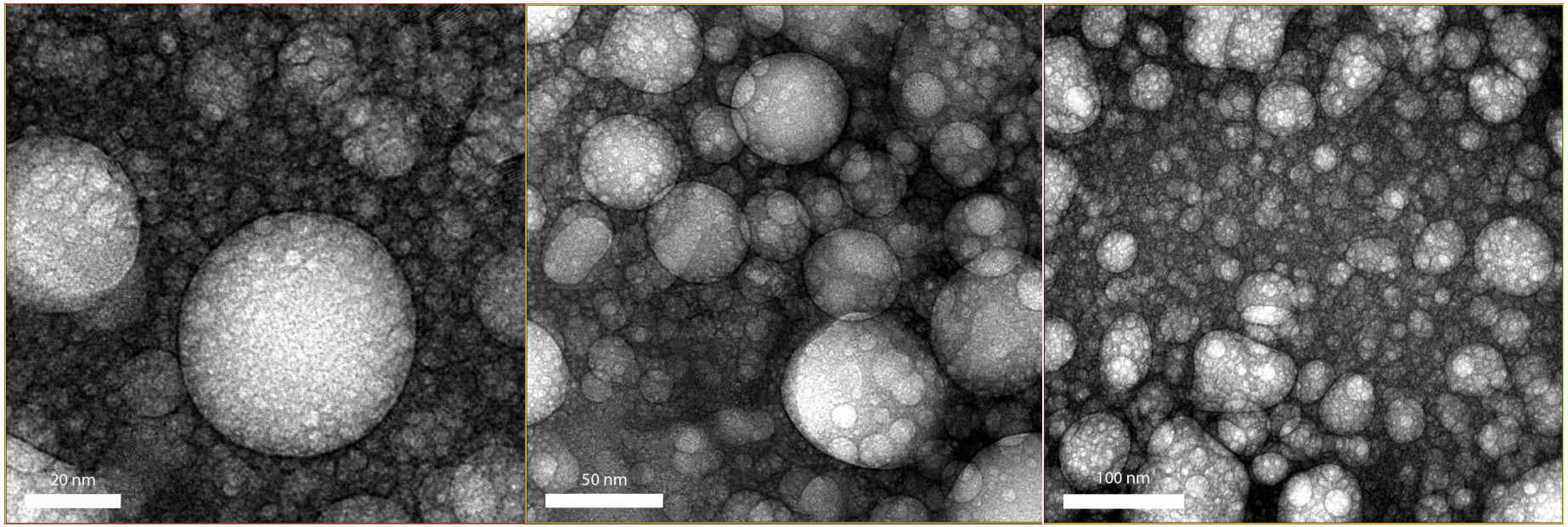


Figure. 3 Morphology of hDFs-exosomes isolated by affinity method under Zeiss Libra 200MC TEM (A.200,000x; B. 100,000x; C.50,000x).

Characterization of exosomes

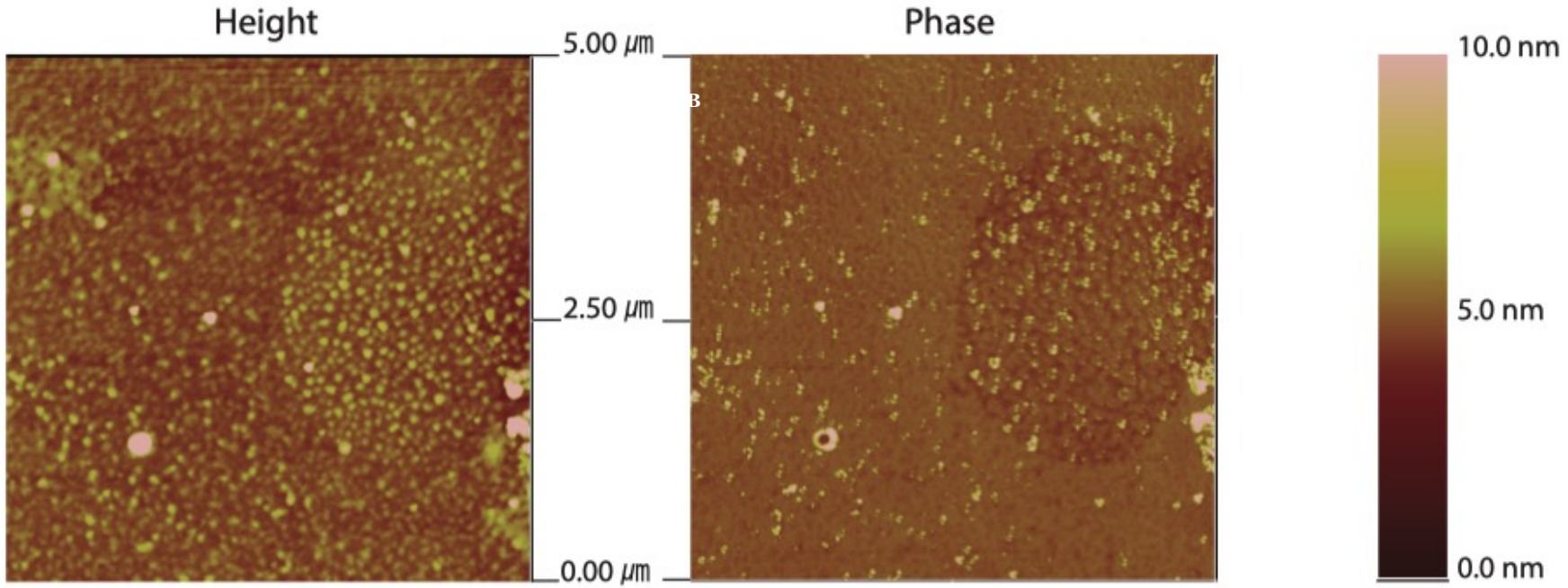
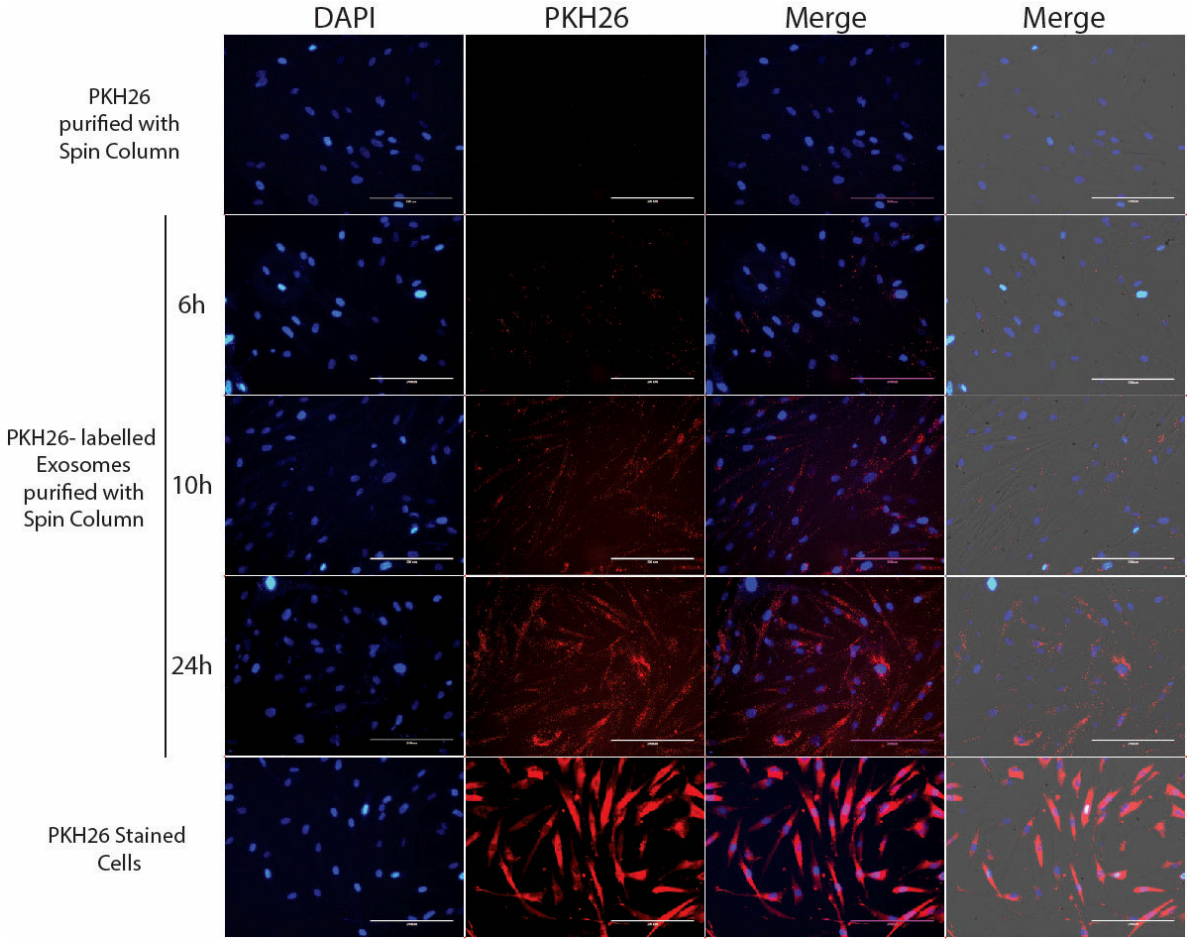


Figure 4. Surface morphology analysis of exosomes by AFM.

Time- dependent cellular uptake of exosomes by their parent cell



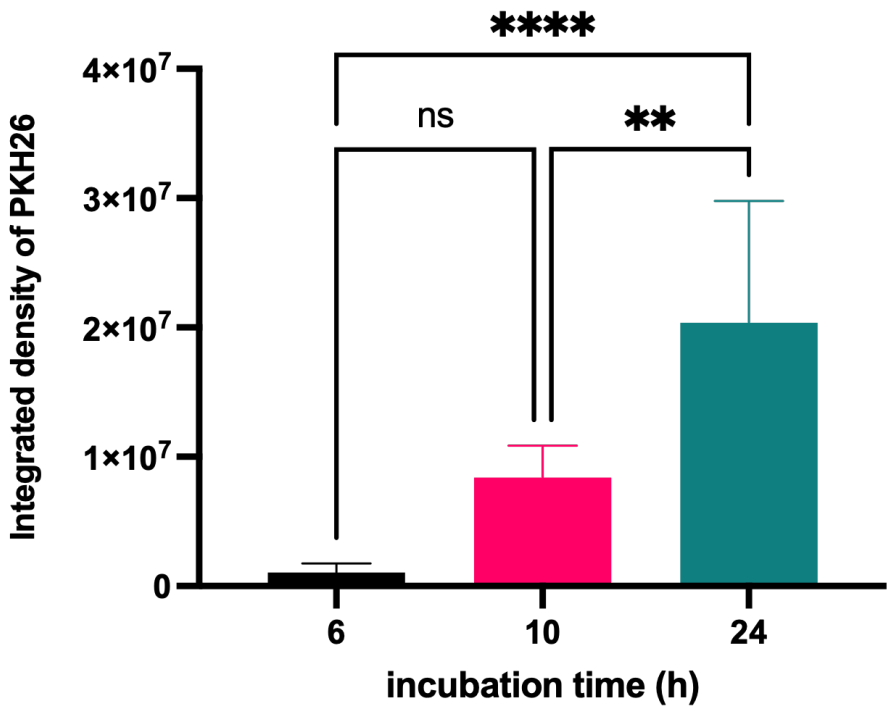
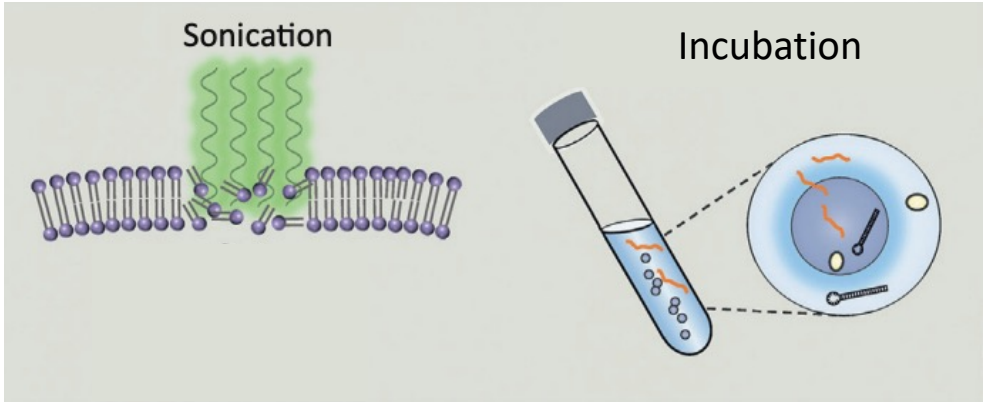


Figure 5. In vitro cellular uptake of exosomes by BJ fibroblast cells.

Encapsulation of Pirfenidone into exosomes



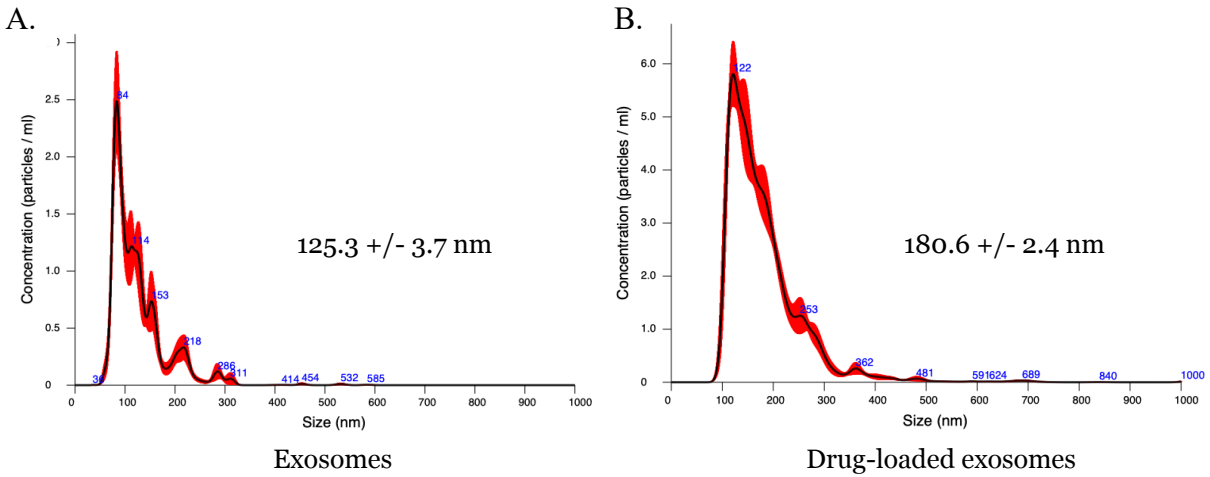


Figure 6. Characterization of exosomes and durg loaded exosomes.

- The highest encapsulating efficiency that can be achieve by sonication is 10.77± 0.516%.

Exosomes and PFD-exosomes promote cell viability

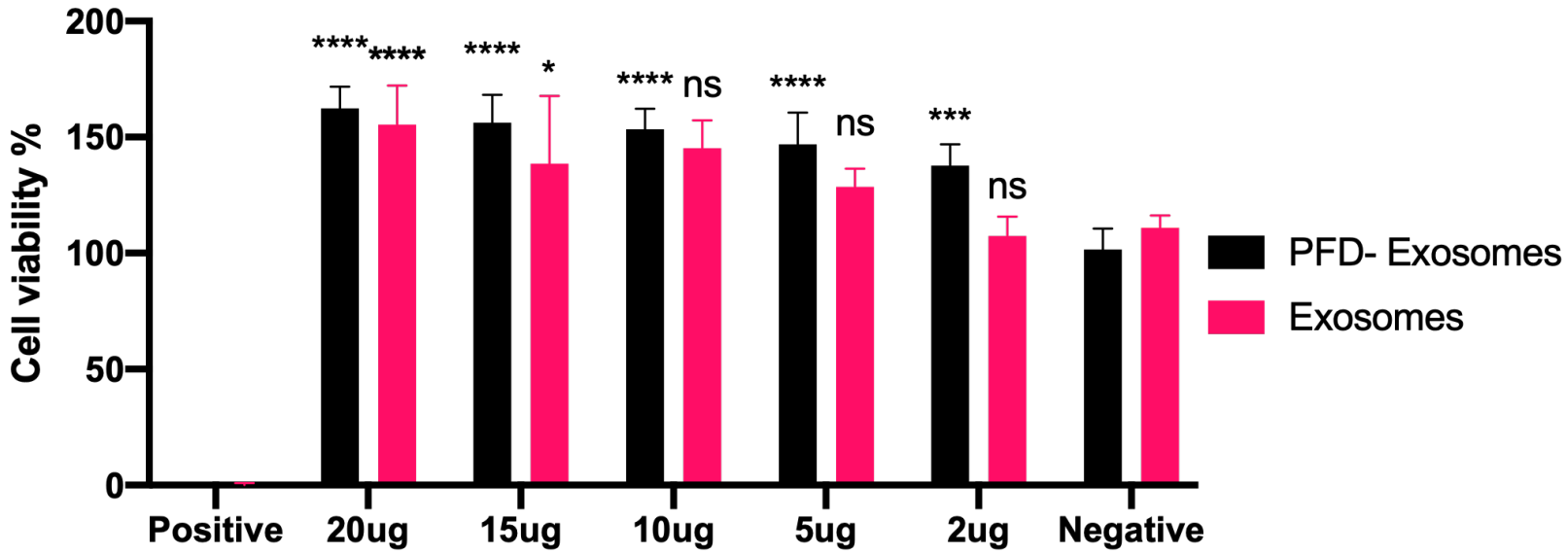
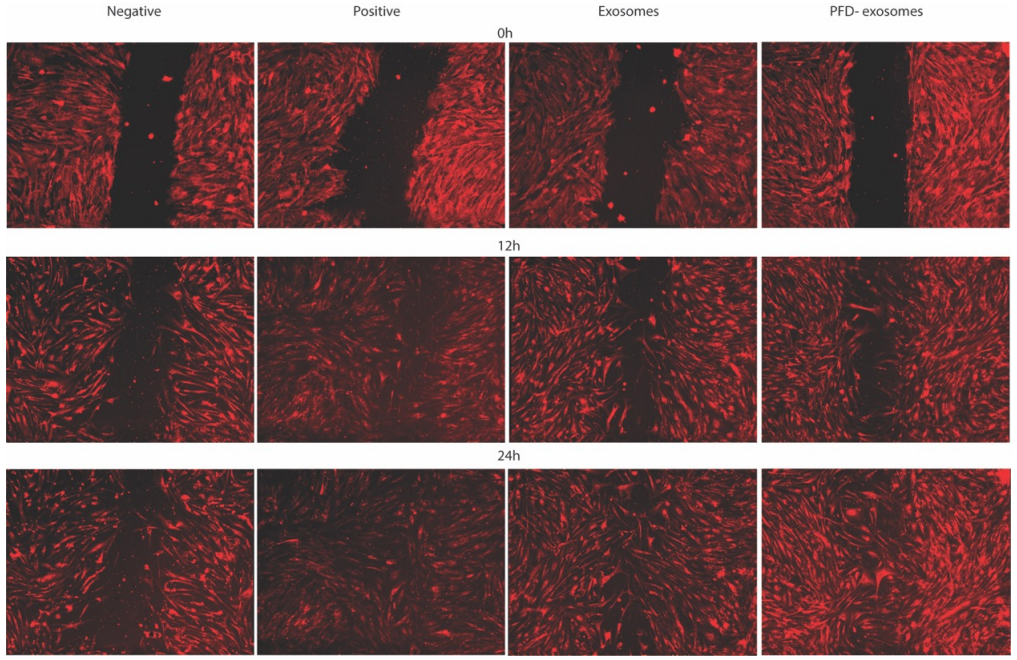


Figure 7. In vitro cytotoxicity of exosomes and PFD- loaded exosomes.

Acceleration of cell migration by exosomes and PFD-exosomes

A.



B.

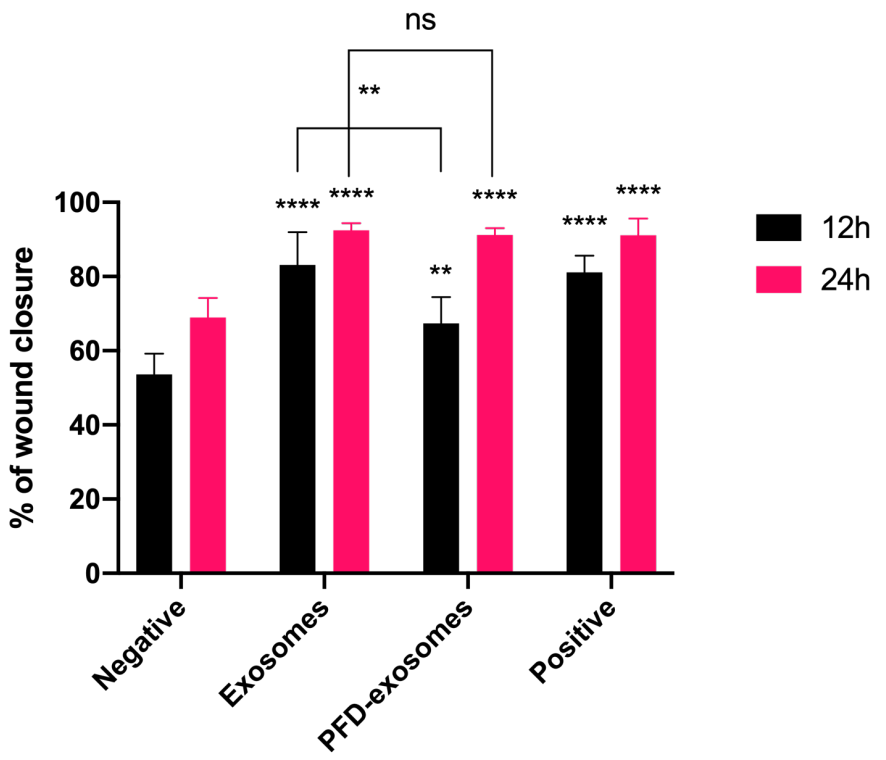


Figure 8. In vitro scratch wound healing studies of PFD- loaded exosomes.

Conclusion

- PFD was successfully incorporated into exosomes as a potential topical therapeutic.
- Both drug-free exosomes and PFD-exosomes were non-cytotoxic to fibroblasts.
- Exosomes and PFD-exosomes showed promising application in enhancing fibroblasts migration and wound closure in vitro.
- Exosomes may not only function as a drug carrier and may have wound healing properties, but further studies are required.

Limitations

- Mechanism of exosomes' uptake by their parent cells cannot be explained.
- Improvement drug loading efficiency.
- Lack understanding the mechanism of exosomes promoting self-proliferation.

Future direction

- In vitro collagen expression of dermal fibroblasts
- Ex Vivo skin permeation studies
- In vivo wound healing study

Acknowledgement

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Thank you!
Any question?
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