Vesicular Delivery of Interleukin-11 gene for osteoporosis treatment

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Background

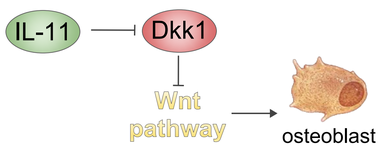
Osteoporosis

- Over 200M people with osteoporosis
- Decreased quality of life

Critical Pathway

- Wnt signaling promotes osteoblastogenesis
- Proteins Dkk1 and Dkk2 are Wnt inhibitors
- Interleukin-11 (IL-11) inhibits Dkk1 and Dkk2

} IL-11 naturally decreases with age



Extracellular Vesicles as Drug Delivery System

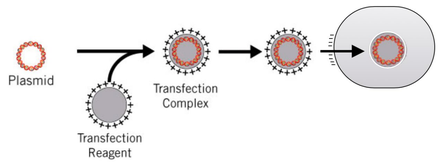
- Bodies that bud off of cell membranes
- Self-origin mitigates immunogenicity
- Surfaces can be conjugated for targeting

Our Solution

- Engineer plasmid containing *IL-11* gene
- Load plasmid into extracellular vesicles (EVs)
- Introduce EVs to bone area to promote osteoblastogenesis

Methods

Adapted Transfection: Plasmid into EVs



Loading Quantification

- Lyse EVs
- Measure genetic content using Nanodrop spectrophotometer

Introduction to Human Embryonic Kidney Cells

- HEK293T incubated with model *GFP* plasmid-loaded EVs

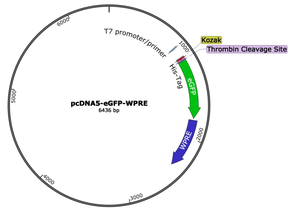
Expression Quantification

- Microplate photometer measures expression of GFP

Results

What We Did

- Loaded model plasmid pcDNA5-eGFP into EVs
- Introduced loaded EVs to HEK293T cells to determine viability as delivery system



Loading Efficiency

Initial Input:
- 40 µL of 167.2 µL used for quantification
- 1500 ng of pcDNA5-eGFP loaded

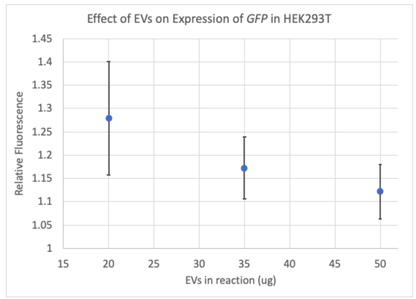
$$\frac{40\ \mu L}{167.2\ \mu L} \cdot 1500\ ng\ plasmid = 360\ ng\ loaded$$

Loaded:
- Nanodrop yielded 6.1 ng/µL concentration
- 15 µL suspension after miniprep

$$6.1\ \frac{ng}{\mu L} \cdot 15\ \mu L = 91.5\ ng \qquad \frac{91.5\ ng}{360\ ng} = 0.254$$

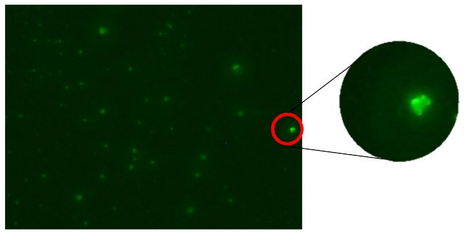
∴ 25.4% loading efficiency

Fluorescence Data



- More data needed to accurately analyze any correlation
- Possibly no dose dependence
- 20 µg of loaded EVs sufficient for observable expression effects

Figure 1. Plate reader results when 20, 35, and 50 µg of pcDNA5-eGFP loaded EVs are incubated with HEK293T cells overnight. Error bars represent std. dev. between duplicates. Data normalized to 1 (divided by neg. control).



- Visible expression of GFP *in vitro*
- EVs successfully deliver plasmid to cells

Figure 2. Microscopy image of HEK293T cells incubated with 20 µg of pcDNA5-eGFP loaded EVs.

Ethics

Experimental Ethics

- Animal testing
- Isolation procedures: human subjects

Application Ethics

- Other uses for osteoblastogenesis
- Athletics

Conclusions

- Transfecting GFP into EVs results in ~25.4% loading efficiency
- Incubation with HEK293T cells produces GFP expression
- Method can be extended to IL-11 expression to treat osteoporosis

Impact and Future Work

Benefits of the Device

- Personalized medical treatment
- Non-invasive approach
- Platform can be modified for wide range of applications

Future Work

- Design *IL-11* encoding plasmid
- Use of $(AspSerSer)_6$ to target bone-formation surfaces
- *In Vivo* animal models
- Downstream processing for purification
- Scale-up for high throughput production

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References

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