

Dose-response analysis after PEP administration on nerve regeneration *in vitro*

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Background

Local modulation of the nerve reconstruction micro-environment has been an area of interest to enhance peripheral nerve regeneration. **Neuromodulators** include:

- **Adipose-derived mesenchymal stem cells (MSCs):**
 - Act through paracrine properties to enhance regeneration
 - Limitations: cell culture, cell instability, tumorigenesis
- **Tacrolimus (FK506): FDA approved immunosuppressant**
 - Accelerates nerve regeneration, reduces fibrosis at nerve reconstruction site.
 - Local delivery to avoid unwanted side effects of drug toxicity.
- **Exosomes (EX)** are nanosized cell-free membrane extracellular vesicle
- **Purified exosome product (PEP)**
 - Blood-derived, lyophilized powder, Off-the-shelf available

Aim

To evaluate the neurite extension after local delivery of **MSCs, PEP or FK506 alone or combined** *in vitro*

Methods

Construction 3D compartmented cell culture model

1. Autografts are harvested from Lewis rats. Allografts are harvested from Sprague-Dawley rats and decellularized according to our *decellularization protocol*.
2. MSC seeding using undifferentiated Lewis rat adipose derived MSCs, Passage 5 according to our *dynamic seeding protocol*.
3. Dorsal root ganglions (**DRG**) serve as the neuronal cells: harvested from Neonatal Lewis rat pups postnatal day 3-7.
4. DRGs are attached to the nerve graft using Tisseel fibrin glue to create DRG-nerve graft constructs.
5. Constructs are placed in the model, isolating the DRG (central nerve) from the nerve graft (peripheral nerve: treatment side).

Outcome measures

- **Confirming stem cell presence and characterization**
 - Immunohistochemical staining against **CD90/DAPI**
- **Neurite extension (μm)** as a measure of nerve regeneration
 - Immunohistochemical staining against **Neurofilament-160**

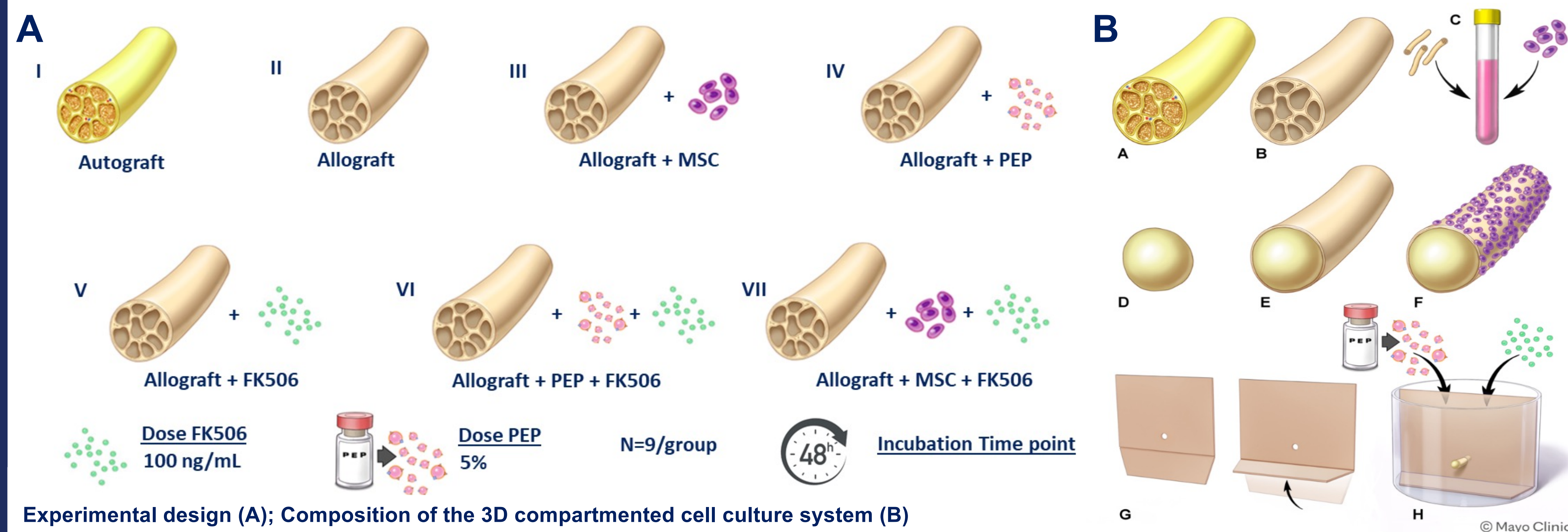
Results

- Stem cell viability was confirmed in all MSC-treated nerve grafts.
- Autograft superior to untreated Allograft (p<0.002)
- Allograft + PEP, Allograft + FK506 + PEP and Allograft + FK506 + MSC superior to untreated Allograft (all p<0.001)
- Allograft + PEP, Allograft + FK506 + PEP and Allograft + FK506 + MSC comparable to untreated Autograft (all p>0.99)
- Allograft + FK506 + MSCs **superior** to Allograft + MSCs (p=0.0012).
- Allograft + FK506 + PEP (longest neurite extension) **comparable** to Allograft + FK506 + MSCs (p>0.99)

Discussion & Conclusion

- The treatment of **PEP and FK506** combined resulted in the longest neurite extension, superior to untreated allografts and comparable to autografts, the current gold standard, isolated PEP treatment, and MSCs and FK506 combined.
- This cell-free exosome product is a novel and promising candidate to enhance nerve allograft regeneration, that can **overcome limitations of current cell-based therapies**, suggesting possible avenues for clinical translation.
- *In vivo* animal models are needed to further investigate the effect of PEP alone and combined with FK506 on nerve regeneration.

Methods



Results

