Neuro-Regeneration of Retinal Ganglion Cells on Bio-Activated Silk Based Scaffolds

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Background

Mature **retinal ganglion cells (RGCs)** connect the retina with brain visual areas, and **do not regenerate after injury**.

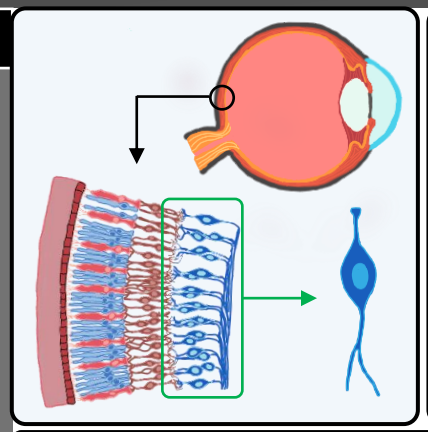
Silk fibroin (Fb) in-vitro assays are used to **screen for trophic factors (TFs)** that can **support RGC neural regeneration.**

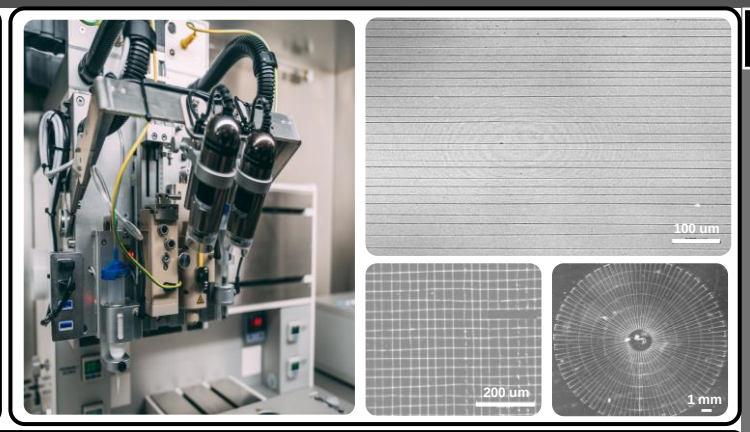
Electrospinning, the manufacturing process for Fb assays, is **technically demanding** and **hinders research reproducibility**.

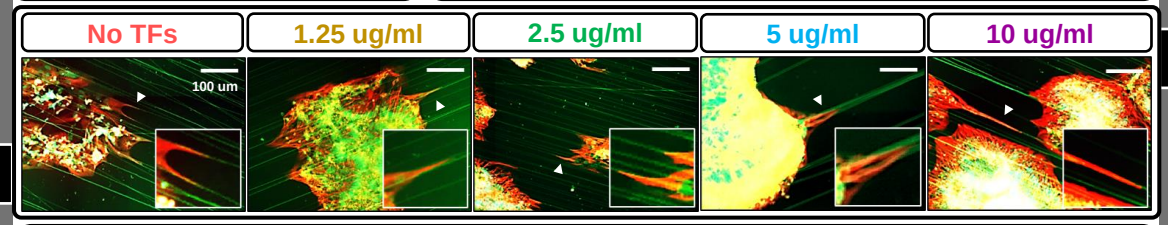
Hypothesis: 3D – Printing technology is an alternative to **modernize and automate** the **manufacturing process of Fb assays**.

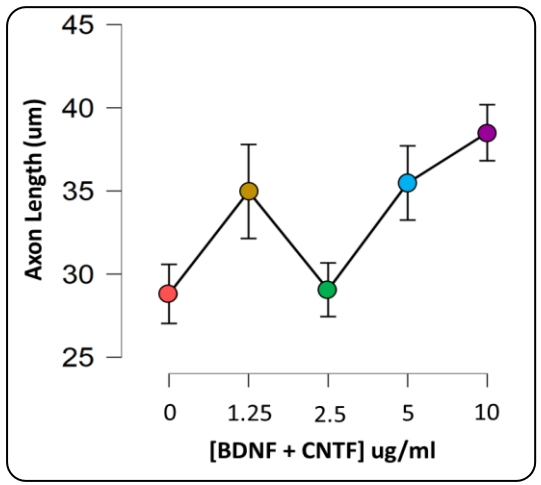
Methods

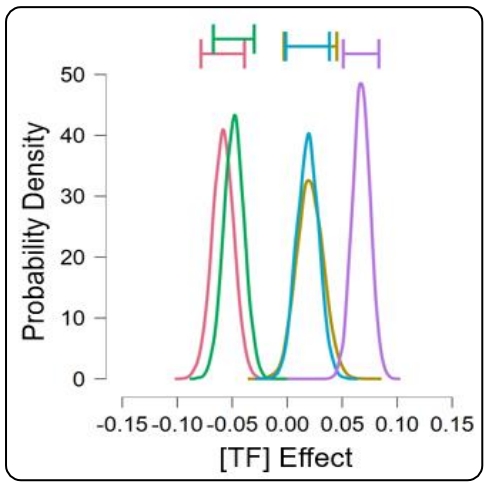
- **8.6% Fb printing solution** prepared with 0.8 mg/ml **fluorescein dye (Green)**.
- Brain derived neurotrophic factor (**BDNF**) and ciliary derived neurotrophic factor (**CNTF**) **TFs were added** [1.25 ug/ml, 2.5 ug/ml, 5 ug/ml, and 10 ug/ml]
- **E11 chick retinal explants** extracted and seeded on each TF condition.
- Immunohistochemistry with antibody against **B3-Tublin (Neurite outgrowth - red)** and **DAPI (Cell nuclei - blue)**
- Statistical analysis was done using **Bayesian inference**.











Results

- Organized and aligned **silk nano fibers (d: ~1 um)** were **3D printed** unto glass coverslips.
- E11 explants **outgrowth co-localized with Fb fibers.**
- Explants seeded on **functionalized scaffolds showed longer outgrowth** compared to our –ve control (0 ug/ml TFs)
- Explant **axonal length** and fiber **[CNTF + BDNF]** are **strongly correlated (BF 1.0 = 5.707 e+21).**

Discussion

3D-Printing can efficiently and autonomously generate Fb based scaffolds for culturing RGCs.

Silk fibers provided mechanical and chemical support for the survival and growth of the seeded retinal explants.

Our results support our hypothesis that our automated silk 3D printing method can accurately and reproducibility engineer bioactivated silk platforms for the study of RGC neuro-regeneration.

Future Directions

- Repeat experiement with mouse RGCs.
- Screen for other TF combinations that may enhance RGC neuro-regeneration further.
- Validate RGC printing modality unto our silk scaffolds.