Delivery of Anti-VEGF Agent and Steroid from Electrospun Scaffolds: Potential Application in Treating Retinal Vascular Diseases

Dhivya Venugopal[1], Sushma Vishwakarma[2,3], Inderjeet Kaur[2], Satyavrata Samavedi[1*]

[1]Department of Chemical Engineering, Indian Institute of Technology, Hyderabad, India
[2]Prof Brien Holden Eye Research Center, L V Prasad Eye Institute, Hyderabad, India
[3]Manipal Academy of Higher Education , Manipal, Karnataka, India
*Corresponding author: samavedi@che.iith.ac.in

Introduction & Motivation

- Retinal vascular diseases are linked to pre-existing conditions (e.g., diabetes), and affect a broad range of age groups ultimately leading to vision impairment/loss.
- Abnormal angiogenesis, excess vascular endothelial growth factor (VEGF) secretion and chronic inflammation are critically implicated in disease pathogenesis. Targeting these conditions via controlled drug delivery can provide avenues to check disease progression.
- Electrospinning is a simple and widely investigated technique to produce micro- or nano-scale fibers. Electrospun fibers offer the ability to load drugs and release them in a controlled manner as desired in specific applications. Nanofibrous matrices produced by electrospinning also resemble the natural fibrous extracellular matrix, which can aid specific cell functions.

Objectives

- Towards the long-term goal of developing treatment modalities for retinal vascular diseases, this work investigates the ability of electrospun meshes in providing controlled drug release followed by preliminary in vitro evaluation using *ex vivo* mixed retinal cultures.
- Accordingly, two different drugs (anti-VEGF agent or corticosteroid) were loaded into polymeric fibers using blend or emulsion electrospinning and release kinetics were investigated. Further, preliminary *in vitro* studies were conducted to confirm the efficacy of the released drugs. The study design is presented in **Figure 1**.

Methods

Polymers: Poly-(caprolactone) (PCL), Poly(lactic-co-glycolic acid) (PLGA)
Drugs: anti-VEGF agent, anti-inflammatory steroid
Cells: *Ex vivo* human mixed retinal cells

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Blend or emulsion electrospinning

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Microscopy | *In vitro* release | Cell viability | I.F. staining for specific markers

Figure 1: Study design and experimental framework

Drug release kinetics

- Successful incorporation and uniform distribution of a bioactive factor via emulsion electrospinning was confirmed by visualizing FITC-BSA within the shell of the electrospun fibers in a poof-of-concept (**Figure 2**).

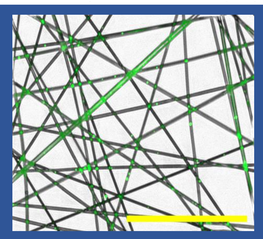

Figure 2: Distribution of FITC-BSA within fibers made by emulsion electrospinning. Scale bar: 100 µm.

- *In vitro* release of the anti-VEGF agent (loaded via emulsion electrospinning) and steroid (loaded via blend electrospinning) was analyzed over 14 days (**Figure 3**).
- While both PCL and PLGA resulted in a small burst release of the anti-VEGF agent initially, PCL provided slower release thereafter. Cumulative release of 37% and 49% respectively was observed over 14 days.
- The choice of PLGA for the steroid was driven by our previous experience with the polymer, which provided controlled cumulative release of 15% over 14 days.

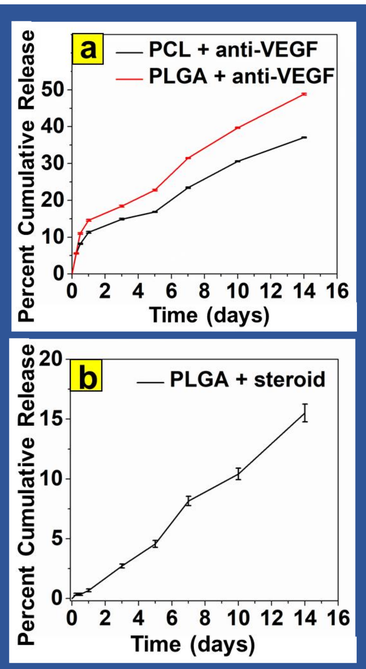

Figure 3: *In vitro* release kinetics of (a) anti-VEGF agent and (b) steroid from electrospun meshes

In vitro cell viability

- The steroid-loaded meshes were tested for their ability to suppress markers of inflammation *in vitro* without compromising cell viability.
- The viability of mixed retinal cells after 4 days of culture on both control and steroid-loaded meshes was higher compared to the no-fiber control (**Figure 4**). Moreover, no significant differences in viability were noted across the control and drug-loaded mesh, suggesting that the released drug was well within the cytotoxic limits.

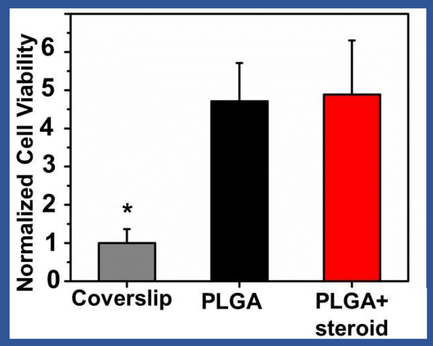

Figure 4: Normalized viability of cells cultured on coverslips, control mesh and steroid-loaded mesh. Asterisk indicates statistical significance compared to the control and drug-loaded samples ($p<0.05$)

Inflammation markers

- Diseased mixed retinal cells cultured on control meshes showed higher expression of the inflammatory marker IL-6 while its expression was significantly lower in the steroid loaded meshes (**Figure 5)**.

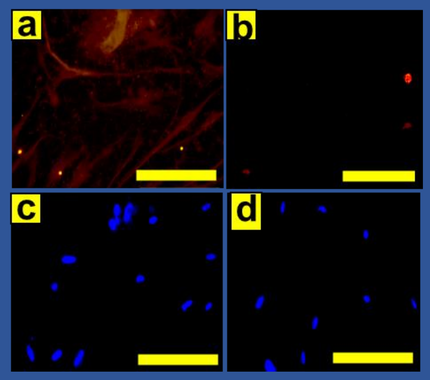

Figure 5: IL-6 expression on (a) control mesh and (b) mesh loaded with steroid. Panels (c) and (d) represent respective DAPI-stained images. All scale bars correspond to 200 µm

- Likewise, the expression of TNF-α too was significantly lower by cells cultured on steroid-loaded meshes as compared to the control meshes (**Figure 6)**.
- Together, these *in vitro* results suggest the initial promise of these drug-eluting electrospun materials in influencing the diseased phenotype of mixed retinal cells.

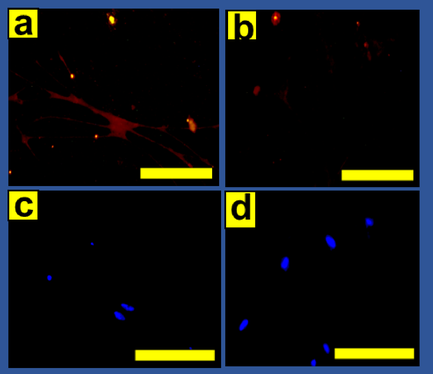

Figure 6: TNF-α expression on (a) control mesh and (b) mesh loaded with steroid. Panels (c) and (d) represent respective DAPI-stained images. All scale bars correspond to 200 µm

Conclusions

- The incorporation of anti-VEGF agent and steroid into electrospun fibers was achieved by blend and emulsion electrospinning.
- Ability of meshes in releasing the drugs individually over 14 days *in vitro* was demonstrated.
- The released steroid was shown suppress markers of inflammation *in vitro* without compromising cell viability.
- The controlled and extended release of the drugs shows promise for exerting long-term biological effects in retinal vascular diseases.

References

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