DEVELOPMENT AND CHARACTERIZATION OF FURFURYL GELATIN BASED ELECTROSPUN FIBROUS MATS FOR USE AS PLATFORMS FOR CARDIAC DISEASE MODELING

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Introduction

- Patients with type-2 diabetes (T2D) are 2-4 times at a higher risk of heart attack/mortality due to functional and structural changes in the myocardium, manifesting in the condition referred to as diabetic cardiomyopathy (DCM) thereby leading to probable heart failure independent of underlying coronary heart disease.
- Since the underlying pathogenesis of DCM is incompletely understood an urgent need for biofabrication of human tissue-on-a-chip models can serve as a basis for the development of novel therapeutic approaches for DCM.
- Furfuryl gelatin(f-gel) has been extensively used for various *in vivo* applications and revealed no toxicity when used for modification of existing implant surfaces and for tissue repair applications [1]. In our earlier published works f-gelatin has been adopted for cardiac tissue engineering applications *in vitro* [2,3].
- Polymer biomaterial based scaffolds must be cell adhesive, biocompatible, possess excellent mechanical fidelity and not impart any effects on the cells and tissue [1]. Electrospinning method was chosen to develop extracellular matrix (ECM) mimicking fibrous structures for its versatility and simplicity.
- Since mechanical fidelity of gelatin based scaffolds is predominantly inferior we hypothesized that blending with polycaprolactone (PCL) would result in mechanically stable scaffolds for retention and viability of cardiomyocytes for a successful DCM model [4].

Objective

*Our **objective** in this study was to adopt visible light cross-linkable furfuryl gelatin (f-gelatin) for electrospinning of fibrous mats using*
1) f-gelatin only; 2) f-gelatin blended with PCL (1:1) and 3) f-gelatin coaxially deposited over PCL fibers
to be evaluated as scaffolds for the development of an in vitro model for studying diabetic cardiomyopathy (DCM).

Materials and Methods

- An in-house electrospinning apparatus was developed for electrospinning 1) f-gelatin only; 2) f-gelatin blended with PCL (1:1) and 3) f-gelatin coaxially deposited over PCL fibers

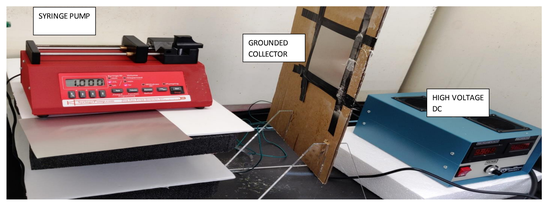


Figure 1. In-house developed electrospinning apparatus with syringe pump(s), grounded collector and a high voltage DC

- Single syringe with 22G needle was used in case of electrospinning f-gelatin and 1:1 fibers while coaxial needle with two syringe pumps was utilized for developing coaxial fibers. For the coaxial scaffolds, PCL was used as core solution and f-gelatin was used as sheath solution to obtain mechanically stable coaxial fibers

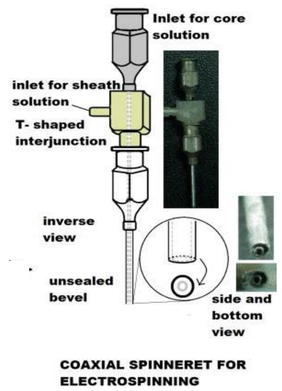


Figure 2. Coaxial needle used for coaxially electrospinning blended fibers of PCL (core) and f-gelatin (Sheath) fibers [5]

Materials and Methods (continued)

- The electrospun film samples obtained through different polymer(s) blends were characterized through scanning electron microscopy (SEM), transmission electron microscopy (TEM) to confirm its surface and internal morphology
- The interaction/non-interaction between the blended polymers were analyzed through differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and Fourier transformed Infrared Spectroscopy (ATR-FTIR)
- The mechanical and structural stability of the electrospun scaffolds were assessed using rheology and SEM.
- The biocompatibility of the scaffolds were evaluated by seeding human AC16 cardiomyocytes cell lines on the scaffolds and flow cytometry
- The diabetic model was created by providing glucose shock to a monolayer of AC 16 cells . DHE and Live–dead assay after 2 and 4 hours was used to confirm the damaging effects of glucose shock treatment

Results

- Electrospun fibrous mats of all three types of scaffolds were obtained from the optimized parameters.
- Average fiber diameter of conventionally blended fibers were significantly lower than the furfuryl gelatin and coaxial fibers
- The difference in viscosity and surface charge density of the polymer solution/s led to these significant differences

Scaffold type	f-gelatin	F-gelatin and PCL (1:1)	Coaxial (f-gelatin>PCL)
Concentration of polymer(s)	10% w/v(f-gel)	5% w/v(f-gel) 5% w/v(PCL)	5% w/v(f-gel) 5% w/v(PCL)
Solvent	1,1,1,3,3,3 hexafluoro-2-propanol	1,1,1,3,3,3 hexafluoro-2-propanol	1,1,1,3,3,3 hexafluoro-2-propanol
Flow rate	0.5mL/h	1mL/h	Core-0.5mL/h Sheath-0.5mL/h
Accelerating voltage	1.5kV/cm	1.5kV/cm	1.5kV/cm
Distance between tip and collector	10 cm	10 cm	10 cm
Average fiber diameter(nm)	760±80 nm	420±110 nm	810±60 nm

Table 1. Optimized parameters of different electrospun fibrous systems

INTERNAL AND SURFACE ANALYSIS OF ELECTROSPUN FIBERS

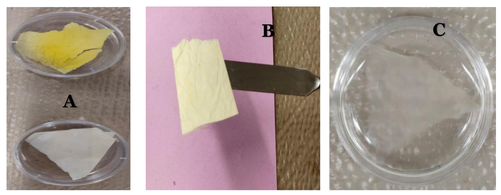


Figure 3. A. Image of uncross linked(top) and crosslinked f-gel electrospun fibers B. Free standing crosslinked f-gel electrospun fibers C.Transclusent nature of f-gel fiber when wert

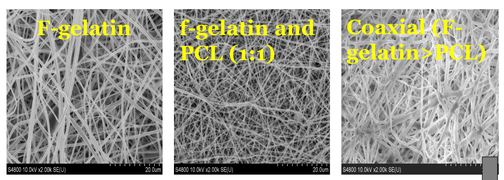


Figure 4. SEM of f-gel and 1:1 electrospun from single syringe pump and coaxial fibers from dual syringe pumps (scale bar 20µm)

- Coaxial fibers show the core-shell structures through TEM
- The diameter of PCL constituted to approximately 40% of the total average diameter of the fiber
- The higher viscosity of f-gelatin solution with respect to PCL solution enabled the formation of coaxial structures and higher share of the average diameter reported

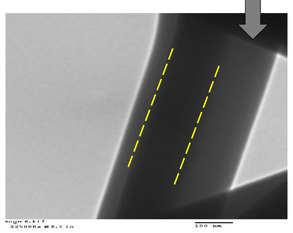


Figure 5. TEM of coaxial fibers from dual syringe pumps (scale bar 100nm)

Results (continued)

NON-INTERACTION OF POLYMERS IN BLENDED AND COAXIAL FIBERS

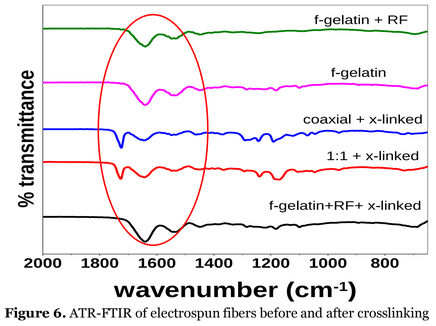
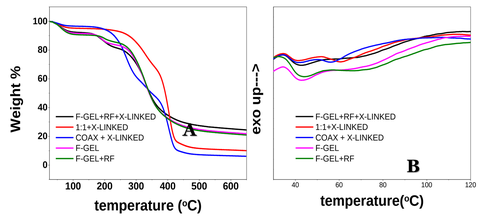


Figure 6. ATR-FTIR of electrospun fibers before and after crosslinking



- Peaks at 1734, 1641 and 1560 cm^{-1} correspond to the C=O stretching, amide I and amide II peaks present in PCL and furfuryl gelatin respectively
- No discernable interactions between the PCL and f-gelatin was observed and inferred
- TGA shows a two-step weight loss for f-gelatin fibers and a three-step weight loss for blended and coaxial fibers
- The first step weight loss corresponds to the loss of water content from f-gel followed by degradation of PCL between 200-300°C and f-gelatin beyond 340°C
- DSC shows crystallization temperature of f-gelatin at 45-50°C and melting temperature of PCL between 55-60°C

STRUCTURAL STABILITY OF ELECTROSPUN FIBERS

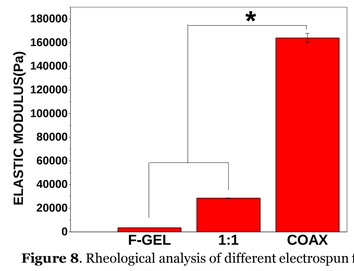


Figure 8. Rheological analysis of different electrospun fibers

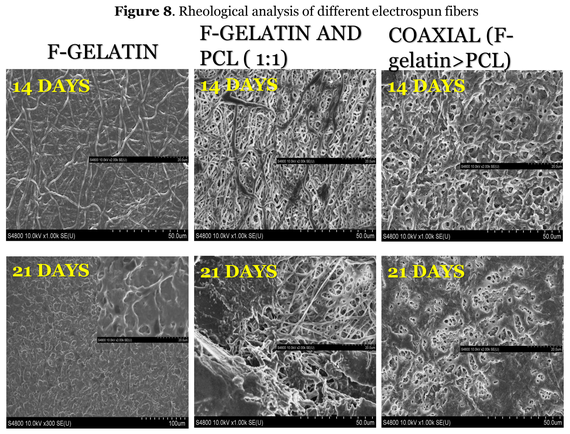


Figure 9. SEM of electrospun fibers after treatment with AC16 media for assessing *in vitro* stability after 14 and 21 days

- Coaxial scaffolds are significantly stiffer and stronger than the 1:1 blended and f-gelatin scaffolds
- In vitro degradation studies in cell growth media confirmed the significantly higher stability of coaxially blended fibers over f-gelatin fibers

Results (continued)

BIOCOMPATIBILITY OF ELECTROSPUN FIBERS WITH HUMAN AC16 CARDIOMYOCYTES

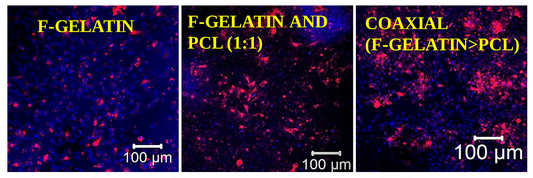


Figure 10. Viability of AC16 cells after 1 day. Blue indicates DAPI staining while red indicates PkH26 staining od the AC16 cells

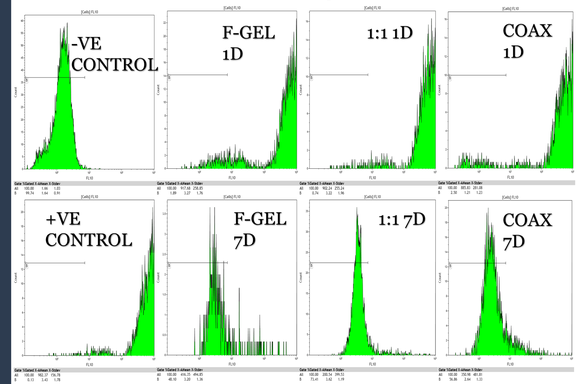


Figure11. FACS of PKH 26 stained AC16 cells seeded on electrospun fibers after 1 day (top panel) and 7 days (bottom panel)

- All electrospun scaffolds showed excellent adhesion and proliferation of PKH 26 stained AC 16 cells after 1 day and 7 days.

DIABETIC CELL STUDY MODEL

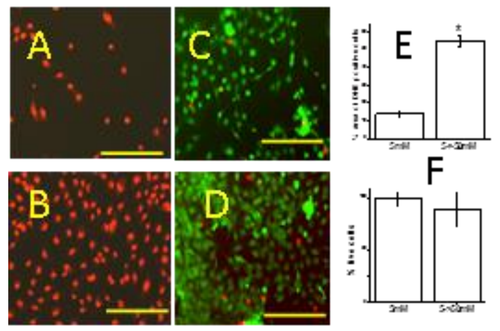


Figure 12. (A) & (B) DHE assay (stained in red) and (C) & (D) live dead assay of AC16 cardiomyocytes with live cells stained as green and dead cells stained as red in 5mM and 50mM glucose concentrations respectively (scale bar 200µm) (E) Mean± S.D % of cells stained with DHE after 4h (F) Mean ±S.D % of live cells after calcein staining

Conclusion

- Parameters for f-gelatin and blended f-gelatin with PCL (1:1 and coaxial) based electrospun scaffolds were optimized and characterized
- SEM and TEM revealed the fibrous nature and coaxial structure of the scaffolds.
- DSC, TGA and FTIR confirmed the non-interaction between PCL and f-gelatin components
- Rheological analysis and i*n vitro* degradation studies established the stability of the blended scaffolds while the biocompatibility of the scaffolds were excellent
- Successful diabetic model can be achieved with the developed electrospun scaffolds as platform with glucose treatment as a hyperglycemic shock for the cells

References

1. Mazaki, T., Shiozaki, Y., Yamane, K. *et al.* A novel, visible light-induced, rapidly cross-linkable gelatin scaffold for osteochondral tissue engineering. *Sci Rep* 4, 4457 (2014).
2. Son TI, et al. Visible light-induced crosslinkable gelatin. *Acta Biomaterialia*. 2010;6(10):4005–4010
3. Kumar, S.A et al. The applicability of furfuryl-gelatin as a novel bioink for tissue engineering applications. *J Biomed. Mat. Res. B* 2019;107(2):314-323.
4. Nagiah, Naveen, et al. "Stem Cell– Laden Coaxially Electrospun Fibrous Scaffold for Regenerative Engineering Applications." *Current Protocols* 1.1 (2021): e13.
5.Raheja,A.,et al. Design of low cost spinneret assembly for coaxial electrospinning. Appl. Phys. Lett. **106**, 254101 (2015)

Acknowledgements

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