National Institute of Technology Rourkela

Synopsis Seminar

Seminar Title	: Development of a silk fibroin/gelatin based composite nanofibrous mat for constructing tissue patch for corneal epithelial regeneration
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Venue	: Seminar room of BM department
Date and Time	: 05 Aug 2025 (11.30 am)
Abstract	: The present work deals with the development of an electrospun silk fibroin (SF) and gelatin (GE) basedpolymeric matrix with optimized composition that resembles the structure and function of the native epithelial tissue. To this end, SF/GE blend spinning solutions prepared with different volume ratios of SF: GE (70:30, 50:50, 30:70) were electrospun, thereby fabricating nanofibrous mats which were designated as $SF_{70}GE_{30}$, $SF_{50}GE_{50}$, $SF_{30}GE_{70}$. The fabricated two-dimensional scaffolds have nanofibrous porous architecture with desired pore network and pore size suitable for corneal epithelial regeneration. The performed Fourier transform infrared spectra confirmed the presence of individual polymers in the polymeric matrix. The nanofibrous mats are hydrophilic in nature, but with varying degrees. Among these, $SF_{70}GE_{30}$ and $SF_{50}GE_{50}$ mats have exhibited favorable hydrophilicity with the respective contact angles of 65.8 ± 1.67 °and 55.23 ± 0.65°. These scaffolds also possess desired porosity of 87.68 ± 0.31% and 88.65 ± 0.49% respectively. However, the tensile strength of $SF_{70}GE_{30}$ (2.74 ± 0.012 MPa) and $SF_{50}GE_{50}$ (2.46 ± 0.018 MPa) scaffolds had a slightly lower tensile strength than the strength of native corneal epithelium ranging 3-5 MPa. An enhanced transparency was obtained with increased GE concentration however, the transparency range of 73-82 % obtained with $SF_{50}GE_{50}$ matches the transparency of natural corneal epithelium. The biocompatibility of the nanofibrous mats was confirmed by the cell attachment, cytotoxicity and cytoskeletal arrangement by culturing rabbit corneal fibroblast cells (SIRC) on the mats. A higher and comparable cell attachment and cell proliferation were evident in $SF_{70}GE_{30}$ and $SF_{50}GE_{50}$ nanofibrous mats than $SF_{30}GE_{70}$. MTT assay revealed that $SF_{70}GE_{30}$ scaffold exhibited the highest cell viability followed by $SF_{50}GE_{50}$ matrix, but the former mat lacks transparency (55-71%) which is an essential property that a scaffold should have for corneal tissue engineering. Moreover, the cytoskeletal arrangement in $SF_{70}GE_{30}$ and $SF_{50}GE_{50}$ represented a denser filamentous actin indicating the initiation of corneal epithelial tissue formation without any insignificant difference. Therefore, considering all the properties, $SF_{50}GE_{50}$ nanofibrous matrix is considered as the most suitable substrate for corneal tissue regeneration. The tensile strength and biodegradability of the SF/GE polymeric matrices were further improved by adding 1-4% (v/v) polycaprolactone (PCL).The resulting tri-polymer complexes were electrospinned and the fabricated matrices were represented as $SF_{50}GE_{50}PCL_1$, $SF_{50}GE_{50}PCL_2$, $SF_{50}GE_{50}PCL_3$, and $SF_{50}GE_{50}PCL_4$. The control $SF_{50}GE_{50}$ was designated as $SF_{50}GE_{50}PCL_0$. Morphologically, the nanofibrous mats have porous architecture with interconnected pores. All the electrospun mats exhibited optimum porosity range of 84.12-88.65 % that are favorable for corneal epithelial regeneration. $SF_{50}GE_{50}PCL_2$ is anticipated to provide superior cell-scaffold interactions during the tissue regeneration process due to its controlled hydrophilicity with contact angle of 63.53 ± 1.52º as compared to other mats. There was no significant change in swelling behaviour noticed upon addition of PCL, thereby controlled swelling behaviour was achieved. The tensile strength of the polymeric matrix increased with increase in PCL concentration, whereas *in vitro* biodegradation was inversely proportional to PCL concentration. The $SF_{50}GE_{50}PCL_2$ nanofibrous mat had respective tensile strength and degradation values of 3.5 ± 0.15 MPa and 54.66 ± 1.52% that match with the strength and rate of corneal epithelial regeneration. The incorporation of PCL into SF/GE polymeric matrix decreased transparency at varied degree, but $SF_{50}GE_{50}PCL_1$ and $SF_{50}GE_{50}PCL_2$ showed the respective transparency of 71-80% and 70-78% which are still matching closely with the required transparency range for corneal epithelium. The *in vitro* cell study performed with SIRC confirmed their biocompatibility exhibiting desired cell attachment, cytotoxicity, and reactive oxygen species (ROS). Among all compositions, $SF_{50}GE_{50}PCL_1$, and $SF_{50}GE_{50}PCL_2$ constructs having lower PCL content expressed superior cell attachment and denser filamentous actin, signifying the initiation of tissue formation. In MTT assay, $SF_{50}GE_{50}PCL_1$ mat exhibited the highest cell viability which was comparable to that shown by $SF_{50}GE_{50}PCL_2$ mat. Overall, the desired tensile strength, hydrophilicity, porosity, controlled swelling and degradation rates, and cell viability, exhibited by $SF_{50}GE_{50}PCL_2$ was found to be superior for corneal epithelial tissue engineering. To improve the antioxidant and antimicrobial properties, different concentrations of curcumin (1 mM, 2.5mM, 5mM and 10 mM) were incorporated into the $SF_{50}GE_{50}PCL_2$ mat which was represented as $SF_{50}GE_{50}PCL_2C_1$, $SF_{50}GE_{50}PCL_2C_{2.5}$ $SF_{50}GE_{50}PCL_2C_5$, and $SF_{50}GE_{50}PCL_2C_{10}$ respectively. The fabricated mats exhibited morphological similarity to the natural extracellular matrix of corneal epithelium and peak shifts in FTIR spectra confirmed the interaction between the curcumin and $SF_{50}GE_{50}PCL_2$ mats. The mats with higher concentrations of curcumin ($SF_{50}GE_{50}PCL_2C_{10}$) possessed higher tensile strength of 4.9 ± 0.14 MPa followed by $SF_{50}GE_{50}PCL_2C_5$ mat measuring strength of 4.3 ± 0.2 MPa. The decrease in transparency with the incorporation of curcumin in $SF_{50}GE_{50}PCL_2C_1$ (69.7-77.1%), and $SF_{50}GE_{50}PCL_2C_{2.5}$ (69.1-77.06%), and $SF_{50}GE_{50}PCL_2C_5$ (68.6-76.5%) mats was insignificant when compared to control (70.7-78.2%) scaffold. Moreover, $SF_{50}GE_{50}PCL_2C_5$ and $SF_{50}GE_{50}PCL_2C_{10}$ mats demonstrated a respective controlled swelling percentage of 246.6 ± 35.11% and 236.6 ± 36.6% and a degradation rate of 43.66 ± 1.52% and 42.66 ± 2.08% respectively which makes them advantageous for use in tissue engineering when implanted *in vivo*. The concentration of curcumin and hydrophilicity were directly correlated, where $SF_{50}GE_{50}PCL_2C_{10}$ mat containing the highest concentration of curcumin had a contact angle of 80.13 ± 1.91 º, which may not be favorable for *in vitro* cell cultures. However, $SF_{50}GE_{50}PCL_2C_5$ with contact angle of 72.6 ± 2 º may

be suitable for the growth and differentiation of corneal epithelial tissues. The antimicrobial and antioxidant properties enhanced at higher curcumin content in $SF_{50}GE_{50}PCL_2C_5$ and $SF_{50}GE_{50}PCL_2C_{10}$ nanofibrous mats which reduced the cellular oxidative damage, and infections, thereby facilitating rapid corneal regeneration. The cytocompatibility of the curcumin incorporated nanofibrous mats was also examined by *in vitro* cell studies, which ensured that the incorporation of curcumin into the polymeric matrix does not negatively affect the cellular growth and attachment. $SF_{50}GE_{50}PCL_2C_5$ construct exhibited higher cell viability, cell attachment, and denser cytoskeleton arrangement, whereas $SF_{50}GE_{50}PCL_2C_{10}$ construct had decreased cell viability due to higher dosage of curcumin, which resulted in apoptosis and cell death. A controlled swelling and degradation rate, desired porosity range, and tensile strength with enhanced cell viability, antimicrobial, and antioxidant properties in $SF_{50}GE_{50}PCL_2C_5$ nanofibrous mats indicated its potentiality for regeneration of corneal epithelial tissues. However, the transparency of the developed nanofibrous mats was compromised in the above case. Therefore, in order to enhance the transparency as well as antibacterial and antioxidant properties, $SF_{50}GE_{50}PCL_2$ mat was loaded with different concentration of quercetin (5 mM, 10 mM, and 15 mM) which were represented as $SF_{50}GE_{50}PCL_2Q_5$, and $SF_{50}GE_{50}PCL_2Q_{10}$, $SF_{50}GE_{50}PCL_2Q_{15}$ respectively. All the mats exhibited desired fiber diameter and pore size with nanofibrous architecture similar to the extracellular matrix of native corneal epithelium. The incorporation of quercetin into the polymeric matrix enhanced the transparency making the mats transparent enough for its usage in corneal epithelial regeneration. Among the fabricated matrices, $SF_{50}GE_{50}PCL_2Q_{10}$ exhibited robust transparency range of 75-85%, adequate tensile strength (3.82 ± 0.1 MPa), and *in vitro* biodegradability ($44.33 \pm 2.51\%$) mimicking the properties of corneal epithelium. The higher concentrations of quercetin in the mats of $SF_{50}GE_{50}PCL_2Q_{10}$ and $SF_{50}GE_{50}PCL_2Q_{15}$ depicted superior antimicrobial property against *S. aureus* and *E. coli*. The *in vitro* cell studies proved that $SF_{50}GE_{50}PCL_2Q_{10}$ mat demonstrated the highest cell viability and cell attachment. The confocal microscopy and flow cytometry results further revealed that mats with higher concentration of quercetin viz. $SF_{50}GE_{50}PCL_2Q_{10}$ and $SF_{50}GE_{50}PCL_2Q_{15}$ illustrated superior antioxidant properties with minimal ROS production. Thus, $SF_{50}GE_{50}PCL_2Q_{10}$ nanofibrous mat exhibiting an improved transparency, tensile strength, antimicrobial, antioxidant, and cell supportive properties, is proven to be an excellent substrate that can used for corneal epithelial regeneration.