CONCLUSION

7. Conclusion

In this study a successful attempt was made to prepare drug loaded biopolymeric scaffold for wound dressing. Freeze drying methodology was opted to fabricate the scaffolds. The whole work was conducted in three parts;

In the first part of the study; two polymers *viz.* silk fibroin (SF) and gellan gum (GG) were used to develop a scaffold for wound dressing. Two different types of hybrid scaffolds were fabricated from SF and GG. First type of scaffold (SF-GG) was fabricated by utilizing the blended mixture of SF and GG whereas second type of scaffold (SF-GG_b-SF) was fabricated by entrapping the beads of gellan gum (GG_b) between the two layers of SF. Scaffolds of pure SF (SF_{sc}), pure GG (GG_{sc}) and GG_b were taken as control. Ciprofloxacin hydrochloride (*cpr*), antibiotic drug was loaded in scaffold during its fabricating to obtain antimicrobial effect in the scaffolds. For SF-GG scaffold; porosity, *cpr* loading efficiency, swelling ratio in simulated wound fluid (SWF), degradation in SWF up to 28 days and compressive strength at 75% compression were 71.5%, 3.1%, 16.85, 2.7% and 0.19MPa respectively, whereas all these parameters for SF-GG_b-SF were 84.5%, 3.7%, 11.90, 3.0% and 0.55 MPa respectively. SF-GG_b-SF releases the *cpr* in a more sustained manner for longer period of time (12h) as compare to SF-GG (6h) through anomalous diffusion which follows 'Korsmeyer-Peppas kinetic model' with R^2 0.976. On the basis of the performance of both types of scaffolds; the SF-GG_b-SF was selected and further evaluated for haemocompatibility, roughness and antibacterial efficacy. SF-GG_b-SF was haemocompatibe with 3.7% hemolysis and its mean absolute roughness (Ra) of the surface was 37.5 nm. The initial antimicrobial activity of SF-GG_b-SF against both *E. coli* and *S. aureus* was good with elimination of more than 98% of bacterial load within 4h but after 24 h of application, the antimicrobial activity of SF-GG_b-SF was drastically reduced. Overall; it was found that swelling and drug loading efficiency of scaffolds can be improved by entrapping the GG_b within SF matrix. But due to poor

degradability, short drug release time, suboptimal roughness level and decreased antimicrobial activity with time; the *cpr* loaded SF-GG_b-SF scaffold cannot be considered suitable for wound dressing. Therefore, some further improvement in the scaffold is required. For these improvements the modified scaffolds were reconstructed by changing the polymer and drug system. The modified scaffolds were fabricated by using SF and chitosan (CS) in place of SF and GG. Silver nanoparticles (AgNPs) were also incorporated in the modified scaffolds along with *cpr* for prolonged antimicrobial effect. For economic feasibility and eco friendly approach, a green synthesis route was opted for the synthesis of AgNPs using leaf extracts of different plants.

The second part of the study was dedicated to herbal synthesis and characterization of AgNPs. Eighteen different plants were screened and the capability of AgNPs synthesis was found in the herbal extracts of *Salvinia molesta* and *Tamarindus indica* plants. Aqueous extracts of leaves of *Salvinia molesta* (AES) and *Tamarindus indica* (AET) were utilized for the purpose of synthesis of AgNPs. Both the synthesis processes (by AES & AET) were studied, optimized and the synthesized AgNPs from both the plant extracts were characterized. Both synthesis processes were found to be photocatalytic in nature. 35 min reaction time, 8 mM $AgNO_3$ concentration and 5.0 % (v/v) of AES inoculum dose were optimal values of process parameters for AES mediated AgNPs synthesis whereas the optimum values of process parameters for AET mediated synthesis of AgNPs were found as; 5 mM $AgNO_3$ concentration, 10.0 % (v/v) of AET inoculum dose and 40 min of sun light exposure time. FESEM-EDX and HRTEM confirmed the synthesis of AgNPs and their relative size. The average size of AgNPs synthesized by AES and AET were 12.46 nm and 32.74 nm respectively. FTIR spectroscopy revealed the involvement of hydroxyl, carboxyl and amino groups of phyto-chemicals present in AES and AET for the biosynthesis of AgNPs. XRD analysis confirmed the crystalline nature and 'fcc'

crystal lattice of synthesized AgNPs in both the cases. Antimicrobial potential of prepared AgNPs was evaluated through disk diffusion method. Smaller AgNPs synthesized by AES were found more effective antimicrobial agent against *E. coli* and *S. aureus* as compare to the AgNPs synthesized by AET. MIC values of AES synthesized AgNPs against *E. coli* and *S. aureus* were 10.50μg/mL and 13.0 μg/mL respectively whereas these MIC values for AET synthesized AgNPs were 16.50 μg/mL and 19.5 μg/mL respectively. On the basis of stronger antimicrobial effect, the AgNPs, synthesized from AES were selected for incorporation in the modified scaffolds for extended antimicrobial effects.

In third part of the work; modified scaffolds were fabricated by SF and chitosan (CS) with incorporated AgNPs and *cpr.* 2% w/v concentration of each polymer (SF & CS) was used for scaffolds fabrication. Three different scaffolds *viz.* S/C/NpCp (2:1), S/C/NpCp (1:1) and S/C/NpCp (1:2) were constructed by SF and CS using different amount ratios of (2:1), (1:1) and (1:2) v/v respectively. These blend polymeric solutions were supplemented with 1 mg/mL of *cpr* and 200 μg/mL of AgNPs before freeze drying. Scaffolds of pure silk (SF_{sc}) and scaffold of pure chitosan (CS_{sc}) were also incorporated in the study as a control. FESEM revealed the interconnected porous nature of all scaffolds with pore size distribution in the range of 100 nm to 180 nm. It was observed that pore size increases with increase the amount of chitosan in blends. S/C/NpCp (1:2), S/C/NpCp (1:1) and S/C/NpCp (2:1) have showed 88.5±2.3, 85.2±1.2 and 81.4±3.6% porosities respectively which indicated that % porosities decrease with increase the amount of CS in the blend. Swelling ratio of CS_{sc} was 6.7 times higher than SF_{sc} in SWF. S/C/NpCp (1:2), S/C/NpCp (1:1) and S/C/NpCp (2:1) showed intermediate swelling ratios lying between the swelling ratios of CS_{sc} and SF_{sc}. Degradation after 28 days of incubation in lysozyme (112 U/mL) containing SWF was found maximum (78.5±3.2%) for CS_{sc} and minimum (4.0±2.2%) for SF_{sc}. S/C/NpCp (1:2), S/C/NpCp (1:1) and S/C/NpCp

(2:1) degrades by 18±1.6%, 27.2±2.1% and 45.2±4.0% respectively after 28 days in lysozyme supplemented SWF. Mechanical studies showed that blending of CS and SF in equal ratio (S/C/NpCp (1:1)) has surprisingly increased both the compressive strength and compressive modulus of blend scaffold due to cross linking between CS and SF components. The XRD and FTIR analysis also supported this cross lining behavior of CS and SF. In SWF at pH 6.0, CS_{sc} showed strong burst release of *cpr* with release of more than 90% of loaded drug within 2.5h whereas SF_{sc} exhibited more sustained release of *cpr* with release of ~77% of incorporated drug in 12h. All the scaffolds release *cpr* through anomalous diffusion which follows Korsmeyer-Peppas model kinetics. On the basis of comparative analysis of results; the S/C/NpCp (1:1) was chosen as suitable scaffold for designing the wound dressing. Before designing the dressing; the presence of AgNPs in the S/C/NpCp (1:1) matrix was confirmed FESEM analysis at higher magnification (60,000 X). S/C/NpCp (1:1) was found to release silver in control manner up to 24 days, therefore, the scaffold was capable to show antimicrobial properties for longer period of time with was confirmed by cell viability studies. S/C/NpCp (1:1) showed only 2.9±0.6% hemolysis with human blood, therefore, was considered as hemocompatible. Mean absolute roughness (Ra) of S/C/NpCp (1:1) surface was 163 nm. After these studies the S/C/NpCp (1:1) was selected to fabricate adhesive wound dressing. Finally the designed wound dressing was evaluated for its wound healing potential through *in vivo* studies in rat as model animal. Designed dressing was found highly effective for wound healing purpose and found to be capable of healing the wound only in 12 days whereas untreated wounds takes 21 days for complete healing. Histological studies revealed that S/C/NpCp (1:1) based wound dressing leads to near native tissue architecture of re-grown tissue at healed site. Overall, the developed S/C/NpCp (1:1) based wound dressing was found effective for accelerated wound healing.