CHAPTER 4

CONCLUSIONS AND FUTURE PERSPECTIVES

Wound infections are the major challenges in the wound care management. Infection can be either due to microbial attack or due to some diseases. This infection can make the wound chronic and exudative leading to life threatening dilemma. These kinds of infection can occur in various kinds of wounds including burn wound, diabetic foot ulcer, etc. Wounds are of different types; wounds with large volume of exudate, wounds with high rate of infection, wounds difficult to heal, etc. Adequate attention and treatments are required for such cases. Currently lot of dressing materials and treatment modalities are there in the wound care management field. Each of them has their own merits and de-merits. Our work was designed to prepare a new wound dressing bandage based on hydrogel material. Special emphasis was made on hydrogels based materials so as to promote moist wound healing. They can provide soothing environment, absorb large volume of exudate from the wound, acts as a barrier against microbes, helps the cells to attach and proliferate in addition to the biocompatible nature.

α-Chitin, β-chitin and chitosan have been selected for the preparation of hydrogel bandages. These polymers are of marine origin and are readily available, biocompatible, biodegradable, etc. We prepared hydrogels using these materials under mild reaction conditions. At neutral pH, the prepared hydrogels did not show antimicrobial property. Hence, we incorporated nZnO in these prepared hydrogels to impart antimicrobial activity as well as to enhance wound healing. We used various concentrations of nZnO to optimize the antimicrobial activity and cell viability. We prepared nanocomposite bandages by incorporating nZnO in the hydrogels of above mentioned polymers by freeze-dry method. The prepared nZnO and bandages were characterized and evaluated in vitro and in vivo.

In vivo evaluation of the β-chitin hydrogel/nZnO and chitosan hydrogel/nZnO composite bandages was performed in Sprague-Dawley rats. The obtained data showed that, wounds treated with the composite bandages were healed faster compared to the control treated wounds. The healed wounds showed intact epidermis,
complete re-epithelialisation and excellent vascularisation as well. The wounds treated with the composite bandages showed promising healing after 2 weeks whereas the control wounds took more than 3 weeks. The wounds treated with composite bandages showed excellent collagen deposition and adequate antibacterial activity in vivo. The major outcomes of the study are follows.

- nZnO were prepared and characterized using XRD, DLS, SEM and AFM. The obtained data showed that the prepared nZnO were having spherical morphology and are having size in the range of 70-120 nm.
- α-Chitin, β-chitin and chitosan hydrogels were prepared using CaCl2 saturated methanol solvent. The prepared hydrogels were purified by dialysis against distilled water for two days.
- nZnO was incorporated in these hydrogels and composite bandages were obtained by freeze-dry method.
- The prepared bandages were micro-porous and flexible. The pore size of the composite bandages were in the range of 200-400µm.
- The prepared bandages were having adequate tensile strength and excellent elongation at break. Among the three tested composite bandages, the tensile strength of α-chitin hydrogel/nZnO composite bandage was lesser in comparison to the β-chitin hydrogel/nZnO and CZBs.
- All the composite bandages showed high swelling ratio. After immersion in PBS for 1 day, the bandages were saturated with the liquid in which it was immersed and thereafter they showed a slight increase upto 7 days. After 7 days, the bandages started to show slightly reduced swelling ratio and reduction found reduced at 21 days.
- In vitro biodegradation data showed that α-chitin hydrogel/nZnO composite bandage degrades faster compared to β-chitin hydrogel/nZnO composite bandages and CZBs.
- Hemostatic potential analysis of the composite bandages proved its faster blood clotting and enhanced platelet activation and platelet adhesion.

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properties. All the three composite bandages showed same kind of result in this analysis.

- Antibacterial and antifungal activity of the prepared composite bandages was assessed against *S.aureus*, *E.coli* and *Candida albicans*. All the composite bandages with higher concentration of nZnO showed higher rate of activity and the activity reduced when the concentration of nZnO was reduced. Control bandages and Kaltostat did not show antibacterial activity.

- Cell viability evaluation was done on HDF cells upto 72 hrs. The obtained result showed that the bandages with higher concentration of nZnO reduced the viability of HDF cells and the obtained viability was about 40-50%. The viability was increased for bandages with lower concentration of nZnO.

- The bandages with lower concentration of nZnO showed excellent HDF cell attachment and proliferation ability compared to the bandages with higher concentration of nZnO.

- Cell infiltration analysis showed that HDF cells were infiltrated to the interior of the bandages upto 100µm and started to proliferate.

- We have conducted *in vivo* evaluation of composite bandages in Sprague-dawley rats except α-chitin hydrogel/nZnO composite bandage. The reason behind exclusion of α-chitin hydrogel/nZnO composite bandage was the inadequate mechanical strength of the same compared to the other two bandages.

- *In vivo* evaluation of β-chitin hydrogel/nZnO composite bandage and CZBs proved the faster healing of excisional wounds treated with the composite bandages compared to the control bandages and Kaltostat treated wounds.

- The wounds treated with composite bandages showed excellent re-epithelialisation, intact epidermis, enhanced collagen deposition and no inflammation.

- The wounds treated with β-chitin hydrogel/nZnO composite bandages showed faster healing compared to the wounds treated with CZBs. The presence of nZnO did not influence the rate of wound healing.
In vivo antibacterial evaluation showed that the bacterial growth was less on the wounds treated with composite bandages with higher concentration of nZnO compared to the bandages with lower concentration of nZnO.

**Future perspectives**

Wound dressings are the main treatment modality in the wound care management. Majority of current wound dressing materials lack the property of adequate exudate absorption and many of them may adhere to wound which can cause trauma. Most of the dressing materials are non-biodegradable and need regular replacement. A myriad of dressings do not possess antibacterial activity as well as hemostatic potential. Lack of flexibility, mechanical strength and wet strength are other de-merits of existing dressing materials. Current dressing materials are not able to maintain a moist environment at the wound/dressing interface. The remedy for all the above mentioned problems is the use of a wound dressing which include all these properties.

Kaltostat™ is a wound dressing material is currently available in the market which is made of alginate. It is a non-woven dressing with adequate flexibility but lacks mechanical strength. The material tends to adhere on to the wounds and cause trauma to the newly forming epithelial layer. The hemostatic potential evaluation data of the material revealed that it is not capable of inducing platelet activation as well as blood clotting. The in vivo evaluation in Sprague-Dawley rats showed the lack of healing ability of the material.
Hydrogel based wound dressings with antimicrobial activity can be used to tackle such situations. Hydrogels based dressings provide soothing effect to the wound, allows transfer of oxygen and acts as a barrier against microbes. Our prepared composite bandages were micro-porous and flexible in nature. They were capable of absorbing large volume of exudates from the wound surface thus prevent wound infection due to exudates. The prepared hydrogel based composite dressing materials were capable of providing sustained antimicrobial activity and excellent blood clotting potential. The prepared composite bandages were cytocompatible and cells attached on to the surface and started proliferation. *In vivo* evaluations of the composite bandage have been performed and the obtained data was promising for the use of these bandages for clinical trial 1, 2 and 3. The major perspectives are given below

**Future perspectives**

- The prepared hydrogels can be used as injectable hydrogel for various types wounds where more soothing effect is required
- Instead of nZnO, other nanoparticles like nano silver, nano TiO$_2$, etc can be used to impart antibacterial activity to the composite bandages
- Antibiotics and antimycotics loaded nanoparticles can be incorporated in to the composite bandages for wound healing applications
- The *in vivo* evaluation of composite bandages can be done on the wounds created on large animals like rabbit, sheep, etc.
- The prepared composite bandages can be used for the treatment of burn wounds and diabetic foot ulcers where large volume of wound exudate has to be removed from the wound surface.
Epidermal growth factors, fibrin, platelet derived growth factors, etc can be loaded in to the composite bandages for the controlled delivery of these at the wound site. This would be helpful for the faster growth of epithelial cells and hence the epidermis formation will be faster. Fibrin will be helpful to enhance the vascularisation.

The prepared bandages can be used as a drug delivery system for various diseases like osteoporosis, skin cancer, etc. using this method, controlled drug release and release at the exact location can be achieved.

The prepared composite bandages can be surface functionalized by plasma treatment and can be used for various tissue engineering applications. Surface functionalization by plasma treatment implies the incorporation of different functional groups (-COOH, -NH2, etc) depending on the process gas (Oxygen, Nitrogen, etc) used. By this treatment the scaffold can be modified without changing its bulk properties.