Hydrogel Films of Chitosan and Keratin for Enhanced Wound Healing

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Abstract

Due to different systemic and local factors, the process of wound healing in humans could be delayed which lead to adverse effects such as chronic consequences sometimes resulting in ulcers. Proper measures, both internal and external measures, should be thus undertaken to facilitate and enhance the healing process effectively. Considering the fibroblast recruitment property of keratin and antibacterial property of chitosan, a composite hydrogel film of keratin and chitosan, to be used as wound dressing, has been developed by using the keratin extracted from raw horns of cattle, biomass wastes, and chitosan prepared by deacetylation of commercially available chitin in combination with gellan gum. For the characterization of keratin, FTIR spectroscopy and SDS-PAGE were performed. The prepared film was characterized by employing swelling, permeability, contamination tests, and MTT assay. The SDS-PAGE of extracted keratin showed a band in the range of human keratin. The film showed characteristics of impermeability, swelling capacity which helps wound healing by retaining moisture and absorption of excess exudates. The film showed no growth of any bacterial colony on its surface confirming its ability to resist any further infections. Likewise, the MTT assay showed cell proliferation was well suited on the prepared film.

Keywords: *keratin, chitosan, gellan gum, wound, SDS-PAGE, MTT assay*

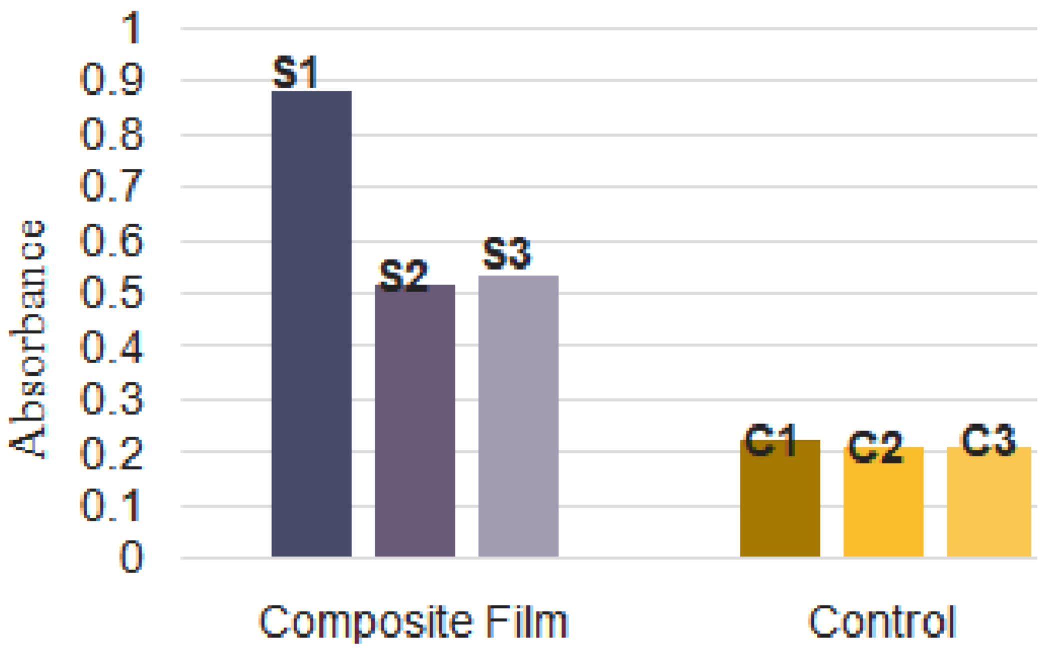

Figure 1: Absorbance of sample film in comparisoan to control

Objectives

To characterize and check the effectiveness of prepared hydrogel film in wound healing application

Methodology

1. Extraction of Keratin

2. Preparation of Chitosan

3. Composite Hydrogel Formation

4. Characterization
- FTIR
- SDS-PAGE
- Solubility test
- Contamination test
- Permeability test
- Swelling test
- MTT assay

Figure 2: Prepared Hydrogel Film

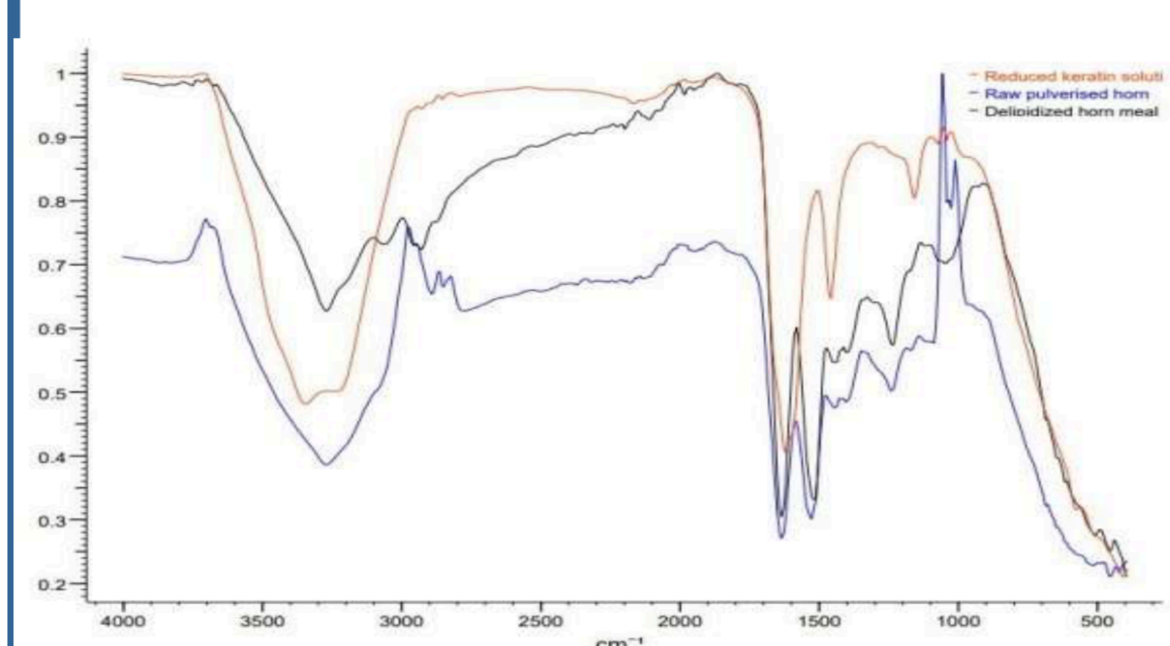

Figure 3: FTIR result of three different samples

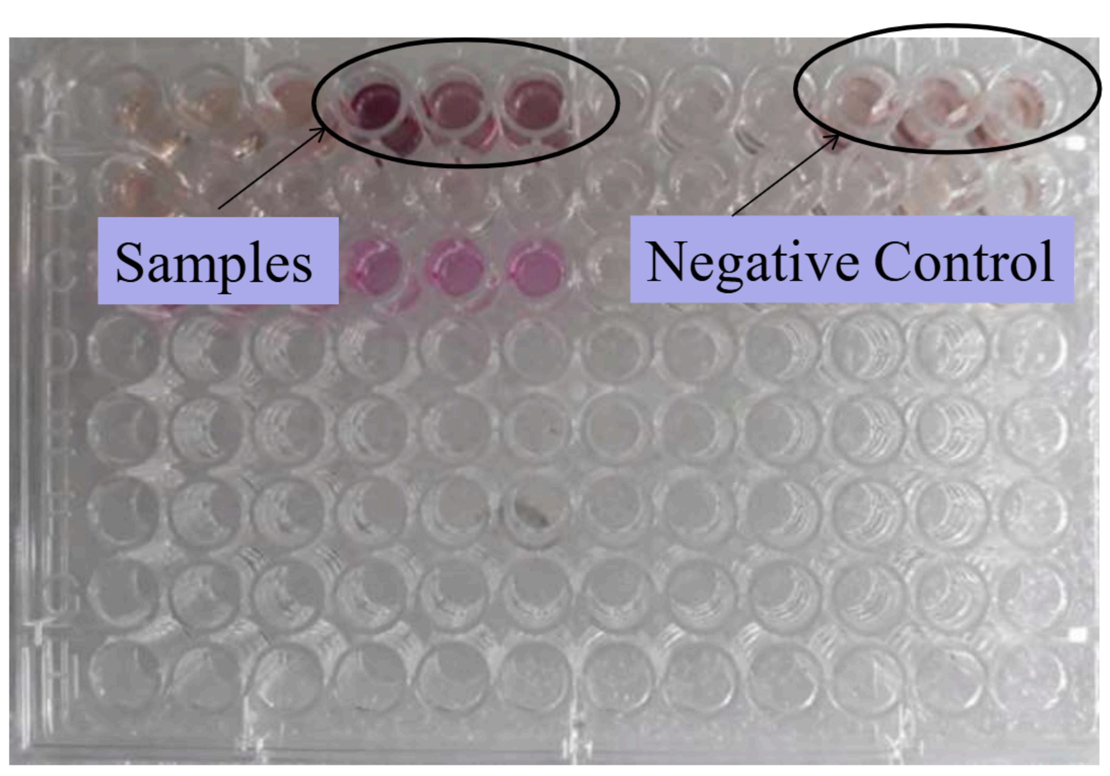

Figure 4: : MTT assay of samples in 96 well plates

Conclusions

FTIR: Based on the spectrum analysis, sample absorption bands were visible which indicates the presence of amide I, amide II and amide III.

Swelling test: The final weights of the swollen sample were higher than its weights before swelling. The ability to swell means the film can absorb exudates up to some esteem and also hold more amount of cell within its dimensions.

Contamination Test: No growth of bacterial colony in the contamination test.

SDS-PAGE: In SDS-PAGE results a band was visible in the range of human keratin.

Permeability test: The volume in the tube containing salt didn't increase in volume. Retention of water helps in keeping the wound moist, hence maintaining the current of injury.

MTT assay: The results of the MTT assay showed great cell proliferation and viability. We can see that the average absorption of composite hydrogel film is better than control cells.

Work in Progress

Overcoming the limitations of this project, the progressing work is more focused on testing and accessing the cell and drug delivery capability of graphene-hydrogel films in the epidermal trauma site. As there is a huge potential of controlling this delivery of contents through electrical stimulation, the project also involves this electrical component.

References

•Singer AJ, ClarkRAF. Epstein 1999_Wound healing. NEnglJ Med. 1999;
•TangL, Sierra JO, Kelly R, Kirsner R S, Li J. Wool-derived keratian stimulates human keratinocyte migration and types IV and VII collagen expression. Experimental Dermatology. 2012.
•Ahmed S, Ikram S. Chitosan Based Scaffolds and Their Applications in Wound Healing .Achiev Life Sci. 2016; 10(1): 27–37.
•Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound healing dressings and drug delivery systems: A review. Journal of Pharmaceutical Sciences. 2008.
•Kakkar P, Madhan B, Shanmugam G. Extraction and characterization of keratin from bovine hoof: A potential material for biomedical applications. J Korean Phys Soc.2014;3(1):1–9.