BioRemedi 2022

Scientific Research Area: Biomaterials and Applications
Preferred Presentation Method: Oral
Student Type: M.Tech
Designation: Postgraduate student
Citizenship: Indian
Country of Birth: India
Country of Residence: India

Development of bromelain-loaded chitosan/alginate bilayer film for the wound dressing applications

Shriram Bhutt B, Mohana Priya S, Tharani K, Monisha Gowri S and Thirugnanam A *
Department of Biotechnology and Medical Engineering; National Institute of Technology Rourkela, Rourkela-769008, Odisha, India.
*Email: hibhutt@gmail.com; *Email: thirugnanam.a@nitrkl.ac.in*

Abstract: Wound healing is a naturally occurring process in our body and comprises various phases like hemostasis, inflammation, proliferation and remodeling. Bromelain, a natural cysteine protease, is a multifunctional molecule with analgesic and anti-inflammatory effects that aid in the treatment of wounds. This study focuses on developing chitosan/alginate bilayer film by incorporating bromelain (0,0.5,1,1.5 and 2% w/v) in the alginate layer via layer-by-layer assembly. Bilayer films were prepared by solvent casting method and were systematically characterized physicochemically, mechanically and biologically in *in-vitro*. Scanning electron microscope (SEM) and Fourier transform infrared (FTIR) spectroscopic analysis determined the surface morphology and functional groups of bromelain in the alginate layer. Further, the swelling (%), tensile strength, wettability and moisture transmission rate of films was also determined. *In-vitro* drug release (pH 7.4) and its kinetics were studied to determine the bromelain release and its mechanism using Korsmeyer-Peppas, Higuchi, Zero-order and Peppas-Sahlin models. Hemolysis assay and cell viability assay in *in-vitro* were performed to assess the hemocompatibility, toxicity of films. The surface morphology from SEM revealed that bromelain was homogeneously dispersed and disruption of alginate bonds by bromelain was studied in FTIR analysis. The high mechanical strength (60.05±2.45 MPa for 0.5 % w/v of bromelain) of the bilayer films decreased with an increase in the concentration of bromelain. The bi-layered films possessed an excellent swelling rate of around 300 - 350% and tend to be slightly hydrophobic (61.71±1.68 to 72.51±3.11° for the alginate layer and 89.84±3.29° for the chitosan layer) due to the bromelain interaction, which reduced the free hydroxyl groups in the alginate layer. The moisture transmission rate decreased with an increase in the concentration of bromelain. The drug release depicted that 2% w/v of bromelain showed an initial burst release, while other concentrations showed a slower release rate. From the drug release kinetics, the Korsmeyer-Peppas model was found to be the closest fit. Bilayer films depicted good hemocompatibility and cell viability above 80%. The study demonstrated that bilayer film incorporated with bromelain (up to 1.5% w/v) might be a potential drug-delivery system for wound dressing applications.

Development of bromelain-loaded chitosan/alginate bilayer film for the wound dressing applications

Shriram Bhutt B, Mohana Priya S, Tharani K, Monisha Gowri S and Thirugnanam A

Department of Biotechnology and Medical Engineering;

National Institute of Technology Rourkela, Rourkela-769008, Odisha, India

INTRODUCTION

- According to the scenario of curing acute wounds, self-healing is insufficient, which profound the usage of biomaterials like films, gels, patches, etc as a transdermal drug delivery system.
- The bilayer films comprising of chitosan (anti-microbial activity, good mechanical property) and alginate (excess exudate removal) are better than other transdermal systems.
- The bromelain, which is a mixture of cysteine proteases derived from pineapple stem and fruit is used in wound debridement and post-surgery recovery.
- Therefore, the present study aims to develop and characterize bromelain-loaded chitosan/alginate bilayer film for wound dressing application.

METHODOLOGY

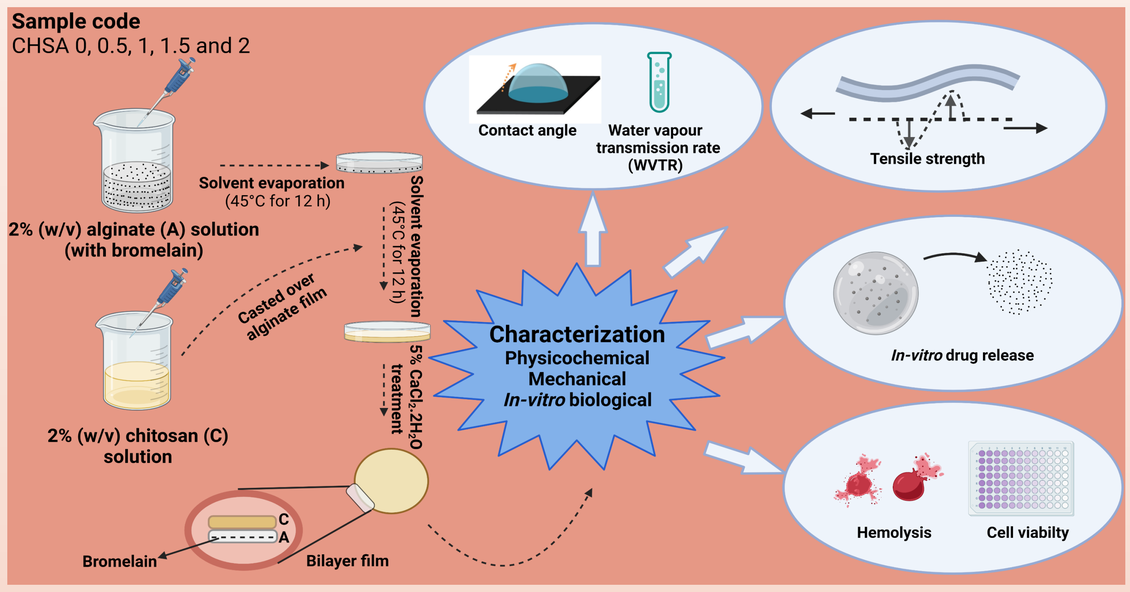

Fig 1. Schematic representation of Bilayer film development

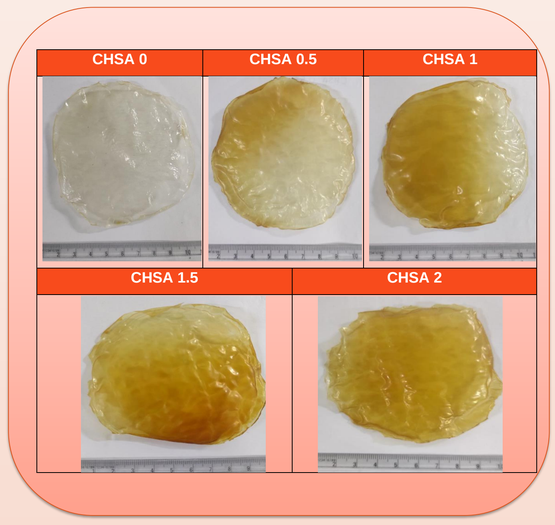

Fig 2. Chitosan/alginate bilayer film

RESULTS AND DISCUSSION

Scanning electron microscopy

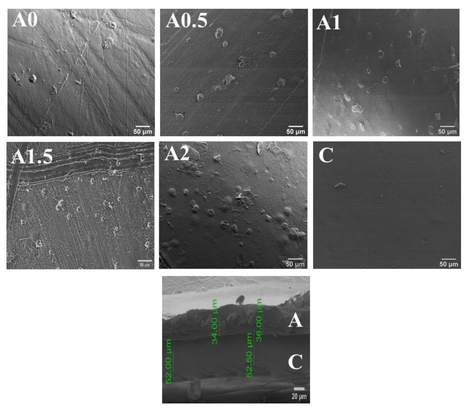

Fig 3. SEM images of bilayer film (surface and cross-section)

- Agglomeration or aggregation of bromelain was observed on the surface of A2.
- Film is homogenous and without voids.

FTIR spectroscopic analysis

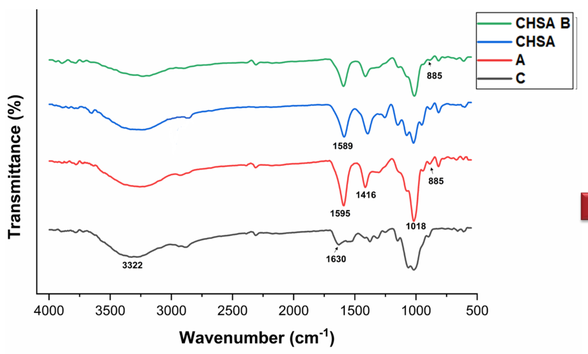

Fig 4. FTIR spectra of bilayer film

- The peak intensity (1595 cm^{-1}) was decreased and slightly shifted to 1589 cm^{-1} due to chitosan and alginate interaction.
- Peak at 1589 cm^{-1} and 885 cm^{-1} decreased in bromelain incorporated film.

Tensile properties

Table 1. Tensile strength and Young's modulus

Sample	Tensile strength (MPa)	Young's modulus (MPa)
CHSA 0	65.05±5.46	930.26±168.74
CHSA 0.5	60.05±8.35	1008.61±69.50
CHSA 1	59.65±6.28	1738.23±52.55
CHSA 1.5	56.15±1.13	1576.42±186.17
CHSA 2	54.56±8.95	1404.81±156.23

- The decreased tensile strength with the increasing concentration of bromelain is due to bromelain interfering with the polymer chains.

Wettability study

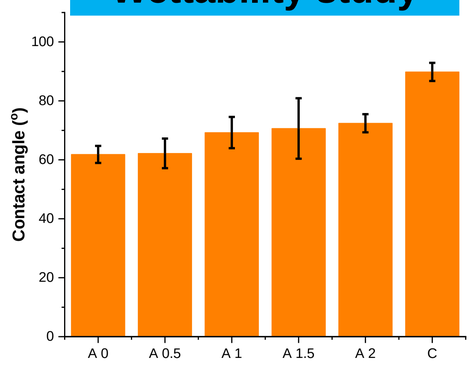

Fig 5. Contact angle of bilayer films

- The increase in contact angle is due to the interaction of hydroxyl groups and the film tends to be hydrophobic with increase in the concentration of bromelain.

In-vitro drug release and its kinetics

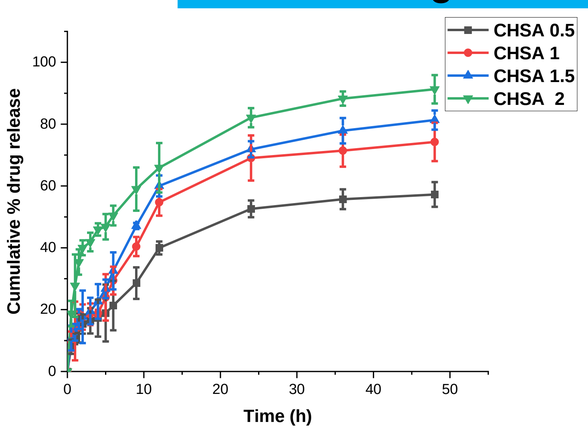

Fig 8. In-*vitro* drug release from bilayer films

- The initial burst release in CHSA 2 is due to more molecules being present on the surface of the film.
- The lower the concentration of bromelain, the release is also slower.
- Korsmeyer-Peppas (KP) model was considered to be the best fit.

Table 2. Kinetic models for drug release from bilayer films

Model	Parameters	CHSA 0.5	CHSA 1	CHSA 1.5	CHSA 2
Korsmeyer-Peppas	k	10.843	9.574	8.493	28.742
	n	0.453	0.666	0.738	0.321
	R^2	**0.983**	**0.975**	**0.985**	**0.991**
Higuchi	k	19.542	9.399	14.944	13.044
	R^2	0.957	0.975	0.953	0.958
Zero-order	k	4.785	2.478	4.132	3.526
	R^2	0.755	0.892	0.958	0.921
Peppas- Sahlin	k_d	0.124	7.292	8.216	8.559
	k_r	10.974	1.813	2.172	19.582
	m	0.236	0.497	0.512	0.188
	R^2	0.982	0.970	0.976	0.988

Swelling study

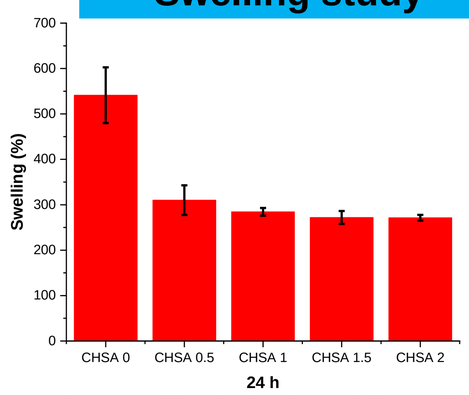

Fig 6. Swelling behavior of bilayer films

- The swelling decreased with bromelain incorporation, due to hydrophobic regions of bromelain and less hydroxyl groups.

WVTR

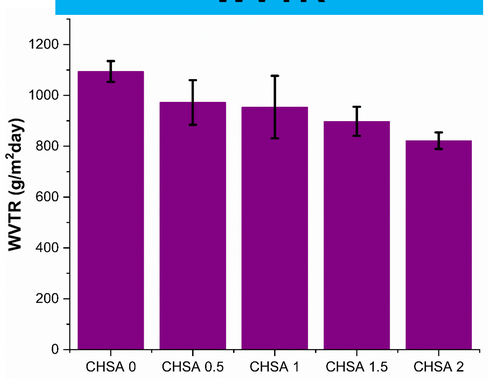

Fig 7. WVTR of bilayer films

- The WVTR correlates with contact angle measurements as the more hydrophobic the material, its WVTR decreases.

Hemolysis study

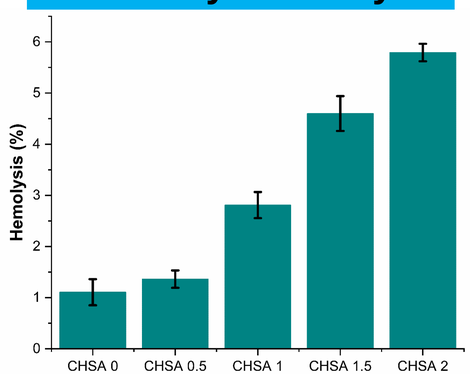

Fig 9. Hemolysis rate of bilayer films

- All bilayer films except CHSA 2 showed a hemolysis rate <5%, which is non-hemolytic and hemo compatible according to ISO 10993-4 standard.

Cell viability assay

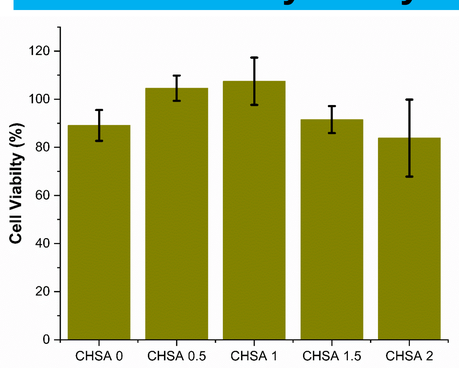

Fig 10. Cell viability of bilayer films in I929 cell line

- All bilayer film exhibited cell viability above 80% but it is starting to decrease from 1.5% of bromelain.

CONCLUSION

- Chitosan/alginate bilayer film showed uniform, homogenous surface and the identification of bromelain incorporation was witnessed.
- The addition of bromelain made the film hydrophobic leading to reduction in swelling, WVTR.
- Highest drug release was observed in CHSA 2
- CHSA 1.5, CHSA 2 showed decrease in cell viability but all were above 80% and CHSA 2 had hemolysis rate >5%.
- From these, it can be concluded that upto 1% of bromelain incorporated in chitosan/alginate bilayer film will aid in wound healing.

REFERENCES

- Thu, H. E., Zulfakar, M. H., & Ng, S. F. (2012). Alginate based bilayer hydrocolloid films as potential slow-release modern wound dressing. International journal of pharmaceutics, 434(1-2), 375-383
- Trevisol, T. C., Scartazzini, L., Valério, A., Guelli Ulson de Souza, S. M. A., Bierhalz, A. C. K., & Valle, J. A. B. (2020). Diclofenac release from alginate/carboxymethyl cellulose mono and bilayer films for wound dressing applications. Cellulose, 27(11), 6629-6642.