Hong Kong Student Science Project Competition 2023

Extended Abstract (Investigation)

Team Number: SBBC075

Project Title: Anti-bacterial non-allergic bio bandages with bio-dressing of hydrogen of chitosan (HC) of black soldier fly (BSF)

Project Type: Investigation

I. Background

Commercially available bandages such as hydrocolloid are neither biodegradable nor anti-bacterial. Chitin is known to be the second most naturally available polysaccharide which could be transformed to chitosan which is known to be anti-bacterial (Hasan, 2018) (Chao, 2019) and haemostatic (Okamoto, 2003) (Hu, 2018). Chitosan can be further converted to hydrogel which is bio-degradable and has good water absorbance. Shells of Hermetia illucens, black soldier fly are readily available sources of chitin as they are made up of about 20% of chitin (Złotko, 2021), Chitosan could be isolated and purified from larvae of black soldier flies. After isolation and purification, Chitosan could be obtained from chitin-rich larvae of black soldier flies *Hermetia illucens*. (Yong, 2022), so hydrogel of chitosan of black soldier fly are potential alternatives of anti-bacterial non-allergic bio-bandages with bio-dressings.

References:

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2. Chao, 2019. Chitosan as A Preservative for Fruits and Vegetables: A Review on Chemistry and Antimicrobial Properties, DUAN Chao, MENG Xin, MENG Jingru, Md. Iqbal Hassan KHAN, DAI Lei, Avik KHAN, AN Xingye, ZHANG Junhua, Tanzina HUQ, NI Yonghao, Journal of Bioresources and Bioproducts, 2019, (4)1: 11-21.
3. Okamoto, 2003. Effects of chitin and chitosan on blood coagulation, Y.Okamoto, R.Yano, K.Miyatake, I.Tomohiro, Y.Shigemasa, S.Minami, Carbohydrate Polymers, Volume 53, Issue 3, 15 August 2003, Pages 337-342.
4. Hu, 2018. Chitosan-Based Composite Materials for Prospective Hemostatic Applications, Zhang Hu, Dong-Ying Zhang, Si-Tong Lu, Pu-Wang Li and Si-Dong Li, Marine Drugs, 2018, 16(8), p.273-297.
5. Złotko. (2021). Isolation of Chitin from Black Soldier Fly (Hermetia illucens) and Its Usage to Metal Sorption. *Polymers (Basel).7;13(5)*, p. 818.
6. Yong. (2022). Chitosan isolated from black soldier flies Hermetia illucens. *Structure and enzymatic hydrolysis Process Biochemistry , Volume 118*, pp. Pages 171-181.

II. Objectives

Commercially available bandages such as hydrocolloid are neither biodegradable nor anti-bacterial. In our investigation, we made anti-bacterial non-allergic bio-bandages with bio-dressings that are the only bio-degradable bandages with bio-degradable dressings available for the moment and at the same time anti-bacterial, non-allergic and haemostatic. Thus, they provide better protection to people with cuts and minor bleeding injuries than commercially available hydrocolloid bandages and wound dressings.

III. Hypothesis

Anti-bacterial black soldier fly bio-bandages and bio-dressings should be bio-degradable as it took 49 days and a month for complete bio-degradation respectively, so they should be better than commercial bandages such as Nexcare Hydrocolloid as the disposal of anti-bacterial non-allergic black soldier fly bio-bandages with bio-dressings would no longer pose burden to landfilling or threat to our environment. Anti-bacterial and non-allergic black soldier fly bio-bandages with bio-dressings are anti-bacterial with degree of deacetylation of DD% (measured using FTIR Spectrum II) 79.5% (due to the presence of chitosan) even without the application of other anti-bacterial agents and hence can provide complete protection of wounds from skin and soft tissues infections and haemostatic (due to the presence of chitosan). After testing and certification based on IS997:2004 and BS EN 13726-1, they should meet many requirements specified.

IV. Methodology

a. Demineralization in excess 2M HNO_3, deproteinization in excess 16.7M NaOH and addition of excess acetic acid to convert black soldier fly chitin to chitosan then to hydrogel.

b. Investigation of the feasibility of improving the water-proof property of black soldier fly hydrogel as bio-bandage by determination of the change in structure of black soldier fly hydrogel before and after roasting at different temperatures and different time using FTIR Spectrum II.

c. Comparison of the absorption of water and strength of black soldier fly hydrogel with commercial hydrocolloid.

d. Investigation of the anti-bacterial effect of black soldier fly hydrogel before and after roasting.
e. Investigation of the biodegradability of black soldier fly hydrogel and roasted black soldier fly hydrogel.
f. Testing and certification of the characteristics of roasted black soldier fly hydrogel as bandages based on IS997:2004 (Tension strength) and black soldier fly hydrogel as bio-dressings based on BS EN 13726-1 (Free-Swell Absorbency).
g. Investigation of the presence of allergens in black soldier fly hydrogel using 3M Clean-Trace ALLTEC60

V. Results

1.1 Degree of deacetylation measured using FTIR Spectrum II

Degree of deacetylation DD% of black soldier fly hydrogel and roasted black soldier fly hydrogel are 79.5% and 73.2% respectively (due to the presence of chitosan). They can serve as haemostatic agents.

1.2 Change in structures and properties of black soldier fly hydrogels roasted at different temperatures and different time

Structural changes took place in black soldier fly hydrogels between 100-120ºC for 15 to 30 minutes when roasting as DD% of black soldier fly hydrogel dropped sharply from 79.7% to 71.1% and 79.2% to 71.1% respectively. Probably condensation of -OH in black soldier fly hydrogel took place which was consistent with the decrease in absorption of water and increase in tensile strength when black soldier fly hydrogel were roasted at 120ºC for 30 minutes in the oven.

1.3 Absorption of water and synthetic blood by black soldier fly hydrogel

Black soldier fly hydrogel without roasting could absorb 18 times of its own mass of water which is much higher than that of commercial hydrocolloid (7 times). On the other hand, roasted hydrogels showed good synthetic blood-proof properties and did not allow synthetic blood to penetrate through.

1.4 Anti-bacterial effect of black soldier fly hydrogels and roasted black soldier fly hydrogels

Black soldier fly hydrogel and roasted black soldier fly hydrogel showed significant anti-bacterial effect. The black soldier fly hydrogel and roasted black soldier fly hydrogel samples showed NO bacterial colonies in all 1/100x, 1/1000x and 1/10000x dilution factor samples. NO oral bacterial colonies were present in drinking water with black soldier fly hydrogel, demonstrating that black soldier fly hydrogel was anti-bacterial, so black soldier fly hydrogel could serve as effective anti-bacterial wound dressings.

1.5 Biodegradability of black soldier fly hydrogels and roasted black soldier fly hydrogels

Roasted black soldier fly hydrogel bandages took 7 weeks for complete biodegradation and black soldier fly hydrogel dressings took 1 month for complete biodegradation. Obviously, anti-bacterial black soldier fly bandages with bio-dressings were biodegradable. On the other hand, the mass of commercial hydrocolloid decreased only by 48.9% in mass after 49 days. It was clear that as typical commercial bandages, commercial hydrocolloids were not biodegradable.

1.6 Certification on IS997:2004 standard

Based on IS997:2004 standard, the load per unit of area of black soldier fly bio-bandages was 371g/m^2 which met the minimum requirement of 36g/m^2, the black soldier fly bio-bandages had stronger tension strength (>20N both in dry and wet conditions) than commercial hydrocolloid. (2.7N dry 2.8N wet) which was comparable with that required (50-67N) and pH of about 4.6 which met the pH range of 4.5-8.

1.7 Certification on BS EN 13726-1 standard

The FSA Free-Swell Absorbency of synthetic blood of black soldier fly hydrogel bio-dressings was 0.81 per 5cm x 5cm dressing which was much higher than that of commercial hydrocolloid (0.299g per 5cm x 5cm dressing) based on BS EN 13726-1.

1.8 Absence of common allergens

3M Clean-Trace ALLTEC 60 showed negative results when tested with roasted and unroasted black soldier fly hydrogel samples. The black soldier fly bio-bandage and bio-dressing should be non-allergic.

VI. If your team will compete the Sustainable Development Award, please indicate the specific sustainable development goal the project is related to, and provide justification for competing for this award. *(Word limit: 300 words)*

The United Nations have announced 17 global goals for sustainable development to be achieved by 2030. The **BSF** wound dressings contribute to twelfth goal, responsible consumption and production. **HC** of **BSF** dressings took a month for complete biodegradation and roasted **HC** of **BSF** bandages took 49 days for complete biodegradation. Obviously, anti-bacterial and non-allergic **BSF** bandages with bio-dressings were biodegradable. Black soldier fly larvae have been used to degrade biodegradable matter to fertilizers. Moreover, black soldier flies take only a short time of 2 weeks to mature, which ensures steady supply of shells and thus responsible production. While black soldier fly bandages do not increase the burden of the

landfill as it is biodegradable, which leads to responsible consumption, the farming of black soldier fly to obtain shells for production of bandages does not affect the ecosystem or cause harm to other species, which also leads to responsible production. On the other hand, the mass of commercial hydrocolloids decreased only by 51.1% in 45 days. It was clear that as typical commercial bandages, commercial hydrocolloids were not biodegradable. The biodegradability of **BSF** bandages makes them environmentally friendly, which takes the responsibility of sustainable consumption and production. (UnitedNations, 2016) We are confident that black soldier fly bio-bandage with bio-dressing can be sustainably developed and meet the needs of the public, making it eligible for marketing.

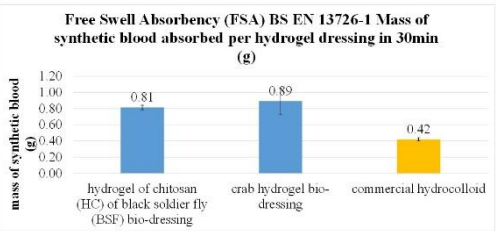
VII. If your team will compete the Social Innovation Award, please list the target group or social issue the project focuses on, and provide justification for competing for this award.

No

VIII. Conclusion

Test and Certification based on IS997:2004 (Left) Samples of bandages (Right)

Serial no. in IS997	Bandage Characteristics/ Mean (standard error)	Israeli Standard	Anti-bacterial, non--allergic hydrogel of chitosan (HC) of black soldier fly (BSF) bio-bandages	Anti-bacterial crab bio-bandages	Commercial hydrocolloid
3	Load per unit of area (gr/m²) min.	36	371 (4)	231 (6)	514 (2)
4	Tension strength in the wrap direction (Newton) min.	50-67	>20	>20	2.7 (0.3)
5	pH	4.5-8	4.6(0.4)	6.1(0.4)	6.7 (0.3)
204	Overall count of micro-organisms https://www.medicinalgenomics.com/wp-content/uploads/2013/04/CFU_Tolerance_European.pdf	European Pharmacopoeia	0,0 (w.r.t. dilution factor of 10^{-3} &10^{-4} oral bacterial; cf. control with oral bacteria: 40,6)	0,0 (w.r.t. dilution factor of 10^{-3} &10^{-4} oral bacterial; cf. control with oral bacteria: 392,62)	40,16 (w.r.t. dilution factor of 10^{-3} &10^{-4} oral bacterial; cf. control: 65,9)



Free-Swell Absorbency based on BS EN 13726-1 (Middle)

Based on the results listed above in 1.6 and 1.7 about testing and certification based on IS997:2004 and BS EN 13726-1, black soldier fly hydrogel bio-dressings performed much better as wound dressings than commercial hydrocolloid. Anti-bacterial non-allergic black soldier fly bio-bandages with black soldier fly bio-dressings are haemostatic, biodegradable and outperform commercial hydrocolloid, so they are for sure eligible for marketing.

Way forward of the research as follows:

1 Primary wound dressing test methods BS EN 13726-1

1.1 Time of blood clotting

1.2 Retention Following Compression (RFC)

1.3 Total Fluid Handling (TFH)

1.4 Moist vapour transmission rate (MVTR)

2 Prick skin tests

3 Testing the haemostatic effect by determination of clotting time of blood of rat

✔ Our project is developed based on previous project and the enhancement is below:

Crab hydrogel bio-bandages;

We obtain chitosan from black soldier fly (**BSF**) instead of crab. Black soldier fly larvae have been used to degrade biodegradable matter to fertilizers. Moreover, black soldier flies take only a short time of 2 weeks to mature, which ensures steady supply of shells and thus responsible production. While black soldier fly bandages do not increase the burden of the landfill as it is biodegradable, which leads to responsible consumption, the farming of black soldier fly to obtain shells for production of bandages does not affect the ecosystem or cause harm to other species, which also leads to responsible production. **BSF** is found to be non-allergic and more eco-friendly than crustaceans such as crab, so the marketing of **BSF** bio-bandages is more socially sustainable.