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優勝作品專輯

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參展科別 **醫學與健康科學**

作品名稱 **Anti-bacterial Crab bio-bandages with Bio-dressings 2.0**

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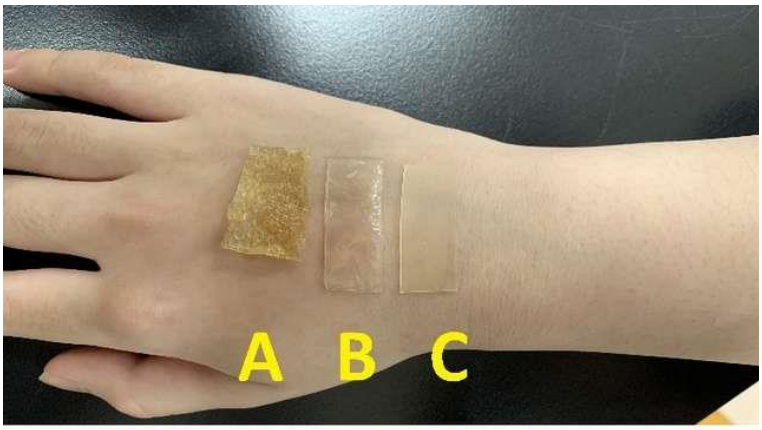
關鍵詞 **biobandage, biodressing, antibacterial**

作者照片

1.Abstract

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. Crab shells are readily available sources of chitin as they are made up of about 25% to 30% of chitin (Pandharipande, 2016), so crab hydrogels are potential alternatives of anti-bacterial bio-bandages with bio-dressings.



A: a bio-bandage (bio-degradable bandage) with a bio-dressing (bio-degradable dressing) obtained from chitin-rich substrate such as crab shells
B: a bio-dressing (bio-degradable dressing) obtained from chitin-rich substrate such as crab shells coated on a commercial waterproof adhesive
C: a commercial hydrocolloid

Fig. 1.1 Samples of bandages

The objectives of this investigation were as follows:

(a) Investigation of the feasibility of improving the water-proof property of crab hydrogels as bio-bandage by determination of the change in structure of crab hydrogel before and after roasting at different temperatures and different time using FTIR.

(b) Comparing the absorption of water and synthetic blood, and strength of crab hydrogels and commercial hydrocolloid.

(c) Investigation of the anti-bacterial effect of crab hydrogel before and after roasting.

(d) Investigation of the biodegradability of crab hydrogels and roasted crab hydrogels.

(e) Testing and certification of the characteristics of roasted crab hydrogels as bandages based on IS997:2004 and BS EN 13726-1.

Results were as follows:

1.1 Degree of deacetylation of DD% of crab hydrogels and roasted crab hydrogels were 82.6% and 72.2% respectively (due to the presence of chitosan), so they could serve as haemostatic agents.

1.2 Change in structures and properties of crab hydrogels roasted at different temperatures and different time

Structural changes took place in hairy crab hydrogels between 100-120ºC for 15 to 30 minutes when roasting as DD% of hairy crab hydrogels dropped sharply from 82.3 to 74.2 and 77.3 to 72.2 respectively. Probably condensation of -OH in crab hydrogels took place which was consistent with the decrease in absorption of water from 32 times to 24 times and increase in tensile strength (cf. 4.4 times stronger than commercial hydrocolloid) when crab hydrogels were roasted at 120º for 30 minutes in oven.

1.3 Absorption of water and synthetic blood by crab hydrogels

Crab hydrogels without roasting took only 14s to start absorbing synthetic blood which is much faster than commercial hydrocolloid (more than 60s). On the other hand, roasted hydrogels showed good synthetic blood-proof properties and did not allow synthetic blood to penetrate through. Anti-bacterial crab hydrogel bio-dressings could absorb about 2.5

times of its own mass of synthetic blood and 33 times of water which were far greater than that of commercial hydrocolloid which was only 17% of synthetic blood and 2.2 times of water.

1.4 Anti-bacterial effect of crab hydrogels and roasted crab hydrogels

Pure chitosan, crab chitosan, crab hydrogels and roasted crab hydrogels showed significant anti-bacterial effect. Among these, the crab hydrogels samples showed no bacterial colonies in all 1/1000x, 1/10000x and 1/100000x dilution factor samples. NO oral bacterial colonies were present in drinking water with crab hydrogels. FEW oral bacterial colonies were present with pure chitosan, crab chitosan and roasted crab hydrogels (cf. commercial hydrocolloid 780; control X 280 with 1/1000 dilution) demonstrating that crab hydrogels were anti-bacterial, so crab hydrogels could serve as effective anti-bacterial wound dressings.

1.5 Roasted crab hydrogel bandages took 42 days for complete bio-degradation and crab hydrogel dressings took a month for complete bio-degradation. Obviously, anti-bacterial crab bandages with bio-dressings were bio-degradable. On the other hand, the mass of commercial hydrocolloid decreased only by 32.9% in mass after 45 days. It was clear that as typical commercial bandages, commercial hydrocolloid were not bio-degradable.

1.6 Basing on IS997:2004 standard, the load per unit of area of anti-bacterial bio-bandages was 342g/m^2 which met the minimum requirement of 36g/m^2, the anti-bacterial 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 7 which met the pH range of 4.5-8.

1.7 The FSA Free-Swell Absorbency of synthetic blood of crab hydrogel bio-dressings was 1.86g 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.

Anti-bacterial crab bio-bandages with bio-dressings fulfill many criteria stated in IS997:2004 and BS EN 13726-1, so they should be eligible for marketing. They are bio-degradable, haemostatic and anti-bacterial, so disposal of them will not pose any threat to the environment and they can serve as good waterproof bio-bandages and bio-dressings.

2.Theory

Chitin from crab shells can be used to produce chitin-derived products, such as chitosan. Chitosan can be made into bioplastic and nanostructured film. Crab shells contains 25-30% chitin. 25% protein, 40-50% calcium carbonate. (Pandharipande 2016) http://ijsetr.org/wp-content/uploads/2016/05/IJSETR-VOL-5-ISSUE-5-1378-1383.pdf

2.1.1 Removal of calcium carbonate by demineralization of carbonates using 2M HNO_3

$CaCO_3(s) + 2H^+(aq) \rightarrow Ca^{2+}(aq) + CO_2(g) + H_2O(l)$

2.1.2 Deproteinization through deacetylation of chitin to chitosan by using NaOH room temperature (RT), 363 and 393 K, hydroxide concentration (2.0 or 10.0M) and time (3 and 24 h) on shrimp chitin deacetylation (Pires 2014)

http://www.sciencedirect.com/science/article/pii/S1876619614000278

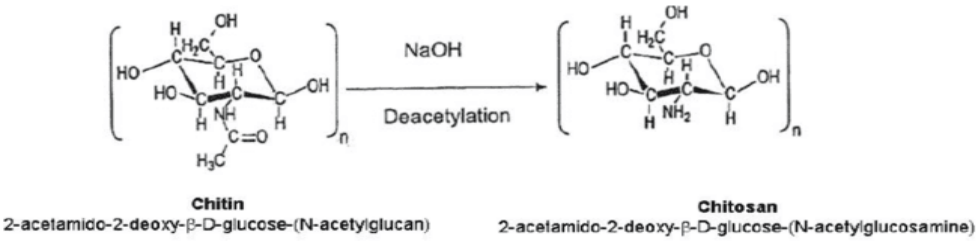


Fig 2.1 Conversion of chitin to chitosan by deacetylation

https://www.researchgate.net/figure/Conversion-of-chitin-to-chitosan-by-deacetylation_fig1_285543611

2.1.3 Water absorption of hydrogel, the salt of chitosan

Chitosan can form a gel based on the neutralization of chitosan amino groups. The formation of the hydrogel then occurs via hydrogen bonds. (Croisier, 2013)

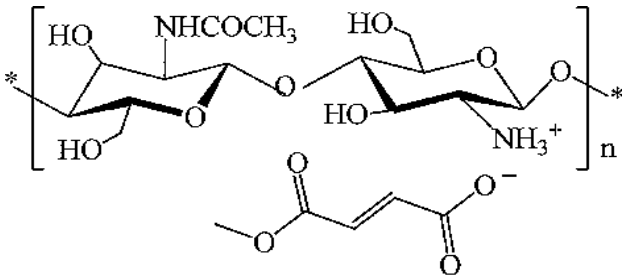


Fig 2.2 Chemical structure of MF-chitosan salt

https://www.researchgate.net/figure/Chemical-structure-of-MF-chitosan-salt_fig3_269711737

Hydrogels usually have high water absorption capacity of 100%, swell in water and retain a significant fraction of water (>20%) within their structure without dissolving. (Buchholz & Graham,1998)

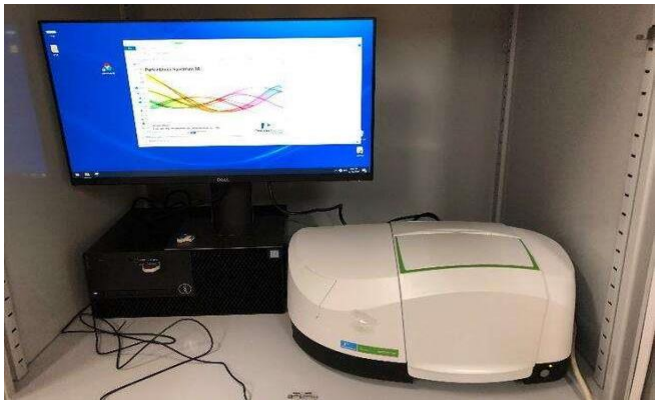


Fig 2.3 FTIR Spectrum Two

2.2 Determination of Degree of acetylation DA% in chitin and degree of deacetylation DD% in chitosan using FTIR

The following FTIR Spectrum Two has been installed in Carmel Pak U Secondary School .

Spectrum Two FT-IR spectrometers feature:

- Standard, high-performance, room-temperature $LiTaO_3$ (lithium tantalate) MIR (mid infra-red) detector with a SNR (signal to noice ratio) of 9,300:1
- Optional temperature-stabilized, high-performance DTGS (deuterated triglycine sulfate) MIR detector with a SNR of 14,500:1. Ideal for low-light, high throughput applications
- Standard optical system with KBr windows for data collection over a spectral range of 8,300 – 350 cm^{-1} at a best resolution of 0.5 cm^{-1}

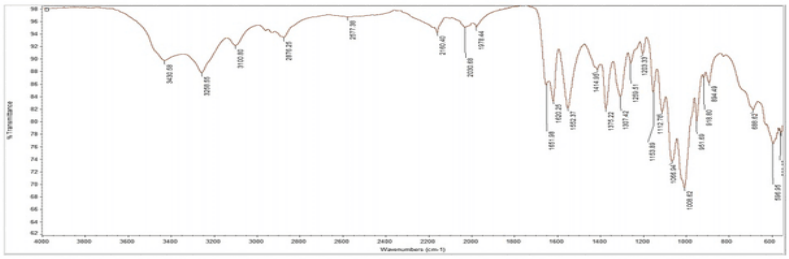


Fig. 3: FTIR spectrogram of the chitin sample 1.

G. Interpretation of FTIR spectra

The interpretation of FTIR analysis of the samples is done for the possible presence of functional groups and the details are given in table 2. The basis of interpretation is the FTIR of standard chitin is reported in literature.

Table 2: Interpretation of FTIR analysis

Sr. No	Standard chitin wavelength in cm^{-1}	Crab chitin wavelength in cm^{-1}		Group
		Sample- 1	Sample -2	
1	3448	3430	3431	OH
2	3300-3250	3258, 3100	3257, 3101	N-H stretching
3	2891	2876	2880	C-H stretching
4	1680-1660	1651, 1620	1619	C=O stretching
5	1560-1530	1552	1552	Amide II band amide II band
6	1340	1375, 1307	1374, 1307	Methyl CH stretch, Amide III
7	1152-1156	1153, 1112	1153, 1112	Glycosidic linkage, C-H stretch
8	1072	1066, 1008	1067, 1008	C-O-C
9	952	951	951	Amide III
10	750-650	688	688	N-H

http://ijsetr.org/wp-content/uploads/2016/05/IJSETR-VOL-5-ISSUE-5-1378-1383.pdf

Fig 2.4 Spectrogram of chitin and interpretation of FTIR spectra (Pandharipande, 2016)

In the FTIR spectroscopy, several equations are described in literature for calculation of absorbance of different bondings

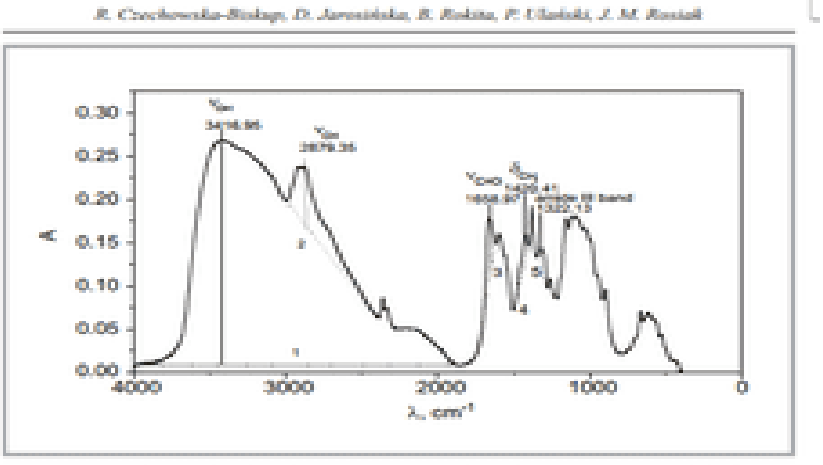


Figure 11. Reciprocal bands and corresponding baselines, based on Duarte et al. [23] (1 - 3) and Braganza et al. [22] (4 - 5) for FTIR spectrum of chitosan sample S2.

and hence the degree of deacetylation. (Biskup, 2012)

Fig 2.5 References bands and corresponding baseline

$$DA\ [\%] = \frac{A_{1655}}{A_{3450}} \times 100/1{,}33\ [17, 23] \quad (15)$$

$$DA\ [\%] = \frac{A_{1655}}{A_{2870}} \times 100/1{,}33\ [23] \quad (16)$$

$$DA\ [\%] = \frac{A_{1655}}{A_{3450}} \times 115\ [17] \quad (17)$$

$$DA\ [\%] = (\frac{A_{1320}}{A_{1420}} - 0{,}03822)/0{,}03133\ [22] \quad (18)$$

where: A_{3450}, A_{2870}, A_{1655}, A_{1420}, A_{1320}, are values of absorbance from baseline 1, 2, 3, 4, 5 to maximum, respectively. In ***Figure 11***, on the basis IR spectrum of chitosan S2, baseline settings and individual bands ascribed for characteristic groups in chitosan are presented.

Fig 2.6 (Biskup, 2012)

Samples	50	60	70	80	90	100
Mussel shell	77.21	83.31	85.75	91	93.22	96.51
Oyster shell	69.68	73.98	80	85.62	90.44	93.27
Prawns shell	40.17	45.78	48.59	51.61	54.22	60.56
Crab shell	54.1	56.49	63.7	69.4	74.16	74.57
Pang scale	50.11	52.58	56.89	62.35	65.77	69.12
Silver scale	47.59	49.16	52.18	56.12	60.1	65.85

Table 4 Degree of acetylation (DA) (%) of chitin samples from sea waste at varied temperature (°C)

https://www.semanticscholar.org/paper/Biopolymer-(Chitin)-from-Various-Marine-Seashell-Alabaraoye-Achilonu/2c78c6353508c9cb7328347e7c7abf533c3a0d02/figure/6

Fig 2.7 DA% of chitin samples from sea waste at varied temperature(Alabaraoye, 2018)

Table 1: Comparison of C1 and C2 based on flow properties.

Commercial Chitosan (C1)
$Degree\ of\ Acetylation = \frac{A(1655)}{A(2897)} \times \frac{100}{1.33}$
$Degree\ of\ deacetylation = \frac{44.65}{94.9} \times \frac{100}{1.33}$
$Degree\ of\ Acetylation = 35.37\%$
Degree of Deacetylation = 100 – Degree of Acetylation
Degree of Deacetylation = 100 – 35.37 = **64.63%**

https://www.sciforschenonline.org/journals/nanomedicine/article-data/IJNN-2-108/IJNN-2-108.pdf

Fig 2.8 DA% and DD% of commercial chitosan (Choudhary, 2016)

2.3 Haemostatic property of chitin and chitosan

2.3.1 Reduction of time of blood clotting

Both chitin and chitosan are found to reduce the blood clotting time as Chitin and chitosan enhanced the release of the platelet derived growth factor-AB (PDGF-AB) and the transforming growth factor-β1(TGF-β1) from the platelets

(Okamoto, 2003)

2.3.2 Formation of Spatial Network Structure

Chitosan (CS) is a linear glycosaminoglycan which makes it easy to construct a network structure, thus promoting the interaction of blood components with chitosan and facilitating formation of strong blood clotting. It follows a gelling hemostatic mechanism. When hydrophobes of HM-CS contacted the blood cells, they anchored into the hydrophobic interiors of blood cell membranes via hydrophobic interactions leading to a three-dimensional gel network was bridged between the chitosan chains and blood cells, which could potentially halt the flow of blood. (Hu, 2018)

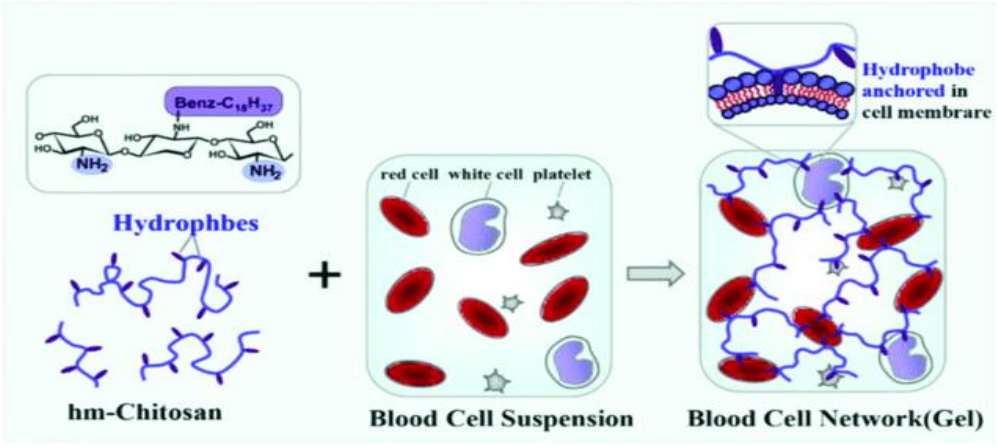


Fig 2.9 Mechanism for gelation of blood by hydrophobically modified (HM) chitosan (CH). https://www.researchgate.net/figure/Mechanism-for-gelation-of-blood-by-hydrophobically-modified-HM-chitosan-CH_fig2_326874991

2.3.3 Synthetic blood

Synthetic blood has the same surface tension as human blood and is suitable for the investigation of the time of absorption by crab hydogels and commercial hydrocolloid.

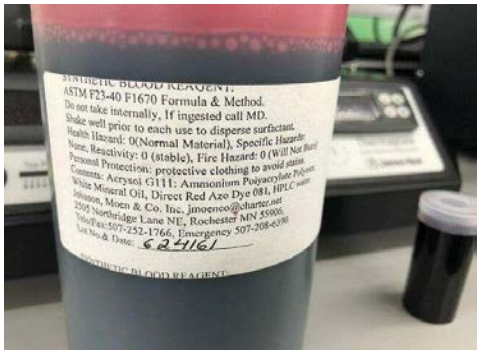


Fig2.10 Synthetic blood

2.4 Anti-bacterial property of chitosan

Chitosan is proved to be anti-bacterial. Three antibacterial mechanisms have been proposed:

i) the ionic surface interaction resulting in wall cell leakage

ii) the inhibition of the mRNA and protein synthesis via the penetration of chitosan into the nuclei of the microorganisms

iii) the formation of an external barrier, chelating metals and provoking the suppression of essential nutrients to bacterial growth

It is likely that all events occur simultaneously but to different extent. (Goy, 2009)

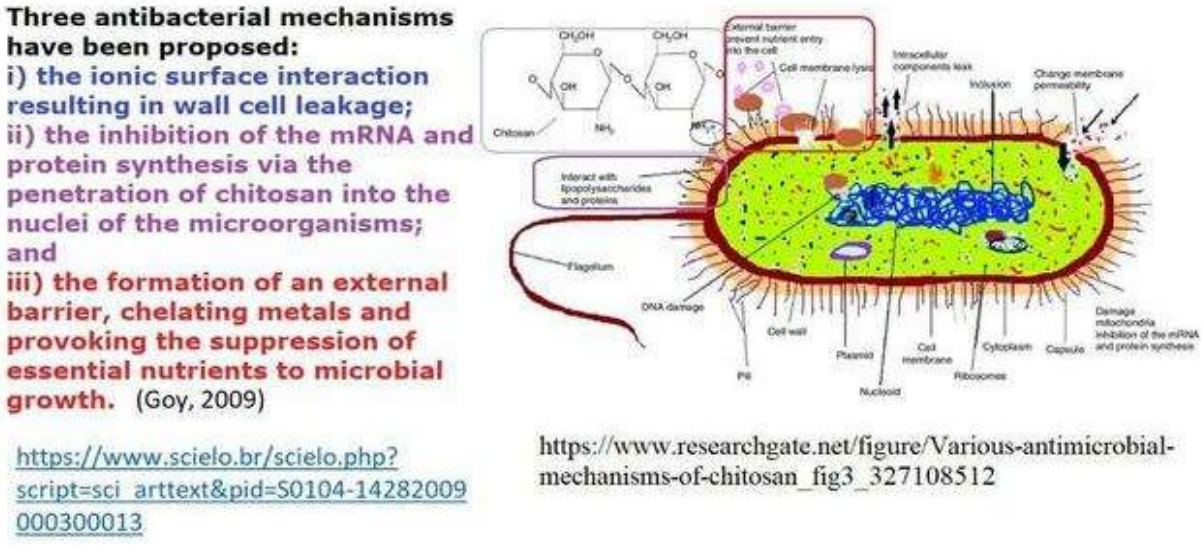


Fig 2.11 Various anti-bacterial mechanisms of chitosan

One evidence about the anit-bacterial property of chitosan is that chitosan coating on fruits and vegetables has been found to be effective for the reduction of a variety of harmful micro-organisms and extend the shelf-life of these products. (Chao, 2019)

2.5 Roasting of hydrogels

Condensation of hydroxyl groups -OH probably takes place during roasting. One such example is the formation of maltose from glucose molecules.

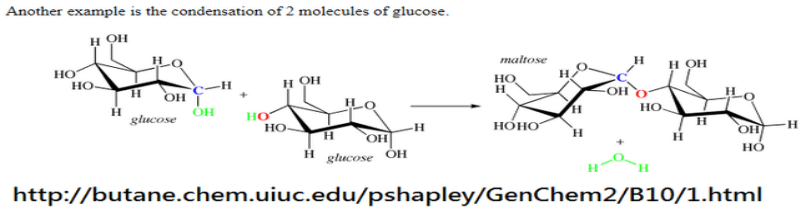


Fig 2.12 Condensation of two molecules of glucose

In the Friedel-Craft alkylation, i.e. condensation of hydroxyl groups, the yield was 21% at 110ºC. (Shinde, 2018)

2.6 Testing and certification of the characteristics of anti-bacterial roasted crab bio-hydrogels as bandages based on IS997:2004 and anti-bacterial crab bio-hydrogel wound dressings based on BS EN 13726-1

2.6.1 Testing and certification of the characteristics of anti-bacterial crab bio-hydrogels as bandages based on IS997:2004

Bandage characteristics Serial no. 3, 4, 4a, 5 and 204 of crab bio-hydrogels and commercial hydrocolloid would be examined as stated in IS997:2004.

SSI 997 (2004)

203. BANDAGE CHARACTERISTICS

In the tests described in Table 3 bandage characteristics will be as detailed in the table

Table 3

Serial No.	Bandage Characteristics	The requirement				Test After
		W&W 18	W&W 20	W&W 24	W&W 27	
3	Load per unit of area (gr/m^2) min.					
	- bandage with fringes	24	27	32	36	Para. 305
	- bandage without fringes	29	31.5	36	40	
4	Tension strength in the wrap direction (Newton) min. Of bandage type 103.1.2	50	60	60	67	IS 915 A
4a	Tension strength in wet condition in the wrap direction (Newton) min. Of bandage type 103.1.2	50	60	60	67	Para. 306
5	pH	4.5 - 8				

204. OVERALL COUNT OF MICRO-ORGANISMS

The bandage is tested as described in European Pharmacopoeia, updated edition.

The number of micro-organisms as described in European Pharmacopoeia, updated edition, for products destined for application to skin.

Fig 2.13 Bandage characteristics http://www.puntofocal.gov.ar/notific_otros_miembros/isr81_t.pdf

2.6.2 Testing and certification of the characteristics of crab hydrogel bio-dressings based on BS EN 13726-1

The majority of wound dressings are applied to remove excess wound fluid (exudate) from the immediate vicinity of the wound. This standard contains a series of test methods which assess absorbency, fluid handling capacity and dispersion characteristics:

Section 3.2 Free-Swell Absorptive Capacity

Dressings are sectioned into 5x5cm samples, weighed and then incubated in artificial exudate at 37°C. The free-swell absorbency following 30 minutes incubation is subsequently calculated. This test is only appropriate for dressings which remain physically intact and which reach their absorptive capacity within 30 minutes under the test conditions.

Free-Swell Absorbency (FSA)

Pre-weighed dressings were soaked in ionic solution at 37°C for 30 min and weighed after removing the excess. Absorbency was calculated as follows:

Absorbency (g/dressing) = wet weight — dry weight

https://www.woundsource.com/poster/assessment-dressing-fluid-handling-comparison-seven-absorptive-foam-dressings

demineralization of crab shell (removal of calcium carbonate using 2M nitric acid

Fig. 3.2 Demineralization

Deacetylation of chitin to chitosan using 16.7M NaOH

Fig. 3.3 Deacetylation

Fig. 3.4 Production of crab hydrogel

3.Methodology

Hairy crab Sapporo 大閘蟹

Commercial hydrocolloid

Table 3.1 Samples under investigation.

3.1.1 Demineralization and deproteinization of hairy crab shells

1. Demineralization was done by adding excess 2M HNO_3 to weighed crab shell samples to remove minerals such as calcium carbonate.
2. Samples were washed, dried and weighed when no bubbles evolved.
3. Deacetylation was done by adding excess 16.7M NaOH to the crab chitin samples.
4. Crab chitosan samples were washed, dried and weighed a day later.

3.1.2 Production of crab hydrogel using acetic acid (vinegar)

1. Add excess acetic acid to the crab chitosan samples till crab hydrogel was obtained. Crab hydrogel samples were washed, dried and weighed.
2. Determination of degree of deacetylation DD using FTIR
3. The absorbance of the N-H bond at about 3450cm^{-1} and the C=O bond at 1655cm^{-1} were measured using FTIR Spectrum Two.

3.2.1 The degree of acetylation DA and degree of deacetylation DD

The degree of acetylation DA and degree of deacetylation DD were calculated using the following formulae:

$DA\% = (A_{1655}/A_{3450})/1.33 \times 100$

$DD\% = 100 - DA\%$

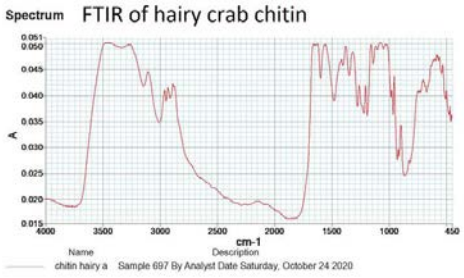


Fig 3.5 FTIR spectrum of hairy crab chitin

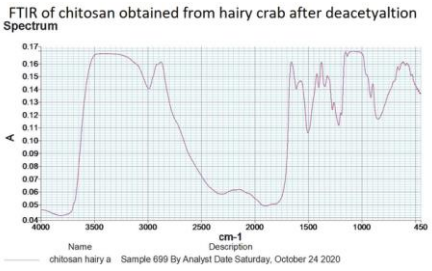


Fig 3.6 FTIR spectrum of chitosan obtained
From hairy crab after deacetylation in an oven at 120°C for 30 mins

3.2.2 Investigating the change in structure of crab hydrogel at different roasting temperature and different time using FTIR Spectrum Two

1. A crab hydrogel sample was roasted in an oven at different temperature.
2. The absorbance of the N-H bond at about 3450cm^{-1} and the C=O bond at 1655cm^{-1} were measured using FTIR Spectrum Two.
3. DD% values at different roasting temperature were compared.
4. The experiment was repeated at 120^{o}C for different time.
5. DD% values were compared.

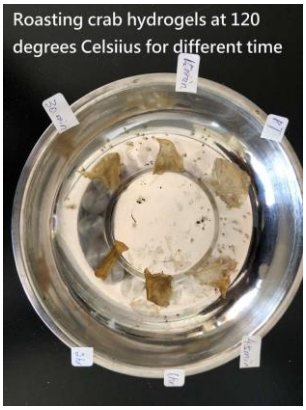


Fig. 3.7 roasted crab hydrogels

3.3.1 Determination of the absorbance of water using different crab hydrogel samples

1. Weighed dried crab hydrogel samples were soaked in water.
2. The mass of crab hydrogel with water was recorded.

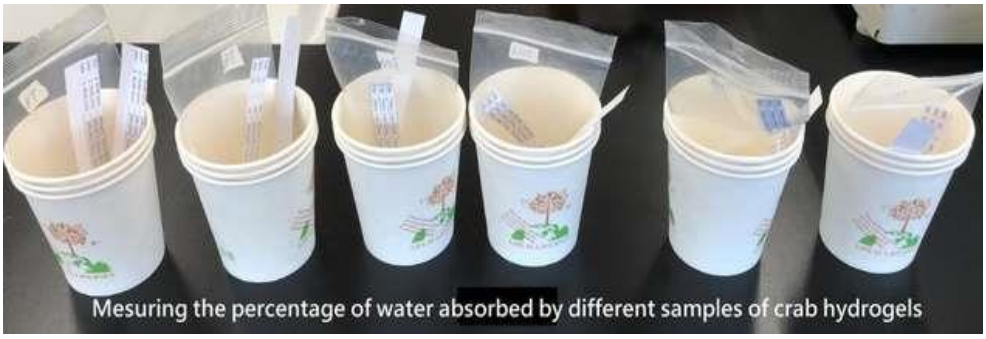


Fig. 3.8 Measurement of absorbance of water using different crab hydrogels

3.3.2 Determination of the time of absorption of synthetic blood by different crab hydrogel samples.

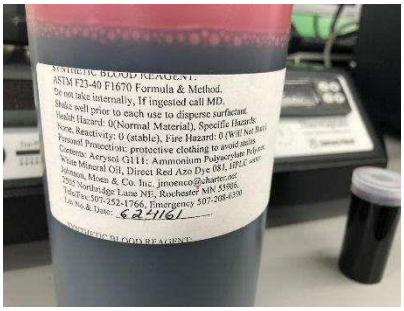


Fig 3.9 Synthetic blood, same surface tension as human blood. Fig. 3.10 Absorption of synthetic blood by crab hydrogels

Time was taken for the absorption of 10 microlitre synthetic blood by different samples.

3.3.3 Determination of the strength of different crab hydrogel samples.

1. The thickness of the sample was measured using a caliper micrometer.
2. The force to punched through the sample using a screw was measured using a Newton balance.

Fig. 3.11 Measuring tensile strength using Newton balance

3.4. Studying the anti-bacterial effect of crab hydrogel

1. About 0.1g of samples were added to 1.0cm^3 drinking water with oral bacteria.

2. After 24 hours, 200 microlitre of the sterilized water samples of different dilutions were spread over agar with culture solution.
3. After 24 hours, the number of bacterial colonies were counted.

samples used as anti-bacterial agents of oral bacterial in drinking water

Fig.3.12 Samples used as anti-bacterial agents


drinking water with oral bacteria collected using cotton swaps

Fig 3.13 Drinking water with oral bacterial

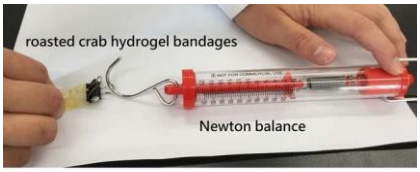
3.5. Comparing the biodegradability of different crab hydrogels.

1. Dry samples of crab hydrogels and commercial hydrocolloid were weighed.
2. Samples were left in soil and water was added to keep the soil wet.
3. Wet samples were weighed two times every week.

Fig. 3.14 Samples in soil for bio-degradation

3.6.1 Testing and certification of the characteristics of anti-bacterial crab bio-hydrogels and commercial hydrocolloid as bandages based on IS997:2004

1. Load per unit area of the samples were calculated by dividing the mass of samples by the surface area.
2. Tension strength of the samples were found using Newton balance.
3. Tension strength in wet condition (soaking the samples in distilled water for 3 hours) of the samples were found using Newton balance.
4. pH of the wet the samples were measured using pH paper.
5. Absorption ability was found by measuring the time for the samples to absorb 10 microlitre synthetic blood.
6. Overall count of micro-organism were calculated by counting the bacterial colonies developed after spreading drinking water with oral bacteria soaked with the samples for 24 hours over agar with culture solution.



Measuring the tension strength of roasted crab hydrogel bandages using a Newton Balance

Fig 3.15 Measuring tension strength using Newton balance

3.6.2 Testing and the certification of the characteristics of crab hydrogel bio-dressings based on based on BS EN 13726-1: Free-Swell Absorbency FSA

1. Mass of samples of dry dressings of 5cmx5cm were weighed.
2. Samples of dressings were allowed to absorb excess synthetic blood for 30 minutes.
3. Mass of the wet samples were weighed.


Measuring FSA Free-swell absorbency of crab hydrogel bio-dressings using synthetic blood

Fig. 3.16 Measuring FSA of crab hydrogel bio-dressings using synthetic blood

FSA = weight of wet samples- weight of the dry samples (g per 5cm x 5cm dressing)

4.Result

4.1.1Percentage of chitin chitosan in hairy crab shells

	chitin	chitosan (after deacetylation)
Percentage % (standard error)	32.0	18.5

Table 4.1 Percentage of chitin chitosan in hairy crab shells

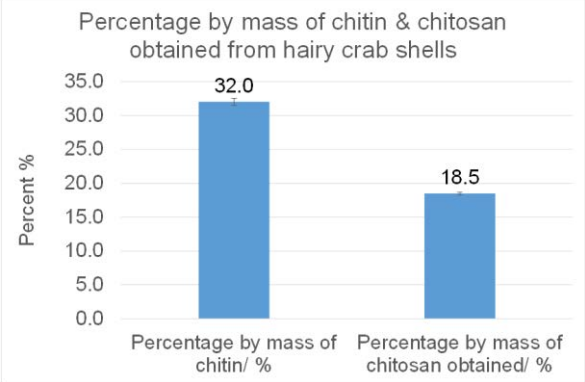
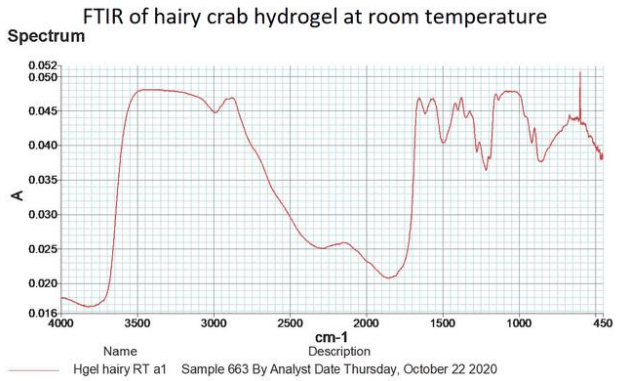


Fig. 4.2 Graph of percentage of chitin chitosan in hairy crab shells

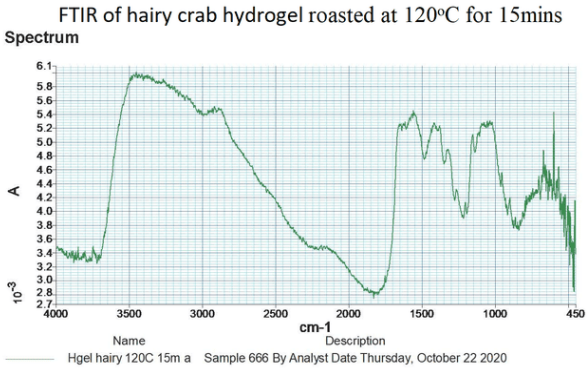
Conclusion: The percentage of chitin in hairy crab shells was 32.1 which was consistent with literature of about 25% to 30%. (Pandharipande, 2016) and the percentage of chitosan obtained was 18.5%.

4.1.2 Investigation of structural changes of hairy crab hydrogels before and after roasting at different temperature and for different time using FTIR.

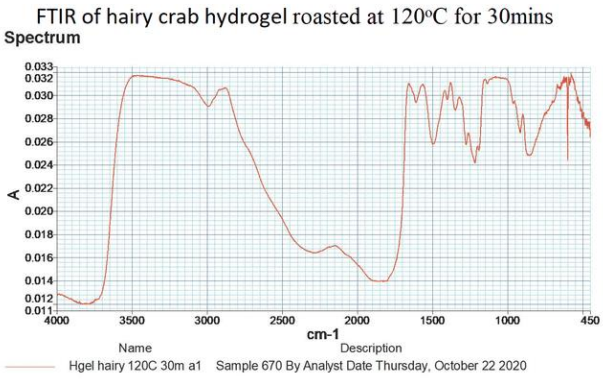
4.1.2.1 Degree of deacetylation DD% of hairy crab hydrogels roasted for different time at 120°C.



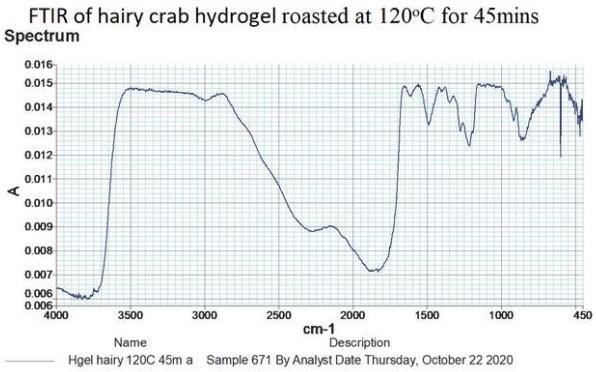
at room temperature (not roasted)



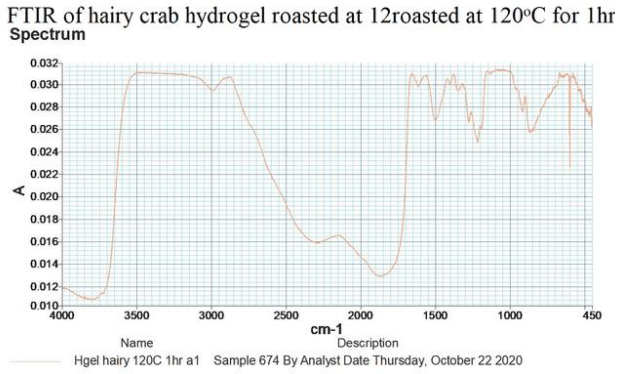
roasted at 120°C for 15mins



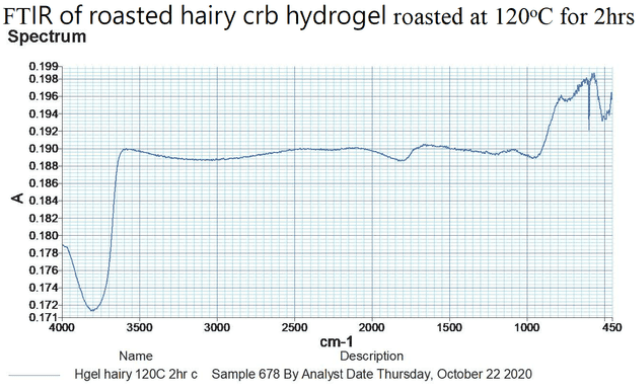
roasted at 120°C for 30mins



roasted at 120°C for 45mins



roasted at 120°C for 1hr



roasted at 120°C for 2hrs

Fig. 4.3 FTIR graphs of hairy crab hydrogels roasted for different time at 120°C

time of roasting/ min	Degree of deacetylation DD% of hairy crab hydrogels roasted at 120 degrees Celsius for different time
0	80.0
15	82.3
30	74.2
45	72.7
60	69.6
120	71.4

Table 4.4 Degree of deacetylation DD% of hairy crab hydrogels roasted at 120 degrees Celsius for different time

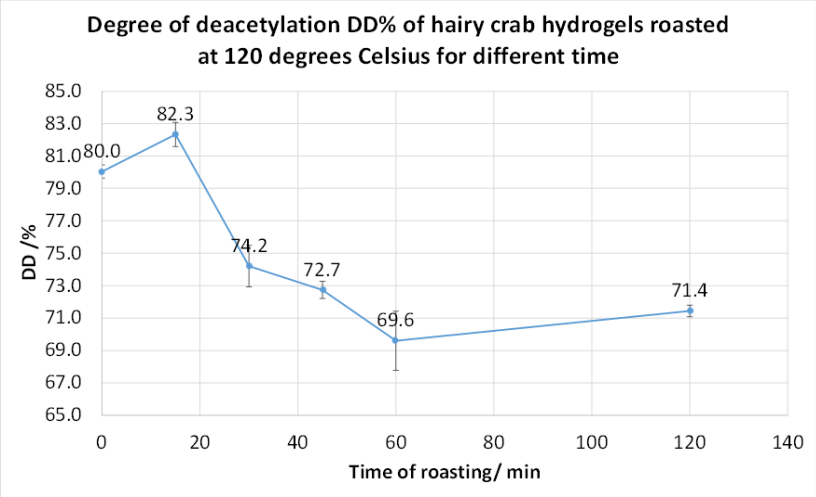
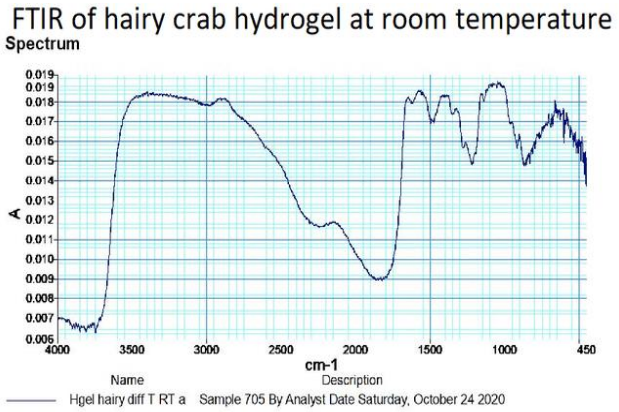


Fig 4.5 Degree of deacetylation DD% of hairy crab hydrogels roasted at 120 degree Celsius at different time

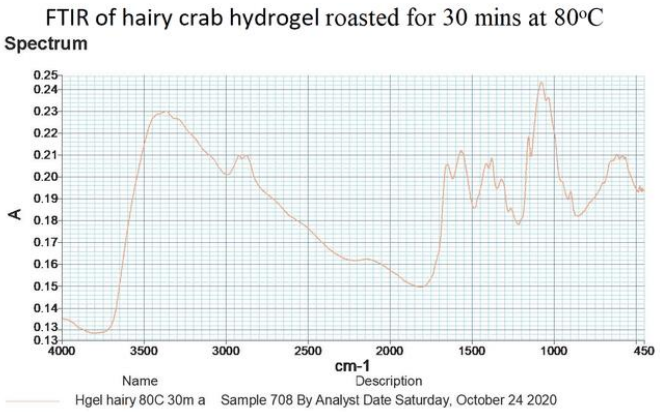
Conclusion: Structural changes took place when hairy crab hydrogels were roasted between 15 to 30 minutes at 120°C as DD% dropped sharply from 82.3 to 74.2.

4.1.2.2 Degree of deacetylation DD% of hairy crab hydrogels roasted at different temperatures for 30 minutes.

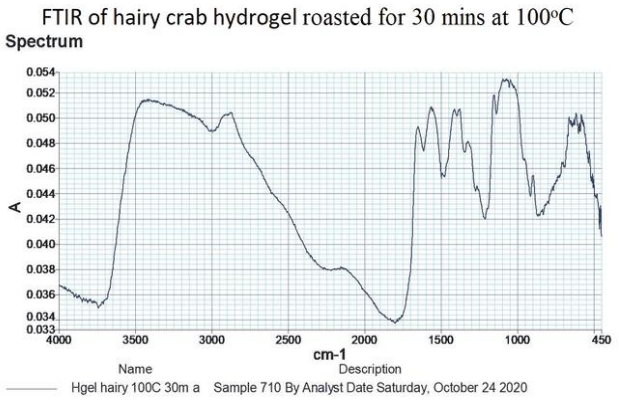
FTIR graphs



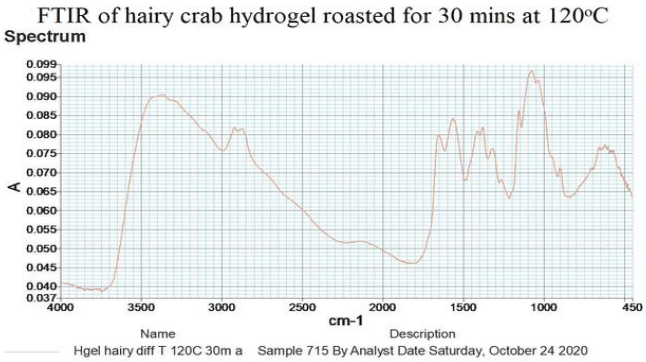
at room temperature (not roasted)



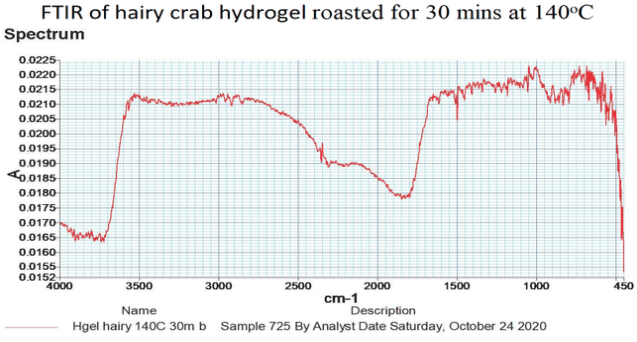
roasted for 30 mins at 80°C



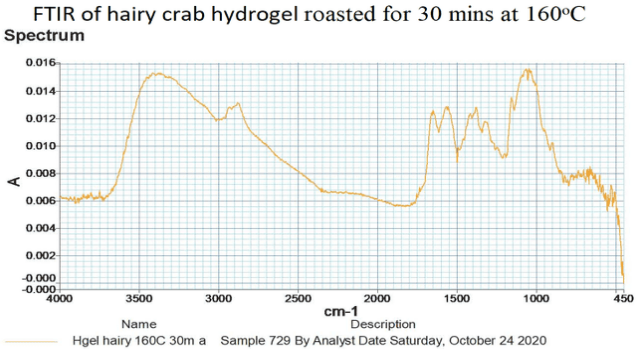
roasted for 30 mins at 100°C



roasted for 30 mins at 120°C



roasted for 30 mins at 140°C



roasted for 30 mins at 160°C

Fig. 4.6 FTIR graphs of degree of deacetylation DD% of hairy crab hydrogels roasted at different temperature for 30 minutes

temperature of roasting for 30 min/ ºC	Degree of deacetylation DD% of hairy crab hydrogels roasted for 30 mins at different temperature
25	**82.6**
80	79.8
100	77.3
120	**72.2**
140	71.8
160	70.7

Table 4.7 Degree of deacetylation DD% of hairy crab hydrogels roasted for 30 mins at different temperature

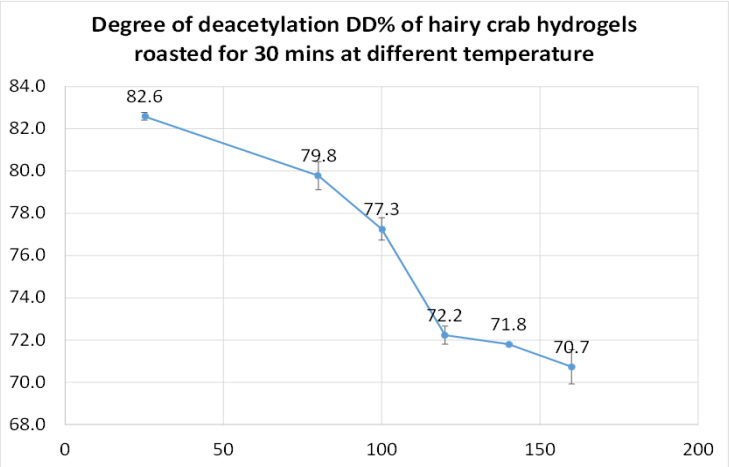


Fig 4.8 Degree of deacetylation DD% of hairy crab hydrogels roasted for 30 mins at different temperatures

Conclusion: Structural changes took place when the roasting temperature was between 100ºC to 120 ºC for 30 minutes as DD% dropped sharply 77.3 to 72.2. To conclude, structural changes took place in hairy crab hydrogels between 100ºC -120ºC for 15 to 30 minutes. Probably condensation of -OH in crab hydrogels took place.

4.2 Comparing the absorption of water and synthetic blood, and strength of crab hydrogels and commercial hydrocolloid.

4.2.1 Investigation of the water absorbance by different crab hydrogels and commercial hydrocolloid

4.2.1.1 Measuring the percentage by mass of water absorbed of crab hydrogels roasted at 120ºC for different time

time of roasting/ min	Percentage by mass of water absorbed by roasted hydrogels at 120ºC at different time %
0	3288
15	3195
30	2339
45	2152
60	2008
120	1206
commercial hydrocolloid	220

Table 4.9 Percentage by mass of water absorbed by roasted hydrogels at 120ºC at different time

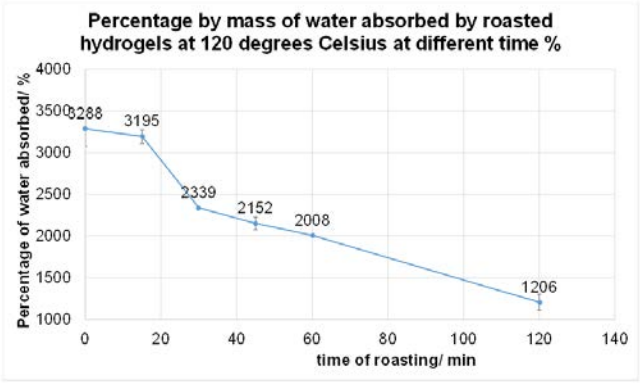


Fig 4.10 Percentage by mass of water absorbed by roasted hydrogels at 120 degree Celsius at different time

Conclusion: Percentage of water absorbed dropped significantly from 3 times to 24 times when the time of roasting was between 15 to 30 minutes. Obviously, structural changes in crab hydrogels took place.

Crab hydrogels without roasting absorbs 33 times which was much higher than that of commercial hydrocolloid (2.2 times). Crab hydrogels could serve as bio-dressings much better than commercial hydrocolloid in terms of absorbency of water.

4.2.1.2 Measuring the percentage by mass of water absorbed of crab hydrogels roasted for 30 minutes at different temperature

temperature of roasting/ ºC	Percentage by mass of water absorbed by roasted hydrogels for 30mins at different temperature %
25	2771
80	2465
100	2317
120	1649
140	1623
160	1463

Table 4.11 Percentage by mass of water absorbed by roasted hydrogels for 30mins at different temperature

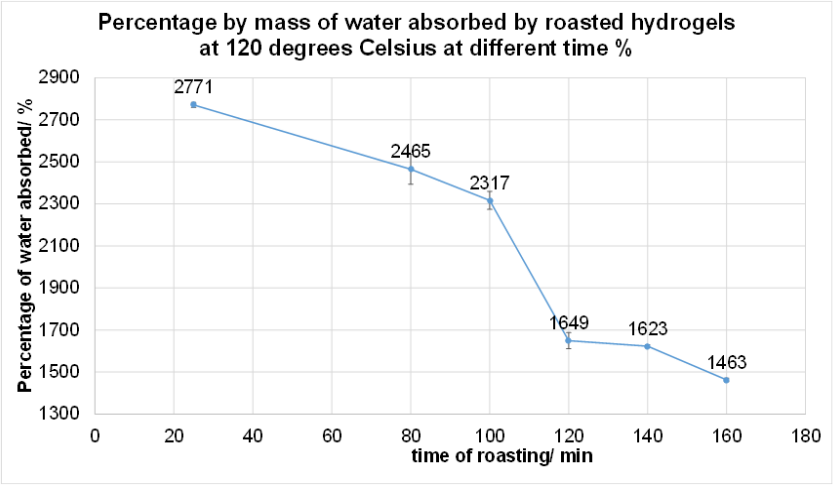


Fig 4.12 Percentage by mass of water absorbed by roasted hydrogels at different temperature

Conclusion: Percentage of water absorbed dropped significantly from 23 times to 16 times when the temperature of roasting was between 100ºC to 120ºC. Obviously, structural changes in crab hydrogels took place.

Thus structural changes in crab hydrogels took place when crab hydrogels were roasted between 100ºC - 120ºC for 15 to 30 minute. Probably condensation of -OH groups took place. The water-proof property of roasted crab hydrogels was much better than crab hydrogels, making roasted crab hydrogels excellent outer layer of wound dressings.

4.2.2 Investigation of the absorption of synthetic blood by different crab hydrogels and commercial hydrocolloid

4.2.2.1 Measuring the time of absorption of synthetic blood by different crab hydrogels roasted for different time, and commercial hydrocolloid

Roasting time/ (min)	Time of starting absorption of synthetic blood by dry sample (s) [60s means more than 60s]
0 (room temp)	14
15	60
30	55
45	60
60	45
120	60
commercial hydrocolloid	60

Table 4.13 Time of starting absorption of synthetic blood by dry sample

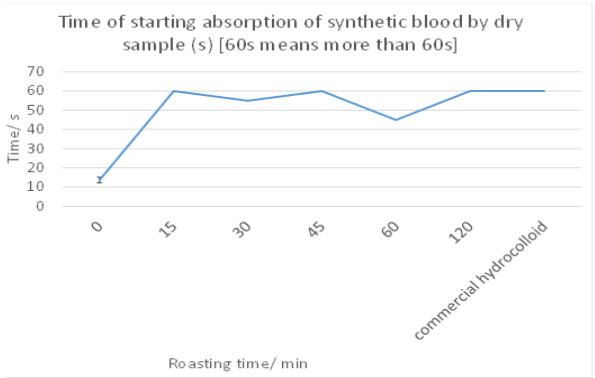


Fig 4.14 Time of starting absorption of synthetic blood by dry sample

Conclusion: Crab hydrogels without roasting took only 14s to start absorbing synthetic blood which is much faster than commercial hydrocolloid (more than 60s). On the other hand, roasted hydrogels showed good synthetic blood-proof properties and did not allow synthetic blood to penetrate through.

4.2.2.2 Measuring the time of absorption of synthetic blood by different crab hydrogels roasted at different temperature and commercial hydrocolloid

Roasting temperature/ degrees Celsius	Time of starting absorption of synthetic blood by dry sample (s) [60s means more than 60s]
room temp	13. 7 (standard error 1.45)
80	60
100	60
120	60
140	60
160	60
commercial hydrocolloid	60

Table 4.15 Time of starting absorption of synthetic blood by dry sample

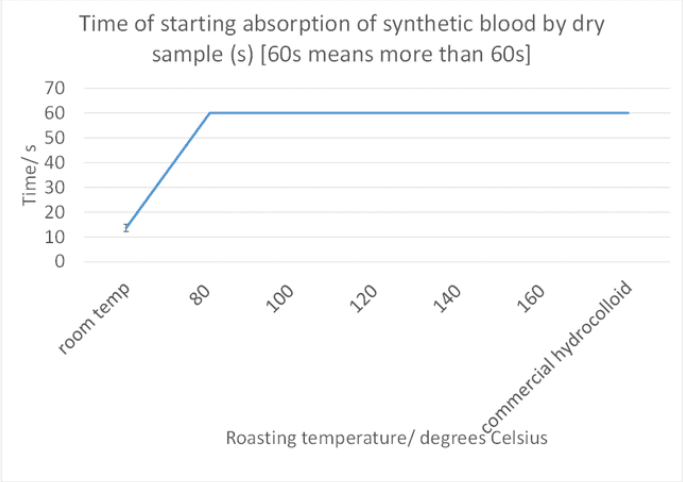


Fig 4.16 Time of starting absorption of synthetic blood by dry sample

Conclusion: Crab hydrogels without roasting took only 14s to start absorbing synthetic blood which much faster than commercial hydrocolloid (more than 60s). On the other hand, roasted hydrogels showed good synthetic blood-proof properties and did not allow synthetic blood to pass through.

Thus crab hydrogels were good wound dressings as they absorbed synthetic blood fast. (synthetic blood has the same surface tension as human blood). Roasted crab hydrogels showed good synthetic blood-proofing properties making roasted crab hydrogels excellent outer layer of wound dressings.

4.2.2.3 Measuring the percentage of synthetic blood absorbed by crab hydrogel bio-dressings and commercial hydrocolloid

	Percentage by mass of synthetic blood absorbed per dressing in 30min %
hairy crab hydrogel dressing	243.0
commercial hydrocolloid	17.0

Table 4.17 Table of percentage of synthetic blood absorbed by crab hydrogel bio-dressings and commercial hydrocolloid

Conclusion: Anti-bacterial crab hydrogel bio-dressings could absorb about 2.5 times of its own mass of synthetic blood which was far greater than that of commercial hydrocolloid which was only 17%.

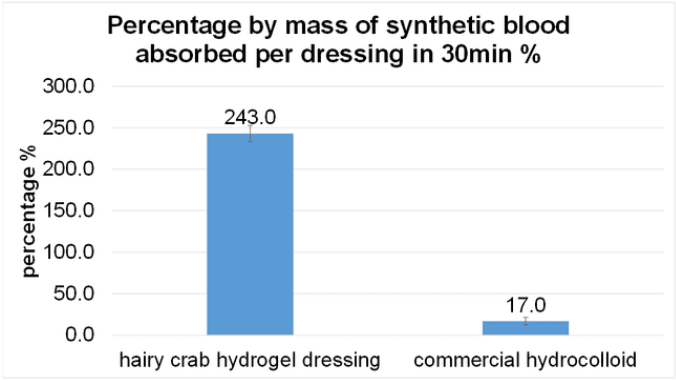


Fig. 4.18 Graph of percentage of synthetic blood absorbed by crab hydrogel bio-dressings and commercial hydrocolloid

4.2.3 Investigation of the strength of crab hydrogels before and after roasting at different time and temperatures.

4.2.3.1 Measuring the average force to punch through crab hydrogels roasted at 120°C for different time

time of roasting/ min	Average force to punch through crab hydrogels roasted at 120°C for different time/ N
0	5.7
15	6.4
30	40.4
45	13.2
60	9.7
120	9.4
Commercial hydrocolloid	9.2

Table 4.19 Average force to punch through crab hydrogels roasted at 120°C for different time

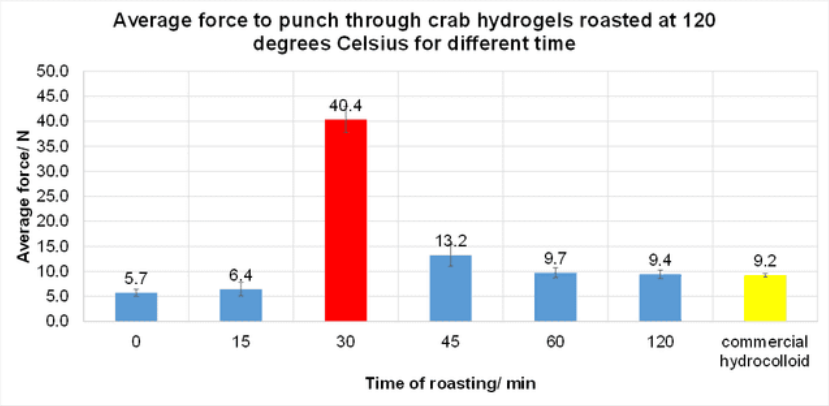


Fig 4.20 Average force to punch through crab hydrogels roasted at 120°C for different time

Conclusion: Crab hydrogels roasted for 30 minutes at 120°C was found to be the strongest. (cf. 4.4 times stronger than Commercial hydrocolloid). Obviously, structural changes took place when crab hydrogels were roasted at 120°C for 15 to 30 minutes.

4.2.3.2 Measuring the average force to punch through crab hydrogels roasted for 30 minutes at different temperature

temperature of roasting/ °C	Average force to punch through crab hydrogels roasted at 120 degrees Celsius for different time/ N
25	5.2
80	4.1
100	5.8
120	30.4
140	6.4
160	6.1
Commercial	9.2

Table 4.21 Average force to punch through crab hydrogels roasted at 120 degrees Celsius for different time

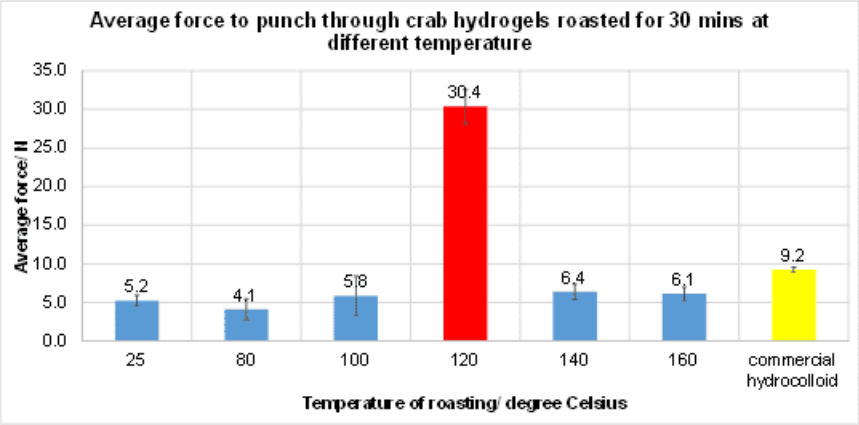
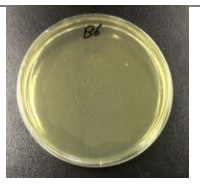
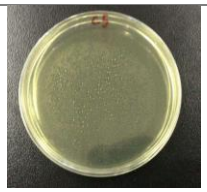
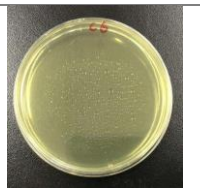


Fig 4.22 Average force to punch through crab hydrogels roasted at 120 degrees Celsius for different time

Conclusion: Crab hydrogels roasted at 120°C for 30 minutes were found to be the strongest (cf. 3.3 times stronger than Commercial hydrocolloid). Obviously, structural changes took place when crab hydrogels were roasted between 100°C - 120°C for 30 minutes.

Thus structural changes in crab hydrogels took place when crab hydrogels were roasted between 100°C - 120°C for 15 to 30 minute. Probably condensation of -OH groups took place. Tensile strength of roasted crab hydrogels was improved making roasted crab hydrogels good bandages.

4.3 Investigation of the anti-bacterial effect of crab hydrogel before and after roasting by counting bacterial colonies of oral bacteria in drinking water

Count of bacterial colonies	mass of sample	A4	A5	A6
		Dilution factor: 1/1000 x	Dilution factor: 1/10000 x	Dilution factor: 1/100000 x
pure chitosan	0.1	 44	 12	 0
crab chitosan	0.1	 failed	 29	 3
crab hydrogel	0.1	 **0**	 **0**	 **0**

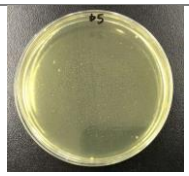
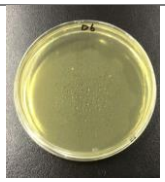
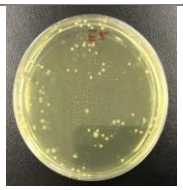
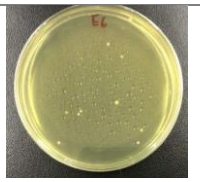
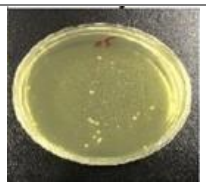
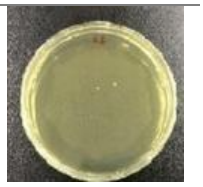
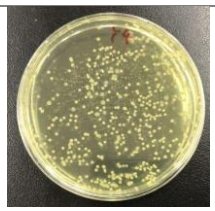
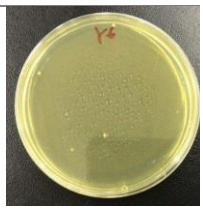
roasted crab hydrogel	0.1	 **0**	 **3**	 **1**
Commercial hydrocolloid	0.1	 **780**	 **90**	 **11**
oral bacteria only (control X)	not applicable	 280	 20	 2
oral bacteria only (control Y)	not applicable	 428	 45	 5

Table 4.23 Count of bacterial colonies of different samples in drinking water with different dilution factor

Conclusion: NO oral bacterial colonies were present in drinking water with crab hydrogels. FEW oral bacterial colonies were present with pure chitosan, crab chitosan and roasted crab hydrogels (cf. Commercial hydrocolloid 780; control X 280 with 1/1000 dilution) demonstrating that crab hydrogels were anti-bacterial, so crab hydrogels could serve as effective anti-bacterial wound dressings.

4.4 Investigation of the biodegradability of crab hydrogels and roasted crab hydrogels.

Percent %	Day 1	Day10	Day14	Day17	Day21	Day24	Day28	Day31	Day35	Day38	Day42	Day45 (dry wt.)
Roasted crab hydrogel dressings	0	NA	NA	-6.64	-8.17	-20.70	-28.76	-30.28	-58.28	-90.74	-100	-100
Crab hydrogel bandages	0	-6.0	-11.5	-41.4	-66.6	-77.1	-91.5	-100	-100	-100	-100	-100
Commercial hydrocolloid	0	-62.5	-68.0	-70.2	-68.6	-69.2	-68.0	-68.6	-68.9	-70.8	-71.8	-32.9

NA not applicable

Table 4.24 Percentage decrease in mass of crab hydrogel dressings, roasted crab hydrogel bandages & Commercial hydrocolloid

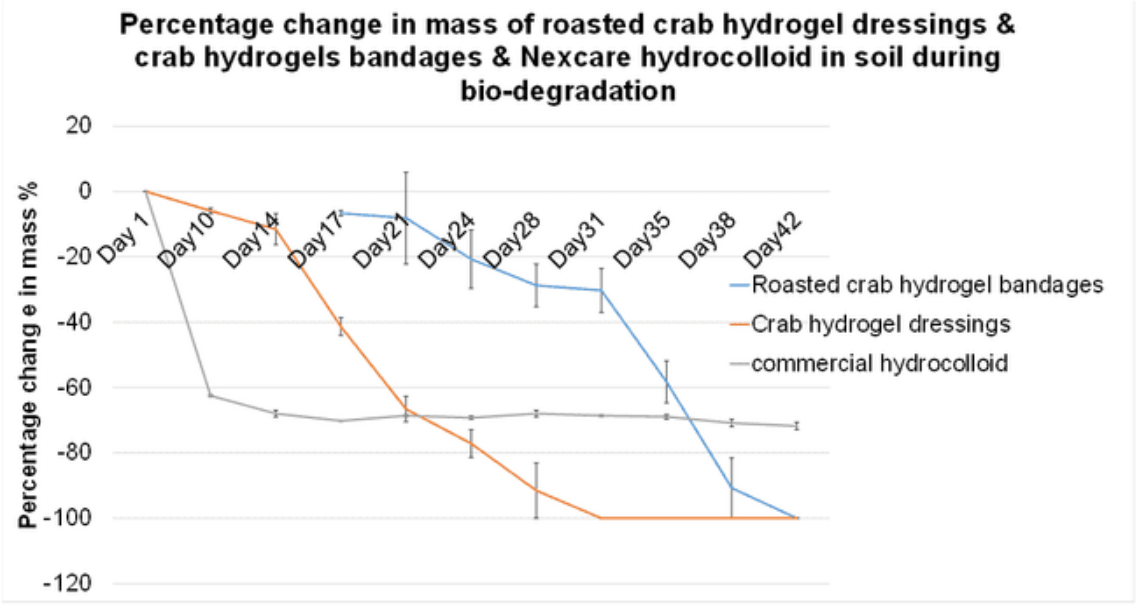


Fig. 4.25 Percentage decrease in mass of crab hydrogel dressings, roasted crab hydrogel bandages & Commercial hydrocolloid

	Day31 (wet wt.)	Day42 (wet wt.)	Day45 (dried wt.)
crab hydrogel dressings	0	0	0
roasted hydrogel bandages	69.7	0	0
Commercial hydrocolloid	31.4	28.2	67.1

Table 4.26 Percentage of mass of samples remained in soil on Day 31, Day 42 and Day 45

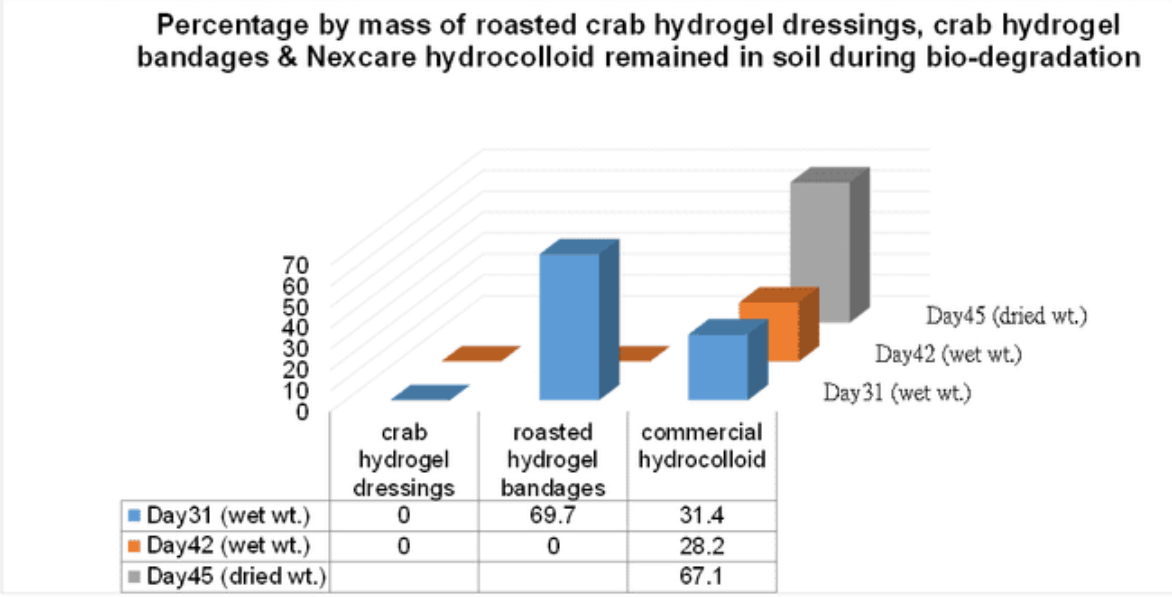


Fig. 4.27 Percentage of mass of samples remained in soil on Day 31, Day 42 and Day 45

Conclusion: Crab hydrogel dressings took a month for complete bio-degradation and roasted crab hydrogel bandages took 42 days for complete bio-degradation. Obviously, anti-bacterial crab bandages were bio-degradable. On the other hand, the mass of commercial hydrocolloid decreased by 32.9% in mass after 45 days. It was clear that as typical commercial bandages, commercial hydrocolloid was not bio-degradable.

4.5 Testing and certification of the characteristics of anti-bacterial crab bio-bandages and commercial hydrocolloid as bandages and wound dressings based on IS997:2004 and BS EN 13726-1

4.5.1 Testing and certification of the characteristics of anti-bacterial crab bio-bandages and commercial hydrocolloid as bandages based on IS997:2004

Serial no. in IS997:2004	**Bandage Characteristics/ Mean (standard error)**	**Israeli Standard**	**Anti-bacterial crab bio-bandages**	**Commercial hydrocolloid**
3	Load per unit of area (gr/m^2) min.	36	342 (18)	514 (2)
4	Tension strength in the wrap direction (Newton) min.	50-67	>20	2.7 (0.3)
4a	Tension strength in wet condition in the wrap direction (Newton) min.	50-67	>20	2.8 (0.0)
5	pH	4.5-8	7 (0.0)	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 (dilution of 1/1000 oral bacterial with crab hydrogels as bio-dressings)	780 (dilution of 1/1000 oral bacteria)

Table 4.28 Comparison of bandage characteristics between Israeli Standard, Anti-bacterial crab bio-bandages and commercial hydrocolloid

Conclusion: The load per unit of area of both anti-bacterial bio-bandages was $342g/m^2$ which met the minimum requirement of $36g/m^2$ based on IS997:2004 standard.

Anti-bacterial crab bio-bandages had stronger tension strength (>20N both in dry and wet conditions) than commercial hydrocolloid. (2.7N dry 2.8N wet) The tension strength of anti-bacterial crab bio-bandages were comparable with that required by IS997:2004 (50-67N).

The pH of anti-bacterial crab bio-bandages were found to be about 7 which met the pH range of 4.5-8 based on IS997:2004.

No oral bacterial colonies were found with anti-bacterial crab hydrogel bio-dressings.

4.5.2 Testing and certification of the characteristics of anti-bacterial crab bio-hydrogel dressings and commercial hydrocolloid as bandages based on BS EN 13726-1.

Mean (standard error)	**Free-Swell Absorbency FSA Mass of synthetic blood absorbed per crab hydrogel dressing in 30min (g)**
hairy crab hydrogel dressing	1.86 (0.040)
commercial hydrocolloid	0.30 (0.057)

Table 4.29 FSA of crab hydrogel dressing & commercial hydrocolloid

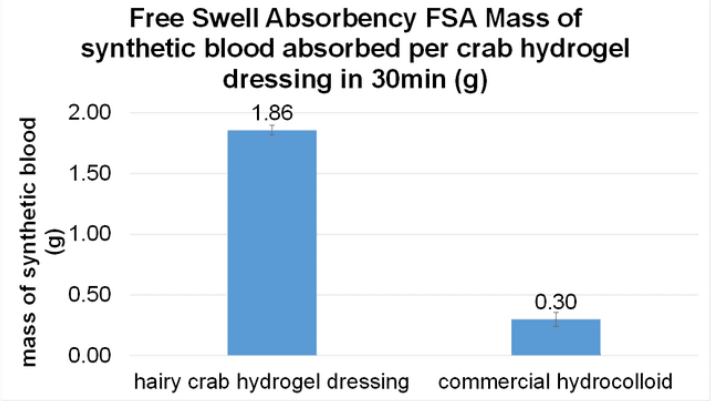


Fig. 4.30 FSA of crab hydrogel dressing & commercial hydrocolloid

Conclusion: The FSA Free-Swell Absorbency of synthetic blood of crab hydrogel bio-dressings was 1.86g 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, so crab hydrogel bio-dressings performed much better as wound dressings than commercial hydrocolloid .

5. Findings

5.1 Change in structures and properties of crab hydrogels roasted at different temperatures and different time

Structural changes took place in hairy crab hydrogels between 100ºC -120ºC for 15 to 30 minutes when roasting as DD% of hairy crab hydrogels dropped sharply from 82.3 to 74.2 and 77.3 to 72.2 respectively. Probably condensation of -OH in crab hydrogels took place which was consistent with the decrease in absorption of water and increase in tensile strength when crab hydrogels were roasted at 120º for 30 minutes in oven.

Percentage of water absorbed dropped significantly from 32 times to 24 times when the time of roasting was between 15 to 30 minutes and dropped significantly from 23 times to 16 times when the temperature of roasting was between 100ºC to 120ºC.

Crab hydrogels roasted for 30 minutes at 120ºC was found to be the strongest. (cf. 4.4 times stronger than commercial hydrocolloid). Crab hydrogels roasted at 120ºC for 30 minutes were found to be the strongest (cf. 3.3 times stronger than commercial hydrocolloid)

5.2 Absorption of water and synthetic blood by crab hydrogels

Crab hydrogels without roasting took only 14s to start absorbing synthetic blood which is much faster than commercial hydrocolloid (more than 60s). On the other hand, roasted hydrogels showed good synthetic blood-proof properties and did not allow synthetic blood to penetrate through. Anti-bacterial crab hydrogel bio-dressings could absorb about 2.5 times of its own mass of synthetic blood and 33 times of water which were far greater than that of commercial hydrocolloid which was only 17% of synthetic blood and 2.2 times of water. Thus, crab hydrogels were good wound dressings as they absorbed water and synthetic blood fast. Roasted crab hydrogels showed good water-proofing and synthetic blood-proofing properties making roasted crab hydrogels excellent outer layers of wound dressings. Also, tensile strength of roasted crab hydrogels was improved making roasted crab hydrogels good bandages.

5.3 Anti-bacterial effect of crab hydrogels and roasted crab hydrogels

Pure chitosan, crab chitosan, crab hydrogels and roasted crab hydrogels showed significant anti-bacterial effect. Among these, the crab hydrogels samples showed no bacterial colonies in all 1/1000x, 1/10000x and 1/100000x dilution factor samples. **NO oral bacterial colonies** were present in drinking water with **crab hydrogels. FEW oral bacterial colonies** were present with **pure chitosan, crab chitosan and roasted crab hydrogels** (cf. **commercial hydrocolloid 780; control X 280 with 1/1000 dilution**) demonstrating that crab hydrogels were anti-bacterial, so crab hydrogels could serve as effective anti-bacterial wound dressings.

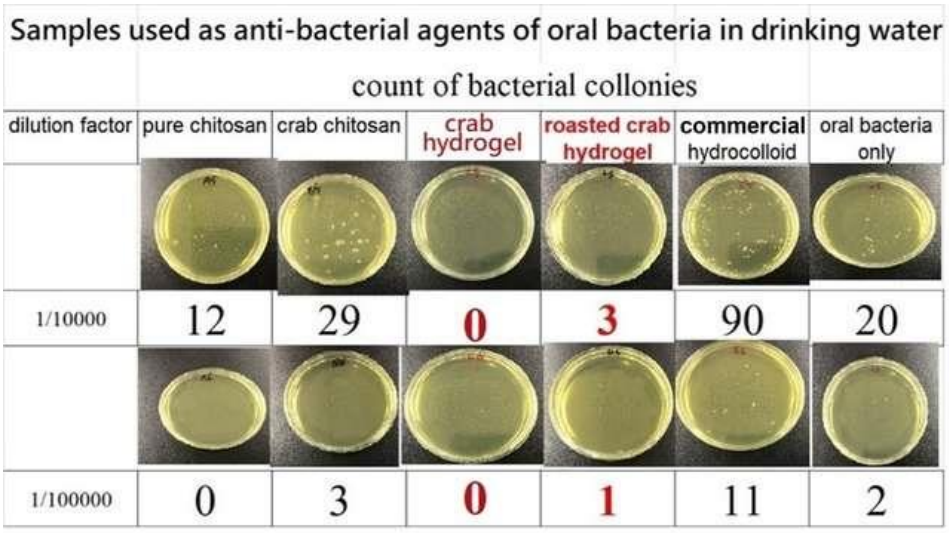


Fig. 5.1 Oral bacterial colonies formed in drinking water with different anti-bacterial agents

5.4 Biodegradability

Crab hydrogel dressings took a month for complete bio-degradation and roasted crab hydrogel bandages took 42 days for complete bio-degradation. Obviously, anti-bacterial crab bandages with bio-dressings were bio-degradable. On the other hand, the mass of commercial hydrocolloid decreased only by 32.9% in mass after 45 days. It was clear that as typical commercial bandages, commercial hydrocolloid were not bio-degradable.


Roasted crab hydrogel (left), Nexcare hydrocolloid (middle) and crab hydrogels (right) on Day 16

Fig 5.2 Samples in soil for bio-degradation

5.5.1 Testing and certification based on IS997:2004

Serial no. in IS997:2004	Bandage Characteristics/ Mean (standard error)	Israeli Standard	Anti-bacterial crab bio-bandages	Commercial hydrocolloid
3	Load per unit of area (gr/m^2) min.	36	342 (18)	514 (2)
4	Tension strength in the wrap direction (Newton) min.	50-67	>20	2.7 (0.3)
4a	Tension strength in wet condition in the wrap direction (Newton) min.	50-67	>20	2.8 (0.0)
5	pH	4.5-8	7 (0.0)	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 (dilution of 1/1000 oral bacterial with crab hydrogels as bio-dressings)	780 (dilution of 1/1000 oral bacteria)

Table 5.3 Testing and certification of the characteristis of crab bio-bandages and commercial hydrocolloid based on IS997:2004

5.6.1 Testing and certification based on BS EN 13726-1

The FSA Free-Swell Absorbency of synthetic blood of crab hydrogel bio-dressings was 1.86g 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. Anti-bacterial crab bio-bandages with bio-dressings fulfilled many criteria stated in IS997:2004 and BS EN 13726-1, so they are eligible for marketing.

6. Discussion

6.1 No bio-bandages commercially available on market

Anti-bacterial crab bio-bandages and crab bio-dressings took a 42 days and a month for complete bio-degradation respectively. The disposal of anti-bacterial crab bio-bandages with bio-dressings would no longer pose burden to landfilling or threat to our environment.

6.2 No wound dressings that are anti-bacterial (without the application of anti-bacterial agents) commercially available on market

Recent advances on anti-bacterial wound dressing were only about applying anti-bacterial agents including chitosan. (Simoes, 2018) Anti-bacterial crab bio-bandages with bio-dressings are anti-bacterial with degree of deacetylation of DD% 82.6% even without the application of other anti-bacterial agents and hence can provide complete protection of wounds from skin and soft tissues infections.

6.3 Testing and certification of anti-bacterial crab bio-bandages with bio-dressings based on IS997:2004

Serial no. in IS997:2004	Bandage Characteristics/ Mean (standard error)	Israeli Standard	Anti-bacterial crab bio-bandages	Commercial hydrocolloid
3	Load per unit of area (gr/m^2) min.	36	342 (18)	514 (2)
4	Tension strength in the wrap direction (Newton) min.	50-67	>20	2.7 (0.3)
4a	Tension strength in wet condition in the wrap direction (Newton) min.	50-67	>20	2.8 (0.0)
5	pH	4.5-8	7 (0.0)	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 (dilution of 1/1000 oral bacterial with crab hydrogels as bio-dressings)	780 (dilution of 1/1000 oral bacteria)

Table 6.1 Testing and certification of the characteristis of crab bio-bandages and commercial hydrocolloid based on IS997:2004

Anti-bacterial crab bio-bandages with bio-dressings fulfilled the above criteria and out-performed commercially available bandages such as commercial hydrocolloid.

6.4 Testing and certification of anti-bacterial crab bio-bandages with bio-dressings based on BS EN 13726-1

The FSA Free-Swell Absorbency of synthetic blood of crab hydrogel bio-dressings was 1.86g 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. Anti-bacterial crab bio-bandages with bio-dressings fulfilled many criteria stated in IS997:2004 and BS EN 13726-1, so they are eligible for marketing.

7.Limitation

7.1 Primary wound dressing test methods BS EN 13726-1

https://www.woundsource.com/poster/assessment-dressing-fluid-handling-comparison-seven-absorptive-foam-dressings

7.1.1 Free-Swell Absorbency FSA

Anti-bacteral crab bio-bandages with bio-dressings showed excellent performance in absorption of synthetic blood with FSA 1.86g per 5cm x 5cm dressing (cf. commercial hydrocolloid 0.30g per 5cm x 5cm dressing). As synthetic blood has the same surface tension as human blood, the bio-dressings should absorb human blood well. However, the effect of anti-bacterial crab bio-bandages with bio-dressings on time of blood clotting is yet to be investigated.

7.1.2 Retention Following Compression (RFC)

RFC were not measured as compression of 40 mmHg was not available in our school laboratory in the measurement the RFC values as follows.
The dressings should be allowed to absorb an amount of fluid representative of that produced by a highly exudating wound over 24 hrs at 37°C. Dressings should be weighed after allowing fluid in excess to drip off (W1) and then compression (40 mmHg) should be applied for 30 sec. After reweighing the dressing (W2), the retention capacity should be calculated as follows:
RFC (%) = (W2/W1) * 100

7.1.3 Total Fluid Handling (TFH)

TFH were not measured as Paddington cups were not available in our school laboratory in the measurement of TFH as follows.
To measure fluid transpired and absorbed, Paddington cups should be filled with ionic solution kept at 37°C and 20% relative humidity for 24 hrs were used. Total fluid handling should be calculated as follows:
TFH (g/m2/24 hrs) = Fluid transpired + Fluid absorbed

7.1.4 Moist vapour transmission rate (MVTR)

MVTR were not measured as Paddington cups were not available in our school laboratory in the measurement of MVTR as follows.
Dressings mounted on Paddington cups should be filled with ionic solution were kept at 37°C and 20% relative humidity

for 24 hrs. MVTR should be calculated as the difference between the Paddington cup weight before (W1) and after (W2) incubation as follows: MVTR (g/m2/24 hrs) = (W1— W2) * (10,000/area of sample)

7.2 Side effects of chitosan on human health

https://www.webmd.com/vitamins/ai/ingredientmono-625/chitosan#:~:text=Side%20Effects%20%26%20Safety&text=Chitosan%20might%20cause%20mild%20stomach,Chitosan%20can%20cause%20irritation.

Chitosan is possibly safe for most people when taken by mouth for up to 6 months. Chitosan might cause mild stomach upset, constipation, or gas. Chitosan is possibly safe for most people when applied to the skin for a short time. Chitosan can cause irritation. Besides, people who are allergic to shellfish are allergic to the meat, not the shell. Proteins such as tropomyosin were believed to be triggering allergic reaction. They were not present in the bandage during deacetylation of crab shells using 16.7M NaOH. (Waibel, 2011) But still, there are concerns about allergic reactions that might come up when anti-bacterial crab bio-bandages with bio-dressings are marketed. However testing of allergic reaction caused by bandages and wound dressings such as skin prick tests that have to be done on human subjects are not available in HKSAR.

7.3 Crab hydrogel dressings as haemostatic agents

As blood clotting experiments involve the use of animal or human fluids which are prohibited in a secondary school laboratory.

8.Further study

8.1 Primary wound dressing test methods BS EN 13726-1

https://www.woundsource.com/poster/assessment-dressing-fluid-handling-comparison-seven-absorptive-foam-dressings

8.1.1 Time of blood clotting

Measurement of the time of blood clotting by different wound dressings could be investigated.

8.1.2 Retention Following Compression (RFC)

Measuring the RFC values of different wound dressings as follows.

The dressings should be allowed to absorb an amount of fluid representative of that produced by a highly exudating wound over 24 hrs at 37°C.2 Dressings should be weighed after allowing fluid in excess to drip off (W1) and then compression (40 mmHg) should be applied for 30 sec. After reweighing the dressing (W2), the retention capacity should be calculated as follows:

RFC (%) = (W2/W1) * 100

8.1.3 Total Fluid Handling (TFH)

Measuring the TFH of different wound dressings as follows.
To measure fluid transpired and absorbed, Paddington cups should be filled with ionic solution kept at 37°C and 20% relative humidity for 24 hrs were used. Total fluid handling should be calculated as follows:
TFH (g/m2/24 hrs) = Fluid transpired + Fluid absorbed

8.1.4 Moist vapour transmission rate (MVTR)

Measuring the MVTR of different wound dressings as follows.
Dressings mounted on Paddington cups should be filled with ionic solution were kept at 37°C and 20% relative humidity for 24 hrs. MVTR should be calculated as the difference between the Paddington cup weight before (W1) and after (W2) incubation as follows: MVTR (g/m2/24 hrs) = (W1— W2) * (10,000/area of sample)

8.2 Prick skin tests

Testing and certification of bandages about allergic reactions should be carried out in well equipped laboratories meeting international standards following strict supervision of allergists especially when human subjects are involved.

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

Determination of Clotting Time (CT), 12 test tubes were arranged in water bath at 37˚C. Control Group: 0.4 ml of blood was collected from each rat in the control group and added to 6 test tubes kept in the water bath. Test group: For the remaining 6 test tubes 0.1 ml of crab hydrogel. 0.4 ml of blood collected from the test group was added to these test tubes. The CT was estimated for both control group and test group. (Ramesh, 2019)

9.Summary

Anti-bacterial crab bio-bandages and crab bio-dressings are bio-degradable as they took 42 days and a month for complete bio-degradation respectively, so they are better than commercial bandages such as commercial Hydrocolloid as the disposal of anti-bacterial crab bio-bandages with bio-dressings would no longer pose burden to landfilling or threat to our environment. Anti-bacterial crab bio-bandages with bio-dressings are anti-bacterial with degree of deacetylation of DD% 82.6% (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, the load per unit of area of anti-bacterial bio-bandages was 342g/m^2 which met the minimum requirement of 36g/m^2 based on IS997:2004 standard. They had stronger tension strength (>20N both in dry and wet conditions) than commercial hydrocolloid. (2.7N dry 2.8N wet) and it was comparable with that required by IS997:2004 (50-67N). Their pH values were found to be about 7 which met the pH range of 4.5-8 based on IS997:2004. The FSA Free-Swell Absorbency of synthetic blood of crab hydrogel bio-dressings was 1.86g per 5cm x 5cm dressing which was much higher than that of commercial hydrocolloid (0.299g per 5cm x 5cm dressing), so crab hydrogel bio-dressings performed much better as wound dressings than commercial hydrocolloid. Anti-bacterial crab bio-bandages with crab bio-dressings for sure are eligible for marketing.

10.Reference

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【評語】090026

This project aims to fabricate a new anti-bacterial crab bio-degradable bandage. The project is well organized, designed, and presented. The team worked coherently and clearly understand the background well, as they were well-prepared for the Q&A. The bio-degradable bandage, despite not being a super novel idea, is still a breakthrough for future application. The team could enhance better if they could focus on bringing more discussion for why their design might be superior to some other available approaches or commercial products.

Additional comments：

1. Similar studies have been reported in several labs.
2. The authors should provide data to prove that your product is better than others.
3. The data should have statistical analysis.
4. Investigation of the feasibility of improving the water-proof property of crab hydrogels as bio-bandage by determination of the change in the structure of crab hydrogel before and after roasting at different temperatures and different times using FTIR.
5. Comparing the absorption of water and synthetic blood, and the strength of crab hydrogels and commercial hydrocolloids.

6. Investigation of the anti-bacterial effect of crab hydrogel before and after roasting.
7. Investigation of the biodegradability of crab hydrogels and roasted crab hydrogels.
8. Testing and certification of the characteristics of roasted crab hydrogels as bandages based on IS997：2004 and BS EN 13726-1.