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## Physicochemical characterisation of biosynthetic bacterial cellulose as a potential wound dressing material

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### SUMMARY

The aim of this study was to characterise the physicochemical and biocompatibility properties of biosynthetic bacterial cellulose (BC) as a potential wound dressing material. The moisture content, water vapour transmission rate (WVTR), thermal stability, cyto- and haemo-compatibility of BC were investigated. Results indicated that the physicochemical properties of biosynthetic BC allow an optimum WVTR to be maintained for improved localised wound moisture levels, good thermal stability and excellent water imbibing capabilities. *In vitro* cytotoxicity and haemocompatibility confirmed the biocompatibility of BC and hence suitability of this material for further exploration into its' application as a wound dressing material.

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### INTRODUCTION

Hydrogels are highly hydrophilic, three dimensional, crosslinked networks of polymers that can be synthesised from a range of synthetic and natural materials, including BC. Unlike plant cellulose, BC is free from biogenic molecules such as pectin, lignin, *etc.* Having a highly crystalline, BC's flexible structure makes it amenable to controlling the release of a range of medicaments, including antimicrobial agents (Abeer et al., 2014). The ability of BC hydrogels to imbibe large amount of aqueous fluids is also advantageous for the management of heavily exudating wounds. The aim of this study was to characterise the physicochemical and biocompatible properties of biosynthetic BC to determine its suitability as a wound dressing material.

### MATERIALS AND METHODS

BC pellicles were biosynthesised, harvested, purified and freeze dried as previously described by Gupta et

al., (2016). The physicochemical properties of the BC were characterised in terms of moisture content, WVTR, TGA, cytotoxicity (MTT assay) and haemocompatibility.

**MTT assay:** DMEM was conditioned with freeze dried (FD) BC pellicle (9.25±0.25cm) overnight at 37°C under constant agitation. HEK293 cells (ATCC) were seeded onto sterile 96 well plates prior to incubation with 200µl BC-conditioned media at 37°C for 24hrs in 5% CO<sub>2</sub>; cell viability was assessed at 595nm using the standard MTT assay. Procedure was conducted aseptically in triplicate.

**Haemocompatibility test:** Using a modified version of the method described by Mohammed (2017), defibrinated whole horse blood (TCS Biosciences Ltd, UK) was washed twice with sterile normal saline (NS) and centrifuged at 3000rpm for 10mins before re-suspending in an equal volume of NS. NS-soaked BC discs (7 mm) were padded dry and incubated with 2mL of washed, NS-suspended horse blood cells

(37°C for 2 hrs, with inversion of samples every 15 mins). Each sample was then centrifuged at 3000rpm for 10mins and the supernatant decanted. Positive (+ve) and negative (-ve) controls were distilled water-suspended and NS-suspended blood cells, respectively. Absorbance (measured at 540nm with NS as blank) and the percentage (%) haemolysis determined as follows:

$$\%Haemolysis = \frac{A_s - A_n}{A_p - A_n} \times 100$$

Where  $A_s$ =abs of supernatant;  $A_n$ =abs of -ve control and  $A_p$ =abs of +ve control.

**Moisture content (Mn):** The wet mass ( $W_w$ ) of BC pellicles was determined before FD and the dry mass

$$M_n = \frac{(W_w - W_d)}{W_w} \times 100\%$$

**WVTR:** Using a covered plastic chamber placed inside 35°C oven with a digital hygrometer (HTC-1 SourcingMap, UK) (adjusted to 50% relative humidity [RH]), the WVTR of BC was investigated using a modified version of the method described by Balakrishnan (2005). Using a saturated magnesium nitrate solution to achieve 50%RH in the chamber, BC discs (4.7cm exposed diameter) were secured onto glass vessels containing 25mL distilled water. WVTR of BC was determined by weighing the complete beaker assembly at set time intervals over 48hrs and calculated as follows:

$$WVTR = \frac{\text{Slope} \times 24}{\text{test Area in m}^2} \text{ g/m}^2/\text{day}$$

**TGA:** Using a Thermogravimetric Analyzer (TGA7, Perkin Elmer, UK) FD BC was heated from 20-500°C at 5°C/min under constant nitrogen flow (20mL/min).

## RESULTS AND DISCUSSION

Two samples of microcapsules were prepared. Sample A contained garlic 2g, polymer EC 1g and HPC 1g whereas Sample B is the formulation of 2g garlic in 1.25g of EC and 1.25g of HPC. The results of both samples regarding %age yield, entrapment The cytotoxicity of BC against an epithelial cell line, as determined by the MTT assay confirmed that biosynthetic BC is cytocompatible with cell viability >98.5%. Moreover, analysis of haemocompatibility indicated <2% haemolysis, that would hence classify the BC as “nonhaemolytic” (Mohamad et al., 2017). As expected, moisture content results showed that

hydrated BC imbibes >99.5% water. This property is extremely beneficial for wound dressing materials as it can confer increased malleability as well as increasing the amount of dissolved oxygen and its extent of permeability; this in turn enhances the epithelialisation process by facilitating continuous aerobic conditions at the wound site (Jadhav et al., 2012). The WVTR reveals a decrease in transmission rate with increasing BC thickness in the order 3360, 2747 and 2505  $\text{gm}^2\text{day}^{-1}$  for 2.1, 2.5 and 2.7mm hydrogel respectively. Reports in the literature confirm that 2500-3500  $\text{gm}^2\text{day}^{-1}$  can provide sufficient moisture level to prevent wound dehydration (Balakrishnan et al., 2005; Jadhav et al., 2012). TGA data (Fig 1) showed distinct percentage weight losses at 100-200°C and  $\approx 350^\circ\text{C}$  which correspond to the removal of water (5-8%) and subsequent decomposition of BC (>60%), respectively.

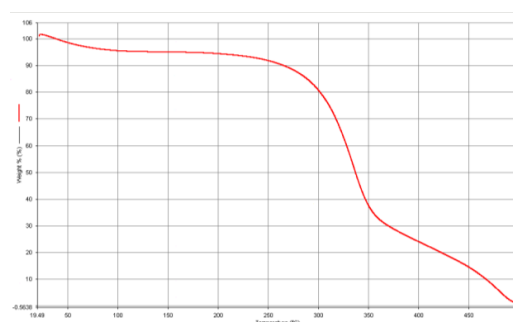


Fig 1. TGA curve of freeze dried BC

## CONCLUSIONS

The results presented here confirm the excellent cytocompatibility and haemocompatibility of biosynthetic BC hydrogels *in vitro*. An analysis of their physicochemical properties also indicate that they are able to maintain an optimum WVTR in accordance with values cited in the literature, display good thermal stability at physiological conditions and have superb water imbibing capabilities, all of which are advantageous for modern wound dressing materials.

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