Bacterial Cellulose Micro-Nano Fibres for Wound Healing Applications

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Abstract

Bacterial cellulose (BC) is cellulose produced by a few limited species of bacteria in given conditions. BC has many remarkable properties such as its high mechanical properties, water uptake ability and biocompatibility which makes it a very desirable material to be used for wound healing. Inherently due to these important properties, the material is very resistant to easy processing and thus difficult to produce into useful entities. Additionally, being rate limited by the dependency on bacterial production, high yield is difficult to obtain and thus secondary material processing is sought after. In this review, BC is explained in terms of synthesis, structure and properties. These beneficial properties are directly related to the material's great potential in wound healing where it has also been trialled commercially but ultimately failed due to processing issues. However, more recently there has been increased frequency in scientific work relating to BC processing into hybrid polymeric fibres using common laboratory fibre forming techniques such as electrospinning and pressurised gyration. This paper summarises current progress in BC fibre manufacturing, its downfalls and also gives a future perspective on how the landscape should change to allow BC to be utilised in wound care in the current environment.

Keywords: Bacterial Cellulose, wound healing, fibres, *Gluconacetobacter xylinum*, fibre production

1. Introduction

As early as in the 19th century A.J Brown, noted that a specific bacterium produced a solid membrane at the surface of his culture when grown in a carbohydrate-rich medium (Brown, 1886). Later studies demonstrated that the material of the membrane produced by these bacterial species were identical to the principle structural polysaccharide of plants, cellulose (Hibbert, 1930). In contrast to plant cellulose, the gelatinous membrane showed incredibly high strength, purity, porosity, a uniform fibre network and enhanced water holding ability (R. Chawla et al., 2009). The cellulose produced by the bacterial genera *Gluconacetobacter* (formerly *Acetobacter*) are commonly called bacterial cellulose (BC), which is in itself a biopolymer. Moreover, BC demonstrates the fascinating ability to enhance

wound healing recovery, revealing the potential to revolutionise the healthcare market (Sulaeva et al., 2015). The cost of wound care for any healthcare provider marks a significant portion of overall expenditure. In hospitals, more than 30% of the beds are occupied by patients having wounds, some of whom who do not require to stay in the hospital for their main disorders (Posnett et al., 2009). With the rise in global average life expectancy, chronic wounds have shown strong correlation with increasing age (Gould et al., 2015).

There is a growing pressure for the development of advanced wound care that has capacity to meet the soaring demands. Although there is an abundance of literature on BC and its applications, there is little on the processing of BC into biomaterials for wound healing, especially in fibrous structures (Carvalho et al., 2019; Picheth et al., 2017; Thomas, 2008). This review focuses on the structure and properties of BC, current progress on its processing for wound care applications and what is necessary to overcome in order to widely use this astonishing material in healthcare settings.

2. Bacterial Cellulose (BC) Synthesis

This cellulose is commonly referred to as “bacterial cellulose” or “microbial cellulose” which is found as a gelatinous membrane at the liquid-air interface of the culture medium (Kamide et al., 1990). BC is produced at certain culture conditions by a number of bacteria belonging to the genus: *Achromobacter, Aerobacter, Agrobacterium, Alcaligenes, Azotobacter, Gluconacetobacter, Rhizobium* and *Salmonella* (Rangaswamy et al., 2015). Yet, the gram negative *Gluconacetobacter xylinum,* has been primary focus in most BC related studies as the cellulose production is far greater in quantity and mass than the other strains, is of extraordinarily high purity and closely resembles that of algal and plant cellulose in its microfibrillar structure (Mikkelsen et al., 2014). Many strains of *G. xylinum* retain the ability to extracellularly produce cellulose in the form of flat, twisting ribbons. *G. xylinum* is an aerobic soil bacterium which belongs to a family of bacteria which are able to ferment carbohydrates into acetic acid (vinegar) (Peggy O'Neill and Cannon, 2000).

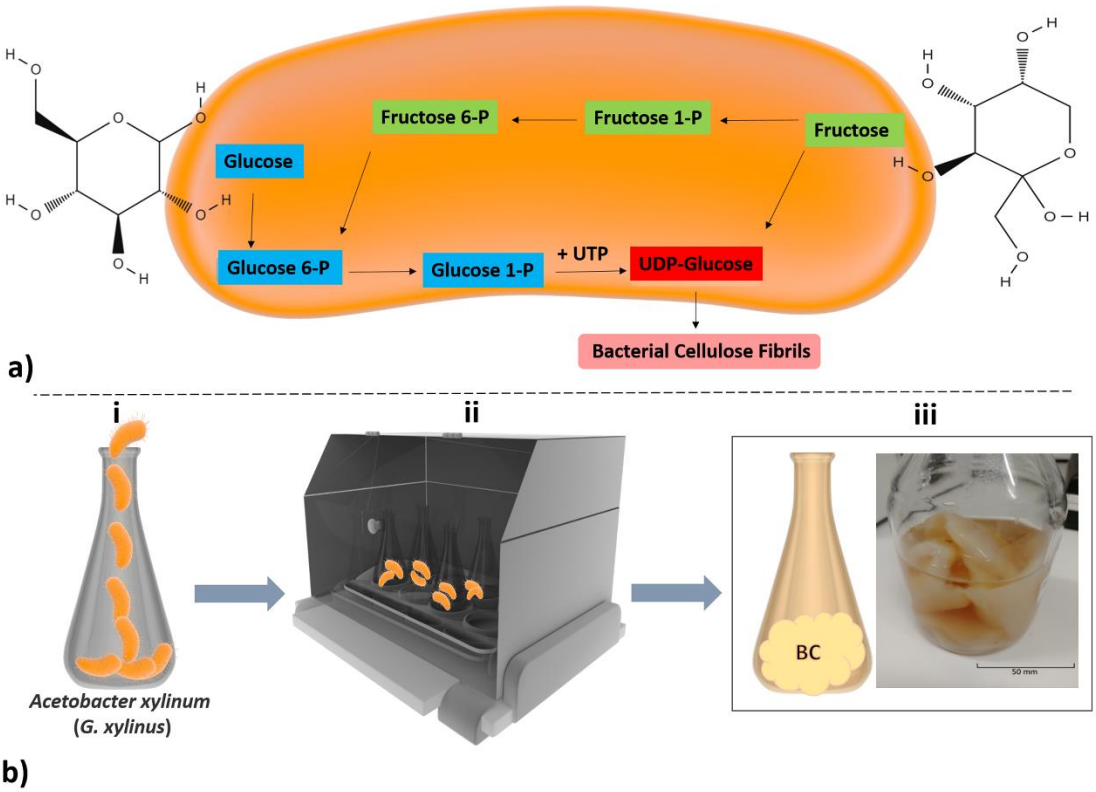

Figure 1: Schematic diagrams of: a) BC fibrils synthesis reaction from glucose and fructose pathways. b) Schematic representation of BC synthesis (i) *Acetobacter xylinum* (*G. xylinus*), (ii) *Acetobacter xylinum* (*G. xylinus*) incubation, (iii) Photograph of bacterial cellulose (BC) gelatinous membrane encased within a 200 mL glass vial and suspended in acetic acid.

The synthesis of cellulose in *G. xylinum* occurs in a multi-step biochemical pathway of reactions beginning with glucose, which is catalysed by multiple enzymes. Cellulose synthesis is considered to be the most crucial enzyme in the BC production process and is responsible to the catalysis of the step preceding the final cellulose production (Ross et al., 1990). The commonly accepted pathway for cellulose production in *G. xylinum* cultures can be summarised as (**Figure 1A**): Glucose (catalysed by glucokinase) → Glucose-6-Phosphate (catalysed by phosphoglucomutase) → Glucose-1-Phosphate (catalysed by UDP-glucose pyrophosphorylase) → UDP-Glucose (catalysed by cellulose synthase) → Cellulose (Klemm et al., 2001).

A single cell of *G. xylinum* has been shown to be able to polymerise up to 200,000 glucose molecules per second into ß-1,4-glucan chains (Hestrin and Schramm, 1954). These chains are extruded into the surrounding medium from the pole of the bacterial rod, which form a single ribbon-like bundle of microfibrils composed of single twisted strands (Ross et al., 1991). This ribbon elongates with the cell envelope at a rate of 2 µm per minute and remains associated during cell division, at the liquid-air interface the suspensions continue with their microfibrillar projections for several hours, giving rise to a cellulosic pellicle (Brown et al., 1976). The fibrils of the ribbons are in close association with the pores longitudinally positioned in the bacterial cell membrane, cellulose biogenesis in *G. xylinum* is one of the best proven examples of unidirectional growth of cellulose microfibrils.

(Zaar, 1979). A single cellulose fibril can be visualised as a cable where the lengthwise strands are D-glucose composed polymeric chains, each chain containing uniformly linked sugar monomers by ß-1,4 glycosidic bonds (Ross et al., 1991).

G. xylinum cultures are characterised as a thick glutinous cellulosic surface mat (**Figure 2)**. This gelatinous membrane (pellicle) is where the embedded cells have direct contact with the liquid/air interface (Schramm and Hestrin, 1954). *G. xylinum* grows and forms cellulose in a range of carbon sources which include glucose, fructose and glycerol (Jonas and Farah, 1998; Mikkelsen et al., 2009; Weinhouse and Benziman, 1974). The growth, metabolism and cellulose production of this bacterium is free from cellulase activity which would otherwise break down the cellulose, this provides a distinct advantage over plant cellulose by being metabolically inert and highly pure (Vandamme et al., 1998).

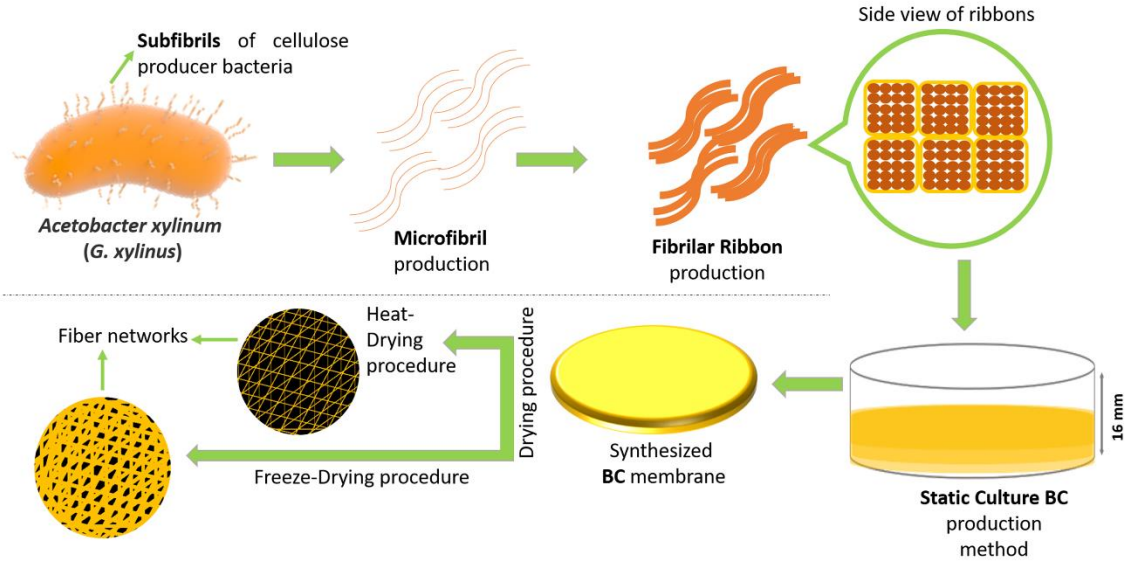

Figure 2: Diagrammatic representation of BC from microfibrils to fibre networks production, step by step in static conditions. Side view depiction of a thick BC gelatinous membrane mat which assumes shape of environment, shown here on a petri dish. The mat contains highly pure network of BC nanofibrils.

Several techniques exist for BC production that demonstrate different degrees of potential for economical and commercially viability as a BC fabrication method. The selection of the cultivation method stringently determines the cellulose microstructure and thus its mechanical and physical properties. Static culture methods (**Figure 2)** employ stationary culture in plastic trays or dishes and have shown to produce a thick and gelatinous BC membrane on the surface of the culture medium which compares with most BC produced and tested (Budhiono et al., 1999; Dudman, 1960). The BC pellicle in a static culture is visible at the surface of the liquid about 2 days from the beginning of the process (Schramm and Hestrin, 1954). An alternative approach to BC cultivation is incorporating an agitated culture such as jar fermenters, horizontal fermenters or internal loop airlift reactors (Kouda et al., 1997; Kouda et al., 1996). Agitated culture approaches can produce cellulose in fibrous suspension forms, pellets, spheres or irregular

masses (**Figure 1B**) (Chao et al., 2000; Naritomi et al., 1998a; Tsuchida and Yoshinaga, 1997).

Static culture systems have been widely investigated and their applications have seen successful commercial applications such as in food and in electronics (Bernardo et al., 1998; Yamanaka et al., 1989). Nevertheless, agitated culture methods are usually deemed more suitable for large scale production due to their higher potential production rates when considering total area of cultivation required. There are, however, many problems that are encountered with cellulose production in fermenters that utilise continuous aeration and agitation. The sporadic presence of non-cellulose producing mutants (*Cel*$^{-}$), leads to the decline in biopolymer production in agitated cultures (Jung et al., 2005; Ross et al., 1991). These mutants are a result of the inactivation of the gene coding for cellulose synthesis (Krystynowicz et al., 2002). In static conditions, cellulose-synthesising *Gluconacetobacter* cells (*Cel*$^{+}$) migrate towards the oxygen-rich medium air interface, where they produce the gelatinous membrane. The membrane limits access to oxygen into the lower depths of the culture and majority of the cells are found in the *Cel*$^{+}$ form. In agitated systems, the uniform aeration leads to preferential growth of bacterial cells instead of cellulose synthesis, in this case the culture is dominated with *Cel*$^{-}$ mutants (Krystynowicz et al., 2002). Furthermore, it was shown that static cultures of *G. xylinum* actually leads to higher yield levels than with swirled cultures, at a period of 2 days following incubation yield was 1.8 x higher in static cultures than with agitated and after 5 days yield was 2.8 x higher in static conditions (Schramm and Hestrin, 1954). Static systems can be less favourable for scale up operations due to the amount of free space required and could limit productivity rate.

Culture conditions can have a marked effect on cellulose production for many different strains of bacteria capable of producing BC (Rangaswamy et al., 2015). Factors such as inoculum density influence the microbial cellulose production, where increasing the concentration of the substance can lead to a reduction in yield, therefore there is an optimum density which needs to be considered. Additionally, there exists an ideal pH range in which both cell growth and cellulose production is the greatest. In tested conditions from pH 3-7, it was found that a pH of 6 led to maximum yield compared to the other pH values (Rangaswamy et al., 2015). Temperature furthermore effects cellulose production where favourable culture temperatures are around 28-30 °C and when temperatures exceed 40 °C, BC production was not observed. Carbon is the sole source of BC production and thus has a significant influence on the yield of BC and its final morphology. Carbon sources such as fructose, glucose, lactose, maltose, mannitol, mannose and sucrose can be utilised to produce BC from different bacteria, maximum yields are usually observed with using sucrose as the carbon source (Eslahi et al., 2020; Wang et al., 2019). Nitrogen is another essential component in cell growth and cellulose production for many bacterial strains, examples of nitrogen sources are: ammonium chloride, ammonium nitrate, ammonium sulphate and peptone. Optimal BC preparation for certain bacteria can result from the use of peptone as the source of nitrogen. On the other hand, cellulose formation from *G. xylinum* and glucose has been observed to be limited by the oxygen concentration of the culture, where negligible BC was produced with nitrogen and maximal amounts where produced with 100% oxygen (Schramm and Hestrin, 1954).

3. Structure of Bacterial Cellulose

Similar to that of plant cellulose, BC shares the same molecular formula $(C_6H_{10}O_5)_n$. The exopolysaccharide-produced BC differs from conventional cellulose in its physical and chemical features. The two cellulose types bear the same chemical similarity being ß-1,4-glucans, but differ in their degree of polymerisation (Yoshinaga et al., 1997). The degree of polymerisation for BC is considerably lower, having a typical polymerisation range between 2000-6000 compared to 13000-140000 of plant cellulose.

BC is composed of twisted ribbon-shaped fibrils approximately 50-100 nm in width and 3-8 nm in thickness (Astley et al., 2001; Brown et al., 1976; Yamanaka and Sugiyama, 2000; Zaar, 1977). It has been shown by X-ray diffraction (XRD), that the size of the microfibrils are associated with its crystallite size (Haase et al., 1974). These ultrafine ribbons have a length of 1-9 µm and form a densely arranged structure stabilised by comprehensive inter-and intra-hydrogen bonding (Bielecki et al., 2005; Esa et al., 2014). The average distance between junction points (pore size) of a typical BC membrane has been calculated to be 0.523 ± 0.273 µm, and the orientation of the segments as the average angle formed between the x-axis and the segments is 85.64 ± 0.56° (J Grande et al., 2008).

The macroscopic structure and morphology of BC fibres are strictly dependant on the cultivation techniques used to produce them (Watanabe et al., 1998). In a static culture, the bacterial cells produce cellulose mats at the surface of the nutrient broth where the interface between the liquid and the oxygen rich air exists. In these conditions, *G. xylinum* cells continuously extrude subfibrils of cellulose from their surface pores which in turn become crystallised into microfibrils, and are forced down deeper through the growth medium (Bielecki et al., 2005). As a result, the cellulose produced in static conditions result in leather-like pellicles which support the population of *G. xylinum* cells. These pellicles consist of overlapping and intertwined cellulose ribbons which form a grid of parallel but disorganised planes (Jonas and Farah, 1998). Comparatively with cellulose produced in agitated cultures, the adjacent strands of the cellulose mats branch and interconnect to a higher degree prevalent in static cultures. In agitated conditions, the increased branching is observable in the form of fibrous strands and irregular granules dispersed thoroughly through the culture broth (Vandamme et al., 1998). Furthermore, the agitated BC interconnect to form a grid-like pattern (Watanabe et al., 1998). The differences in morphology between cellulose produced by agitated and static conditions also contribute to differing levels of crystallinity, crystallite size and the content of cellulose I_α. The schematic BC microfibril model, physical properties and biomedical application areas are shown in **(Figure 3).**

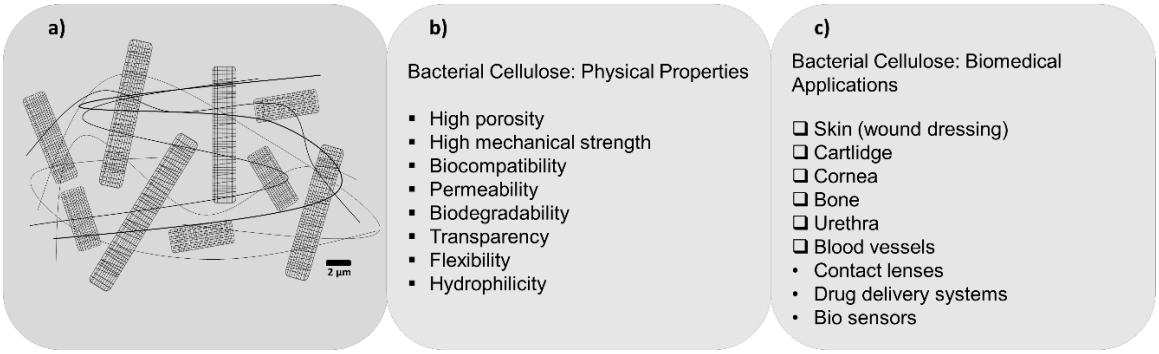

Figure 3: a) Schematic diagram of BC microfibrils, showing a unique structure that isn't commonly found in cellulose, b) Physical properties of BC (Hussain et al., 2019), c) Biomedical applications of BC (Gallegos et al., 2016; Portela et al., 2019).

Further differences between agitation produced BC and statically produced BC are obvious when viewed using a Scanning Electron Microscope (SEM). Statically produced BC have fibrils with a more extended morphology with fibrils stacked above one another in a crisscross pattern. Conversely, strands of agitation produced BC reveal an entangled and curved physiology (Johnson et al., 1989). Compared to plant cellulose, BC has a unique characteristic in its crystalline structure. Native cellulose consists of cellulose Iα and cellulose Iβ crystalline structures, where cellulose Iβ is the major component, approaching approximately 60% in composition. (VanderHart and Atalla, 1984; Yamamoto and Horii, 1993). Interestingly however, BC contains 60% cellulose Iα (Atalla and Vanderhart, 1984).

Another key difference between plant cellulose and BC lies in their morphological structures. In plant cellulose, several cellulose molecular chains assemble to form microfibrils. This assembly subsequently leads to the development of high-order bundles and clusters called fibril lamella and fibre cells (Shoda and Sugano, 2005). Plant cellulose forms a complex structure with impurities such as lignin and hemicellulose. Contrariwise, BC is secreted by *G. xylinus* cells fashioned into a ribbon-like structure composed of microfibril bundles. The fibre diameter of these ribbons are over a hundred times thinner than that of plant cellulose (Guhados et al., 2005). Due to the special ultrafine reticulated structure of BC, there are many unique characteristics that become apparent in their potential and current applications, these are discussed in the next section.

4. Properties of Bacterial Cellulose

BC has a wealth of useful properties that allow it to be used in a wide range of applications, especially in industry and healthcare. The properties are dependent on the structural features as mentioned previously. When the BC pellicle is chemically purified and dried on a flat substrate, a thin and translucent cellulose membrane is established. This membrane holds a plethora of unique properties due to its fine and continuous network of crystalline microfibrils, both in its dried and wet (never-dried) state (Shibazaki et al., 1993).

BC has been discovered to have the highest Young's modulus of any two-dimensional organic material, at a staggering stiffness value of 15 GPa. The

extraordinarily high stiffness arises from the strong interfibrillar binding in the network of its ultrafine fibrils and also owning to its high crystallinity (Yamanaka et al., 1989). The effect of sodium hypochlorite (NaClO) and sodium hydroxide (NaOH) on the stiffness of the BC was investigated, the Young's modulus of the BC sheets further increased to 23 GPa at a 0.5% concentration of NaClO and approached 30 GPa at a concentration of 5% NaOH (Nishi et al., 1990). Therefore, the mechanical properties of BC can be further improved with the treatment of alkaline or oxidative solutions, which can be beneficial in many industrial applications where greater stiffness is required. Post-processing of BC allows its mechanical properties to be tailored by exposing it to different chemical treatments, this is especially useful in applications where a highly specific stiffness is desired such as in tissue engineering and cellular wound healing (Chen et al., 2015; Wang et al., 2012).

BC shows further favourable mechanical properties with high tensile strength, afforded by its highly crystalline structure and fine diameter network of fibres which work together in unison with tensile loads. With a density of 1600 kg/m^3, BC microfibrils have an individual Young's modulus of 138 GPa and a tensile strength of more than 2 GPa (Dobre et al., 2010; Nishino et al., 1995). Aramid fibres, a class of heat-resistant and highly strong synthetic fibres used in body armour fabric and ballistic composites, show similar tensile strengths to that of BC, proving how much strength there is in its dense nanofibre network (Young et al., 1992). BC has shown good potential in material reinforcement in various composites which gives the newly formed composite greater mechanical properties (Gindl and Keckes, 2004; Yano et al., 2005).

Tissue engineering is a rapidly growing field which aims to restore, repair or maintain the function of various vital tissues and organs (Stock and Vacanti, 2001). Biomaterials have been widely used as tissue engineering scaffolds where an ideal material would successfully mimic the extracellular matrix and be able to guide the necessary cells towards effective tissue reformation. Being a natural polymer, BC proves to retain a high level of biocompatibility as shown by studies which show the *in vitro* and *in vivo* biocompatibility of BC. Especially, implantations of BC within rat models have successfully demonstrated biocompatibility with the absence of macroscopic indications of inflammation in response to the implant within the animal (Helenius et al., 2006). Absence of fibrotic encapsulations together with the absence of giant cells point towards good biocompatibility of the material in *in vivo* conditions. The results here are not surprising given that cellulose-based materials are generally considered biocompatible and thus invoke negligible inflammatory and foreign body responses (Miyamoto et al., 1989).

BC pellicles demonstrate a high level of chemical purity due to the absence of hemicellulose, lignin, pectin and other biogenic compounds (Song et al., 2009). Removal of hemicelluloses and lignin from cellulosic materials require difficult post processing which adds time and cost and would otherwise pose an economic burden in the manufacturing industry (Frederick et al., 2008). The energy requirement for the purification of BC is considerably lower than that of other cellulosic materials, allowing for a reduction in processing costs and chemically-intensive processes which can form hazardous waste products (Gea et al., 2011). Compared to plant and other cellulose sources, BC offers a more economical (in

terms of purification) and environmental source of cellulose which is unfortunately limited by its production rate.

Due to the nature of its ultrafine fibre network, BC has a very large surface area per unit mass, which gifts it the ability of having a very large water holding capacity. BC can hold up to 200 times its own dry mass in water, the majority of this liquid is not bound to the polymer and can be easily released via gentle pressing (Lin et al., 2009; Schrecker and Gostomski, 2005; Shezad et al., 2010). The excellent water holding capacity and water release rate of BC make it suitable as wound dressings. Capillary forces are responsible for holding the water in the cellulose pore structure where water is bound to the cellulose fibrils with hydrogen bonding (Gelin et al., 2007; Ul-Islam et al., 2012). Despite its high water holding ability, the actual BC fibres are very hydrophobic which permits it to be used in a wide range of civil and industrial applications (Feng et al., 2002; Marins et al., 2011; Yuyang et al., 2006).

XRD analysis on static-culture produced BC shows that this material has a crystallinity index of 50% (Krystynowicz et al., 2002). Cellulose produced by bacteria grown in agitated cultures have shown to acquire a reduced crystallinity compared to those produced in stationary cultures (Czaja et al., 2004). The movement and rotation in agitated cultures cause an external force of disturbance to the fibril crystallisation process, leading to lower crystallinity (Yan et al., 2008). Due to its high crystallinity however, BC has an incredibly low solubility and thus is limited in its processability (Hu et al., 2014). It is insoluble in most common solvents that are used in the manufacturing industry which limits its potential applications in these fields. A few solvents have been found to dissolve BC such as lithium chloride with N,N-dimethylacetamide, sodium hydroxide/urea aqueous solutions and some ionic liquids (Lu and Shen, 2011; Phisalaphong et al., 2008; Shen et al., 2010). These solvents however pose problems in terms of processing costs, health and safety issues due to toxicity, environmental devastation and can also negatively alter the properties of the BC (Aral and Vecchio-Sadus, 2008; Qin et al., 2014). On the other hand, the low solubility of BC can be advantageous in applications where the stability of the material in response to various gas and liquids is crucial, such as in air or water filtration systems (Kosmider and Scott, 2002).

Cellulose, being the most abundant natural homopolymer, shows excellent biodegradability from both plants based and bacterial sources. BC is completely biodegradable in a wide range of environmental conditions, which makes it a promising candidate in environmental protection, biomaterial and tissue engineering applications (Li et al., 2009; Wan et al., 2009). Another considerably attractive advantage of BC is its ability to be physically moulded into any form or size during synthesis (Bäckdahl et al., 2008). This mouldability does not come at the expense of causing any notable alteration to its physical properties. For example, BC grown in a petri dish will take up the shape and volume of the dish and will be formed into a circular gel-like pellicle. A summary of the properties of BC relating to wound healing can be found in **Table 1**.

Table 1: Table summarising the key properties of BC and its relevance to wound healing.

Property	Advantage	Benefits to Wound Healing	References
Biodegradability	Bandage for chronic wounds potentially doesn't need removing	Reduction of pain from bandage removal	(Hu and Catchmark, 2011; Laçin, 2014)
ECM Resembling Matrix	Biomimetic structure promotes prompt wound healing	Cells of the wound response can be guided to become more efficient	(Svensson et al., 2005; Wu et al., 2014)
Excellent Biocompatibility	Reduces complications with immune rejection	Risk of fibrotic scarring is lower	(Helenius et al., 2006; Torres et al., 2012)
High Stiffness	Great Durability	Allows bandage to withstand some trauma	(Lin et al., 2013; Nakayama et al., 2004)
High Tensile Strength	Resistance against tearing as a wound dressing	Provides mechanical protection against external trauma	(Naritomi et al., 1998b; Wan et al., 2009)
High Water Uptake Ability	Maintains moist environment and flow of wound exudate	Allows for a more efficient recovery process and management of osmotic environment of cells	(Lin et al., 2009; Schrecker and Gostomski, 2005; Ul-Islam et al., 2012)
Large Surface Area	Increased interactions with cells in the wound response	More efficient cellular interactions leading to a healthier recovery	(Iguchi et al., 2000; Nishi et al., 1990)

5. Wound Healing

The unique structural and mechanical properties of BC make it suitable for use in a variety of applications such as in food, electronics and medicine (Fontana et al., 1990; Jagannath et al., 2008; Shibazaki et al., 1993). However, out of all the applications, BC has revealed outstanding potential in wound healing and wound care products. The benefit of advanced wound care products and services that address infection and recovery times will function to revolutionise the healthcare industry, its impact would be remarkable for the entirety of the human population. As mentioned previously BC has valuable properties such as its high crystallinity, water holding and absorption capacity, low solubility in solvents and high tensile strength (**Figure 3B**). These features are all beneficial for skin repair materials.

A good wound repair material has the important characteristic to be able to absorb exudate during and after application and removal. Currently available wound care materials have traditionally showed good absorbance and permeability such as with gauzes which adhere to desiccated wound surfaces, but on removal can cause trauma and damage to the wound site (Boateng et al., 2008). When considering the properties of BC to current wound care materials, BC shows

incredible promise in overcoming the downfalls associated with current dressings. Consequently, BC membranes have been used as either wound dressings or skin substitutes. The membrane produced by the bacteria can be directly used from the culture by simply washing the pellicle with water. BC can also be processed further if need be to suit the exact wound healing application.

In the late 20th century, BC was first used as a temporary skin substitute and biological dressing under the trade name BioFill®, now known as Dermafill™ (Fontana et al., 1990). The product was intended to treat patients suffering from various skin wounds as a result of burns, dermabrasion, cuts and ulcers. Since then, many other BC based products have been commercially available for topological application for wound recovery. Studies show that the use of BC membrane-based dressings establish superiority to conventional materials in reducing wound pain, retaining exudate, accelerating and facilitating re-epithelialisation, reducing total healing times, diminishing infection rates and reducing visible scarring (Czaja et al., 2006; Czaja et al., 2007; Fontana et al., 1990). Moreover, due to the translucency of the BC dressing, it is remarkably simple and easy to inspect the wound, without interference or removal of the membrane from the patient.

During the wound healing process, correct moisture levels are required for efficient recovery times. Having a high-water holding ability, BC allows for the wound site to have the ideal moisture conditions. Furthermore, due to the network of its nanofibres, the membrane will prevent infection by creating a physical barrier that will prevent bacteria infiltrating into the wound site preventing the risk of infections (Kaewnopparat et al., 2008; Shezad et al., 2010). The heating of the skin in burn victims causes the breakdown of the semi-permeable membrane associated with the lipoprotein layer in the outermost layer of the skin (stratum corneum) (Jelenko et al., 1968). When the stratum corneum is destroyed, there is a substantial evaporative loss of water which is associated with a large degree of heat loss which can lead to hypermetabolism in burn patients (Lamke et al., 1977). The high-water absorptivity, water retention and vapour transmission features of BC creates an environment where the wound exudate is locked into the dressing whilst also preserving proper wound moisture during healing.

Owing to a multitude of hydroxyl groups, air-dried BC allows the for exceptional water vapour permeability which can be hugely beneficial in wound dressings (Fu et al., 2013). Using air-dried membranes allows for breathable dressings which permit the passage of water vapour through the material. Studies show that an ideal moisture content of a wound environment is one of the most important factors of successful wound healing (Fleck and Simman, 2010). Experimental values of controlled water vapour tests on wound re-epithelialisation and contraction enhancement show that in the case of a dressing with a water vapour transmission rate of 2028 ± 237.8 g/m^2. 24h was found to be in the optimal timescale for healing. (Xu et al., 2016).

A necessity for wound dressings is its competence in maintaining structural integrity between the time period of application and removal, especially when applied near joint areas where movement can cause failure of the dressings. The tensile strength of a BC membrane has been experimentally calculated to be approximately 15 MPa with 32% elongation at break, the addition of chitosan can increase the Young's modulus (Lin et al., 2013). The tensile strength of BC

membranes is also dependant of culture conditions and post treatment which can be found to as high as 260 MPa (Kim et al., 2011; Yano et al., 2008). The elongation at break of 32% for the BC membrane reveals a high degree of toughness. These properties allow BC to be extremely suited in a wide range of wound dressings for different wound sites. For example, BC is both mechanically strong and flexible and can thus be produced and be given to patients with knee wounds where their movement will not be restricted and the dressing will not fail.

Cytotoxicity and cell attachment testing on BC membranes have shown that BC maintains high fibroblast viability which is highly desired in a dressing material as cell toxicity would be a major concern for any material that comes in contact with an open wound (Moreira et al., 2009). BC additionally accommodates high level of cell attachment due to its ultrafine network of nanofibers, this feature is especially useful in the progression of wound healing where enhanced cell attachment would play a role in healing acceleration (Diegelmann and Evans, 2004). Furthermore, the ultrafine network presents a high surface area to volume ratio that has potential in cell seeding which can facilitate faster wound regeneration.

The bio-absorbability of BC allows enhanced restoration of the targeted tissue in a wound environment. Bioabsorbable BC has been developed and tested in pH conditions that are commonly found in wound environments (Hu and Catchmark, 2011). It was shown that by incorporating BC with different cellulases, that the degradation rate of the material could be controlled. This permits modified BC to be able to degrade through a function of a predetermined and configurable time.

BC has shown similarity to the human carotid artery in its stress-strain response curve (Bäckdahl et al., 2006). The resemblance to soft tissue could be due to the comparable architecture of the carotid artery and BC, but this finding also suggests that BC can be formed to be biomimetic towards tissue and skin. Numerous publications that BC is also similar to skin, making it suitable as a skin substitute material or a temporary wound treatment dressing (Ciechańska, 2004; Fu et al., 2013; Lee and Park, 2017). An ideal wound dressing system would present similarity to the autograft skin in structure and in functionality (Jones et al., 2002). By mimicking native soft tissue, wound care materials made of BC could prove to improve patient compliance.

Given its highly nano-porous structure, BC allows for the incorporation of pharmaceuticals and antibiotics into a wound, whilst simultaneously serving as an effective physical barrier against potential infections with its filter-like mesh of microfibrils. Porous fibres for the delivery of active pharmaceutical ingredients is not a new concept, drugs can be easily incorporated into the BC dressing to be released at a controlled or delayed release rate (van de Witte et al., 1993).

When BC grows in its native conditions, it takes the form of the surrounding environment such as the petri dish. The membrane remains highly mouldable even after extraction from the growth medium. Wounds come in different shapes and sizes and can occur at any part of the body and therefore should not be thought of as a flat surface. The mouldability of BC allows it to be placed on any wound irrespective of where it may be on the patient. BC-based wound dressings can be made to be extremely conformable to the exterior or wounds and allow great levels of comfort that is not experienced by standard gauzes.

6. Bacterial Cellulose Processing (fibres)

There has been an abundance of work focusing on the improvement of static culture methods for producing BC (Çakar et al., 2014; De Wulf et al., 1996; Vandamme et al., 1998). From an industrial point of view however, the fact remains that these culture systems are inefficient as they are labour intensive and have a long turnaround time. Johnson & Johnson, a major pharmaceutical company, attempted the commercialisation of BC as early as in the 1980s. The company supported a pioneering series of investigations into the application of BC for different types of wounds, but details of any clinical trials have never been published, and many companies have failed to introduce a commercial wound healing product which incorporates the benefits of BC due to the many difficulties associated with the efficiency of large-scale fermentation (Ring et al., 1986a, b).

Commercial production of BC was again investigated in the 1990s by a number of large Japanese companies and governmental organisations aiming to efficiently mass produce BC (United and Congress, 1993). The $45 million effort from these companies resulted in many patents and publications, however there was no indication of commercial success. The 1990's was also the decade when fundamental studies on BC biosynthesis was carried out in Poland. The government-backed initiative lead to successful clinical trials continuing through to the new millennium (Czaja et al., 2006). The study also led to the discovery of an efficient strain of *Gluconacetobacter,* which is able to produce cellulose in nutrient mediums which were more economical (Krystynowicz, 1997). Therefore, there was a shift in focus to unearthing strains of *Gluconacetobacter* which would result in higher yields and production rates of BC. The discovery of more efficient bacterial strains allows for advancement into fermentation scale up with promise of commercialisation.

The major obstacle preventing commercialisation is the efficiency of the current production technologies. Manufacturers of BC based artificial skin have been varying concentration of carbon sources, surface/volume ratios of the cultures, and duration of fermentation in the effort to scale production (Czaja et al., 2006). Unlike other bacterial polysaccharides, BC cannot feasibly be synthesised economically in large stirred-tank fermentation systems. Agitated microbial cultures have been shown to have a reduction in cellulose yield and a loss of attractive properties such as crystallinity.

Until very recently, a different approach to BC manufacturing has been on the rise with numerous publications from both academia and industry. The endeavour to form BC into a secondary fibrous form via highly controlled fibre forming techniques has seen a rise. Fibre forming techniques such as electrospinning have been utilised to create ultrafine fibres with BC that can be used in a wide range of potential applications such as drug delivery, tissue engineering and wound healing (Abeer Muhammad et al., 2014; Mohd Amin et al., 2012; Svensson et al., 2005). The benefit of being able to process BC into fibres are vast. The ability to produce continuous nano- and micro-fibres from BC allows for the fabrication of bandages from small amounts of raw material. Furthermore, this allows for the tailor ability of fibre morphology and also allows for potential industrial scale up of BC manufacturing which requires less raw or pure BC.

6.1. Electrospinning

Electrospinning is an electrohydrodynamic technology in which a polymer solution is fed through a needle that is connected to a high voltage power supply (Luo et al., 2012). The solution becomes charged as it flows through the needle and the electrical stresses overcome the surface tension of the polymer solution (Deitzel et al., 2001). The droplets emerging from the tip of the needle converge into a conical shape (Taylor cone) as a result of the balance between various forces, and a polymer jet is ejected from the apex of this cone (Kim and Reneker, 1999). It is this jet that leads to the production mechanism as the solvent subsequently evaporates and in its stead leaves dried, uniform fibres (Feng, 2002). The technology is summarised by (**Figure 4**).

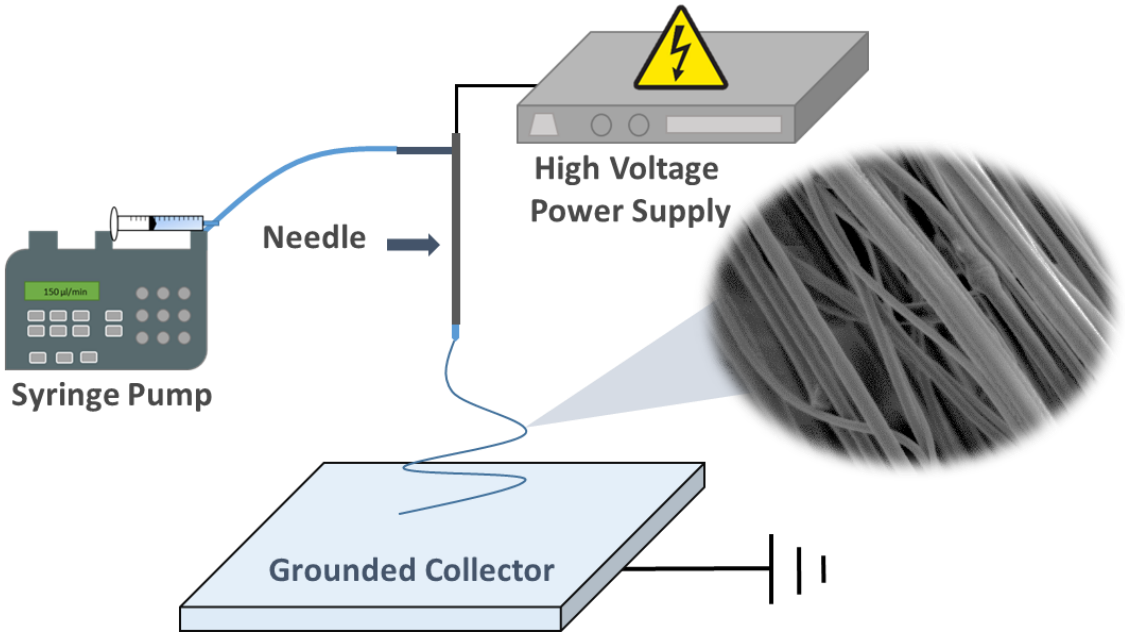

Figure 4: Schematic representation of the electrospinning setup showing a syringe pump where polymer solution is fed through the needle, upon contact with a high voltage electric field, a Taylor cone appears, and fine fibres are formed produced as a result.

Being one of the more established laboratory fibre forming techniques, much attention has gone into forming fibres via this facile technique. BC nano whiskers have been used to improve the mechanical properties of other fibres which are produced by other polymers. The improvement of mechanical properties mainly depends on the extent of BC nano whiskers dispersion in the fibres within the matrix. These whiskers are high aspect ratio (length to diameter ratio) cellulose crystal suspensions, extracted from the cellulose source and reveal a needle like structure under SEM (Bercea and Navard, 2000). They are identified as whiskers due to their elongated shape and their high crystallinity achievement, by creating mixtures of these crystal suspensions with polymer lattices, there is a drastic enhancement of mechanical properties at even a low weight fractions (Favier et al., 1997). BC whiskers can also be obtained by acid hydrolysis of the BC microfibrils, forming highly crystalline rod-like particles (Dufresne, 2000).

Blends of BC and Poly(ethylene oxide) (PEO), a water soluble polymer have undergone electrospinning with aqueous BC solutions of 5 wt% (Park et al., 2007). The solution was able to form fibres such as the PEO would, the BC whiskers-reinforced fibres showed a significant increase in Young's modulus, percentage extension at break and maximum stress. Furthermore, ethylene vinyl alcohol (EVOH) fibres were also spun with electrospinning, XRD studies showed that the BC whiskers had a highly crystalline structure (73.1% crystallinity index) compared to untreated BC membranes (Martínez-Sanz et al., 2011). There is an abundance of polymers used in biomedical and tissue engineering that suffer from poor mechanical properties, therefore, electrospinning of BC has shown to have great potential in composite material reinforcement (Gindl and Keckes, 2004; Pommet et al., 2008; Wan et al., 2009).

More recently, improvements in the portability of electrospinning devices have allowed for point-of-need spinning of fibrous constructs with great potential in wound healing applications (Sofokleous et al., 2013). The ability to directly spray an active patch onto a wounded patient allows for the control of fibre morphology, patch thickness, material choice, easy transport and storage of nanofibrous products and gives complete control over wound coverage and thickness. Polycaprolactone (PCL) was used as a carrier polymer along with 8 differing ratios of BC to generate BC-PCL composite nanofibres which could be exploited in use as emergency point-of-need wound care using a novel electrohydrodynamic gun (Aydogdu, M. O. et al., 2018). BC was processed into fibres after being suspended in dimethylformamide (DMF) and subjected to ultrasonication to form a gel-like solution that could be mixed with the PCL polymer solution. BC shows only slight solubility in DMF, but the sonication process reduces the particle size of the BC membrane to improve solubility.

From the electrohydrodynamic gun study on BC, it was found that the increase in BC content from 5 to 10 wt% resulted in an increased frequency of beads in the fibres (Aydogdu, Mehmet Onur et al., 2018). However, it was also observed that the bead count could be reduced by increasing the carrier polymer concentration. Other experimental studies show that the main factors which contribute to bead formation in electrospinning are to do with solution properties such as: low molecular weight, low concentration, low viscosity, high surface tension and low charge density (Fong et al., 1999). The solution properties of the BC-PCL solutions where experimentally measured, it was found that the increase of BC content from 5 to 10 wt% actually increased viscosity and electrical conductivity but only slightly increased the surface tension of the solution. The increased presence of beads in this case may be due to the rise in surface tension seen from the addition of BC, other than the other measured solution properties.

An important property of BC is it's biocompatibility and ability to mediate cellular interactions similarly to that of native tissue in numerous instances (Bäckdahl et al., 2006; Torres et al., 2012). The produced BC-PCL fibres where tested with Saos-2-human osteosarcoma cell line which had osteoblastic characteristics (Rodan et al., 1987). In an MTT assay after 72 hours, all BC-PCL fibrous samples showed cell viability in excess of 75%. It was found that by increasing the PCL concentration, the cell viability increased, possibly due to the increase in fibre diameter favoured by the cells. In the case for 5 and 15% PCL, cell viability increased with increasing BC content, however due to the cell viability of PCL

alone being very high, it is difficult to determine whether any increase in cell viability was due to an increase in BC content. Nonetheless, it can be concluded that a BC-PCL composite system is very capable of retaining an acceptable level of cell viability.

The cellular interaction with the BC-PCL scaffolds were observed by SEM. Cells appeared to cover the scaffold and fill the spaces in the nanofibre matrix. Here were two dominant cell morphologies that could be determined from the micrographs, the cells along the axial length of the fibres depicted an elongated morphology whilst globule-shaped cells where seen at the intersections of the fibres. The presence of the elongated cells indicated that cytoskeletal rearrangement may have taken place which has been previously reported to activate nearby receptors which affects gene expression (Curtis and Wilkinson, 1997). The ability for a material to absorb water is an important factor in a wound dressing, a high swelling ratio permits exudate absorption and the efficient exchange of nutrients and waste (Martin, 1997). All BC-PCL samples showed a high level of water uptake in swelling tests whilst the sample with the highest concentration of BC and polymer showing the highest swelling percentage.

Nerve tissue engineering is a popular topic in biomedicine due to the limited regeneration capacity of native nerves. A study into the production of nanofibrous scaffolds for enhancing peripheral nervous system neural tissue regeneration and neurite outgrowth was carried out using a BC-PCL polymer mix (Altun et al., 2019). When a gap larger than 3 cm between peripheral nerves occurs, axon regrowth is extremely difficult, nerve tissue engineering thus provide scaffolds that aid this crucial regeneration (Monaco et al., 2017). Here a concentration of 5% (w/w) BC was dissolved in a 50:50 solvent ratio of chloroform and DMF, dissolution required ultrasonic agitation of 5 hours over a period of 15 days. The dissolution process was captured optically every 3 days: days 1-3 showed no disintegration of the BC, days 4-6 showed slight disintegration, days 7-9 illustrated decomposition of the BC particles, at days 10-12 the dissolution process continued where whisker-like structures where observed, day 15 showed good dissolution (**Figure 5**). Mechanical strength is important in nerve tissue engineering as the constructs must be able to withstand the forces and motion of everyday interaction and movement where nerves will stretch and contract. The addition of BC into the fibrous scaffold doubles the tensile strength from 14.6 MPa to 29.3 MPa. The average diameter of the produces fibres for the PCL scaffolds was 527 nm and for the BC-PCL scaffolds there was a range of 70-120 nm.

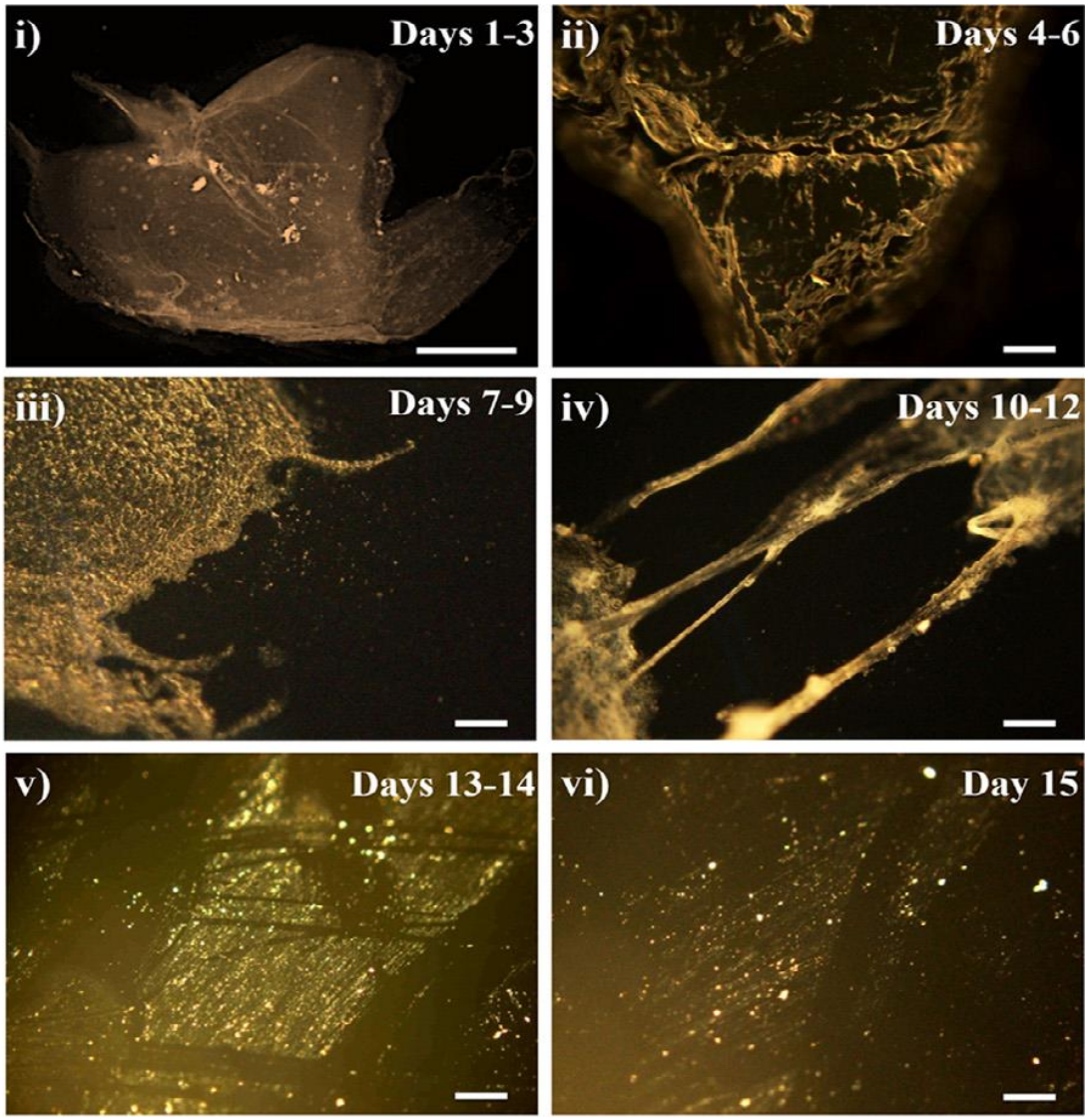

Figure 5: BC dissolution process is illustrated using optical microscope images: (i) Days 1–3, (ii) Days 4–6, (iii) Days 7–9, (iv) Days 10–12, (v) Days 13–14 and (vi) Day 15. Scale bar = 1 mm (Altun et al., 2019).

The hybridisation of fibre scaffolds with hydrogels improves mechanical durability and alters its biocompatibility and functionality (Kouhi et al., 2019). A concurrent electrospinning/electrospraying technique was utilised to produce fibrous hydrogel of keratin/ tragacanth gum-conjugated BC hydrogel (Azarniya et al., 2019). The setup was centred around a rotating mechanical mandrel where two separate electrohydrodynamic setups could deposit onto it, on one side was an electrospinning needle and on the other was an electrospraying needle. The benefit of this arrangement is that hydrogel particles can be uniformly embedded into the fibre network without having an effect on its porosity or diameter distribution. The hybrid product would act as a temporary skin substitute, in order to cope with the mechanical durability demands, BC was incorporated into the fibrous mats at different concentrations. In this work a concentration of 1,3 and 5 wt% BC was prepared in a solution with keratin and PEO where acetic acid was used as the solvent. The produced fibrous mats without BC had an average fibre diameter of 243 ± 57 nm. With the addition of BC, it was noticed that there were fibre breakdowns and a higher number of inter-fibre bonds present which may be

the result of BC affecting the solvent evaporation rate. The formation of fibre branches when BC was added can be explained by the theory that the surface of a conductive fluid jet can undergo statistic equilibrium undulations via the combined effects of surface tension and electric Maxwell stresses (Yarin et al., 2005). Remarkably, the average fibre diameter was reduced to 150 ± 43 nm when BC was added at 1% and subsequent higher conditions did not yield much change in the fibre diameter.

Hydrophobicity is an important characteristic to consider for materials in wound healing and in tissue engineering as it can affect biocompatibility of protein adsorption and cellular interaction with the material (Pertile et al., 2010). The keratin-based nanofibers produced without BC were hydrophobic and had a water contact angle of 126°. The addition of BC saw the hydrophobicity to significantly reduce and at 1 wt% BC, the water contact angle was 83°. This enhanced hydrophobicity of the fibres and is due to the hydrophobic nature of BC via its highly porous nonwoven network of nanofibrils. The incorporation of BC into the fibres also shows a significant enhancement in mechanical strength. At only 1% BC concentration and compared to keratin-PEO fibres, there is an increase from 7.1 MPa to 13.3 MPa in the tensile strength,123 MPa to 250 MPa in the elastic modulus and reduction in the elongation at break from about 15% to 10%. The enhanced mechanical durability of the BC-reinforced fibres is probably afforded by the reorientation of the BC fibrils and the entanglements between the keratin-PEO fibres (Astley et al., 2003). Furthermore, the interfacial cohesion between the BC and the keratin-PEO fibres in addition to the reduction in fibre diameter from the inclusion of BC can also be responsible for the improved mechanical properties (Wan et al., 2009). The study also carried out *in vitro* cell studies with the fibres, it was found that keratin-BC fibrous composites had an acceptable level of cytocompatibility as assessed through MTT assays where there was over 90% cell viability in L929 fibroblast cells (Azarniya et al., 2019).

6.2. Pressurised Gyration

Pressurised gyration is a hybrid fibre forming technique which combines solution blow spinning with centrifugal spinning to form low diameter fibres with a rapid production rate and can be used to generate bandage-like fibrous mats (Ahmed et al., 2019; Heseltine et al., 2018; Mahalingam and Edirisinghe, 2013). The setup consists of an aluminium vessel with multiple small apertures on its exterior which is connected to a high-speed motor and a gas inlet. The vessel rotates at high speeds and gas is infused simultaneously into the vessel which drives the polymer solution out through the orifices forming a polymer jet (Ahmed et al., 2018). The polymer jet gives rise to fibre production much like electrospinning as the solvent evaporates. This technique not only allows for very high throughput of production, but also allows you to control final fibre morphology by varying the rotation speed and the magnitude of applied gas pressure (Alenezi et al., 2019). Orientation of fibre bundles to generate mats of wound dressings can be manufactured in this way.

BC fibres blended with poly(methyl methacrylate) (PMMA) at several different ratios have been successfully formed with pressurised gyration to produce biocompatible fibrous scaffolds (**Figure 6**) (Altun et al., 2018a). 5 and 10 wt% of BC solutions were made in a 50:50 wt:wt ratio in DMF and tetrahydrofuran (THF).

The BC was subjected to ultrasonication for an hour in order to form a gel that could be spun using pressurised gyration. The ratio of BC:PMMA was altered and physical properties were determined along with further tests including SEM imaging, fourier-transform infrared spectroscopy (FT-IR) and cell proliferation studies. Solution viscosity and surface tension was discovered to have increased with elevating BC-PMMA wt ratios, similar with electrospinning, these parameters fundamentally alter fibre formation in pressurised gyration. SEM imaging showed greater particle count on the fibres with higher ratios of BC-PMMA, indicating that these particles were caused by the higher BC content. The FT-IR spectra on the BC-PMMA fibres confirmed presence of BC on the fibres as the profiles were consistent with that of pure BC and PMMA.

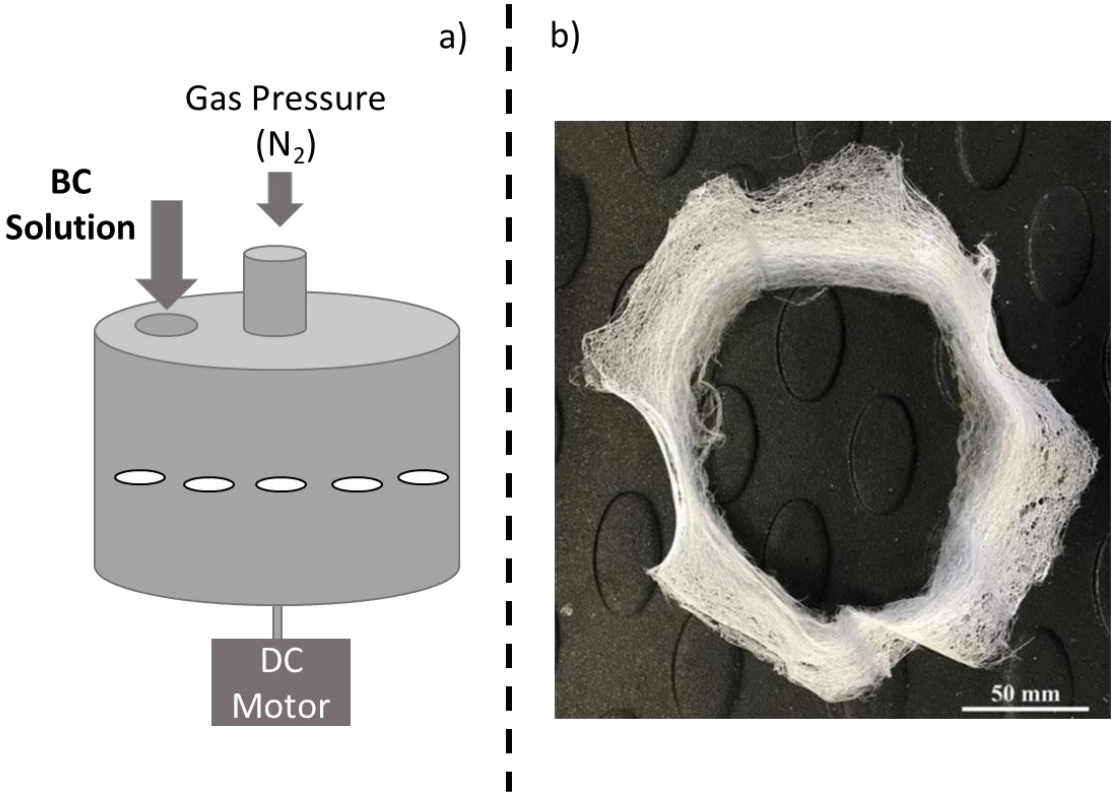

Figure 6: Schematic representation of a) pressurised gyration setup, b) Photograph of the bandage-like fibrous mat produced from the 5:50 (wt ratio) BC:PMMA blend.

Having applications in wound healing the scaffold must be biocompatible, non-toxic and must allow for adequate cell attachment, migration, proliferation and differentiation (Sachlos and Czernuszka, 2003). BC-PMMA scaffolds produced by pressurised gyration where investigated and found to be biocompatible with no indication of toxicity to the tested Saos-2 cell line. Adding BC to the BC-PMMA fibres increased cell viability compared to just solely using PMMA fibres. BC-PMMA scaffolds with 5 wt% BC were considered appropriate for wounds dressing applications because they retained cell viability of over 85%. The produced scaffold demonstrated cell spreading and proliferation of DAPI stained cells, the scaffolds showed enhanced metabolic activity compared to the control (**Figure 7**). MTT assays demonstrated that the scaffolds of 5 wt% had improved metabolic

activity and proliferation of the seeded cells compared to the 10 wt% BC. Furthermore, preliminary mechanical tests on the scaffolds revealed that the BC-PMMA fibres had lower stiffness and higher ductility, the tensile strength of 5:50 BC-PMMA was 2.6 times greater than PMMA fibres produced by electrospinning.

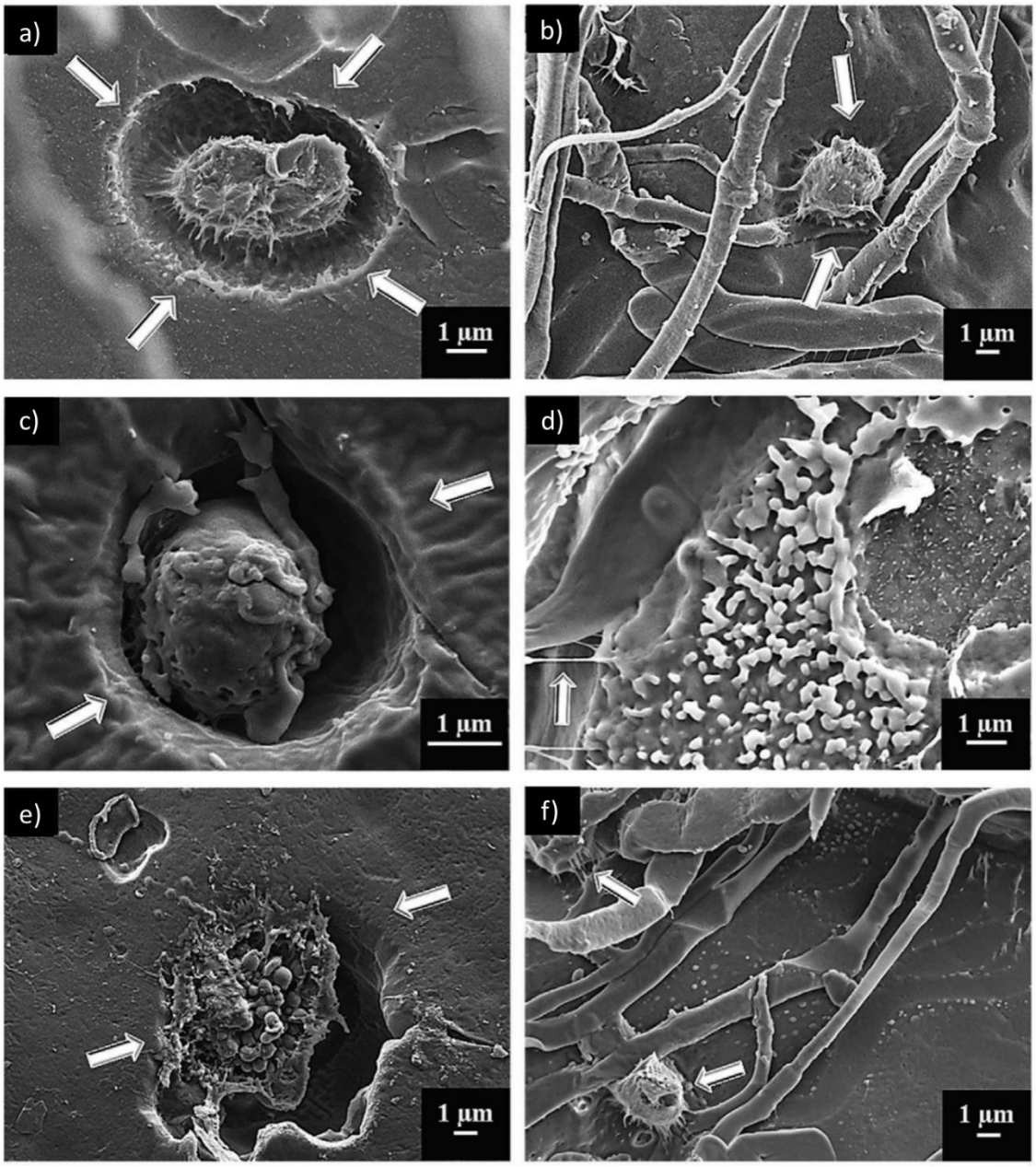

Figure 7: Scanning electron microscopy images of the BC:PMMA scaffold samples 72 hours after incubation with Saos-2 cell line with ratios of: a) 5:30, b) 10:20, c) 5:40, d) 10:30, e) 5:50, and f) 10:40. Arrows indicate embedded cells and their extension (Altun et al., 2018a).

Bandage-like polymeric structures were also produced using pressurised gyration using BC and PMMA blends with the addition of metallic antimicrobial nanoparticles (Altun et al., 2018b). In this study, BC was incorporated into a polymer solution of PMMA using sonication in a 50:50 solvent mixture of DMF and THF. Additionally, two types of nanoparticle mixtures were also added; one using Cu-Ag-Zn/CuO and the other including Cu-Ag-Tungsten carbide. The study

showed that BC-PMMA bandage-like fibres could be produced at a high yield with pressurised gyration and that these fibres can have antimicrobial nanoparticles incorporated for improved mechanical properties, higher water uptake ability and lower cell cytotoxicity.

An investigation into the maximal loading of BC in binary and ternary blends of fibres was carried out with an emphasis on production yield and mechanical properties by (Aydogdu et al., 2019). Poly(lactic acid) (PLA) and PCL fibres were created with and without blends of BC, eventually an optimised composite of PCL-PLA-BC was also created. For pure PLA fibres, there was a 92% yield, and the addition of BC into the polymer matrix caused a deterioration of yield down to 54% at only 10 wt% BC. It was observed that a huge fall in yield occurs as a result of higher BC loadings, as attested to by many other articles (Altun et al., 2018b; Aydogdu et al., 2019; Azarniya et al., 2019). Pure PCL fibres had a yield of 87% and saw a drop to 61% yield when loaded by 10 wt% BC. PLA and PCL composites were also produced and tested to compare the ternary behaviour of the different polymer systems. The 90:10 PLA-PCL blend had a very high yield of 97%, which also showed that these polymers worked very well as composites.

A BC concentration of 30 wt% was deemed the highest concentration whilst maintaining an acceptable level of yield (> 30%) and mechanical integrity. The BC in the polymeric solution also caused an increased frequency of beads within the fibres. As expected, the addition of BC to the solutions lead to an increase in viscosity and thus caused thicker fibres to be formed in the presence of BC.

With an increasing concentration of BC in PLA binary systems, the ultimate tensile increases with each 10% increment. PLA alone has a tensile strength of 2.3 MPa, at 10 wt% BC concentration the tensile strength is 3.8 MPa, 20 wt% it's at 5.4 MPa and at 30 wt% it is 6.5 MPa. At 40 wt% BC concentration, the PLA fibres lose mechanical integrity and the tensile strength drops to 2.3 MPa as the BC content increases. This drop in tensile strength corresponds with the reduced fibre count and yield with high BC levels which impairs the integrity of the bandages. The results for the stiffness of the PLA-BC binary system follows the same trend. The stiffness of PLA increases from 10 wt% to 30 wt% of added BC, it then falls sharply at 40 wt% and continues to drop.

The mechanical behaviour of the PLA-BC binary polymer system follows a similar trend with the PLA-BC polymeric fibres. With 100% PCL, the tensile strength is around 2.3 MPa, the addition of 10 wt% BC creates an increase in tensile strength to about 2.7 MPa. PCL proves to be a superior carrier of BC compared to PLA when comparing tensile strength as 50 wt % BC shows the highest value at around 6.7 MPa. At a 100% concentration of PCL, the Young's modulus is around 23 MPa, the addition of BC at 10 wt % causes an increase of stiffness to about 27 MPa and at a 40 wt % concentration of BC the stiffness drops to ~ 12 MPa.

This study then focused on the production of PCL and PLA fibres with BC loading, ultimately to design an optimised ternary polymeric system with a mixture of PCL, PLA and BC. The optimised ternary sample consisted of 70 wt% mixture of PLA and PCL and 30 wt% BC, it had a higher tensile strength than both PCL and PLA at around 9 MPa and had a high stiffness of around 19.6 MPa. It showed that BC can be used in binary and ternary polymeric systems to produce fibres that can benefit from the mechanical characteristics of multiple polymers.

6.3. Bacterial Cellulose Solutions

Due to the large number of inter- and intra- molecular hydrogen bonds, BC is very difficult to process into solution, which is a necessity in order to generate fibres using major methods such as electrospinning. BC is an especially insoluble material and does not dissolve in common organic solvents such as acetone, chloroform and DCM. Experimental results show that BC has partial solubility in 8.5 wt% aqueous sodium hydroxide (NaOH) solution (Łaskiewicz, 1998). Even then, temperatures of -5°C are required, only about 20 wt% of the cellulose is dissolved and the degree of polymerisation of the BC source must be low too. The solubility of BC in NaOH solution can however be further increased when 1 wt% urea is added. Even then, BC is not completely soluble in these conditions, and the use of such acids and chemicals can lead to toxic production environments and hazardous industrial waste.

High molecular weight BC was discovered to be soluble in a binary solvent system of lithium chloride/N,N-dimethylacetamide (LiCl/DMAc) (Shen et al., 2010). It was also found that the type of BC membrane and how it was formed had a large effect on its solubility with these solvents. BC samples with large grains in their microstructure were more prone to form large gels during the swelling stage of dissolution which hindered additional diffusion of the solvent into the fibres. The samples that showed good solubility were those that were in powdered form, having much higher surface area to volume ratio. There are several activation procedures that can improve the initial solubility of cellulose and BC including treatment with liquid ammonia, freeze drying and swelling in water followed by solvent exchange in dimethylacetamide (Morgenstern and Berger, 1993; Rohrling et al., 2002). These activation steps are thought to induce inter- and intra-crystallite swelling, increase accessibility and break of hydrogen bonds. Temperature was also found to have a marked effect on dissolution where temperatures below 45°C caused difficulty in dissolution and activation temperatures over 60°C showed greater dissolution.

BC with a high degree of polymerisation (6500) was dissolved in 1-n-butyl-3-methylimidazolium where temperatures of 80°C and 12 hours of mechanical stirring were required (Schlufter et al., 2006). The dissolution by 1-n-butyl-3-methylimidazolium was found not to significantly degrade the polymer chains. The ionic liquid, 1-allyl-3-methyl-imidazolium chloride was also used to dissolve BC but a transition from cellulose I to the cellulose II allomorph was observed with the resulting electrospun fibres (Chen et al., 2010).

Although solubility of BC has been observed with some ionic liquids, the case remains that these solutions would pose an obstacle in the mass production of BC fibres and other derivative wound care materials. Firstly, the acute toxicity of these liquids is a great concern at both the factory level and through run-off. For example, the toxicity caused by 1-butyl-3-methylimidazolium chloride was investigated in zebrafish and it was found to cause oxidative damage as well as DNA damage (Zhang et al., 2017). Furthermore, the economics of such solvent systems, binary and otherwise, increase the costs to the end consumer with higher processing expenditures and prolonged manufacturing times. High temperature

processing of BC increases energy input during manufacturing which is both environmentally and economically detrimental.

7. Future Developments and Conclusions

The secondary processing of BC has proven to be difficult. Due to its nature, large scale production of BC in wound care materials is not feasible. Therefore, by reprocessing the BC into secondary fibres and blends, there can be a more commercially feasible methods of mass-producing for the healthcare market. The answer may lie in fibre forming techniques such as electrospinning and pressurised gyration, these methods allow for the tailoring of the fibre structure to best suit for wound healing applications.

However, the solubility of BC has played a major obstacle in forming spinnable solutions. Work needs to be done to discover solvents that can dissolve the BC membrane in a non-toxic and economical manner, as well as to not remove the fundamental properties of high utilisation value. Spinnable solutions can then be processed into fibres, added to blends containing other natural polymers which can have antibacterial and pro-wound healing effects.

An alternative approach into forming BC solutions can be to use mechanical force, whereby the BC membrane is broken into smaller particles or fibrils which may improve its solubility in several solvents. Such an approach has been used to spin BC-PMMA scaffolds as discussed previously where high frequency ultrasound has been used to form a gel-like spinnable solution within a carrier polymer. As discussed earlier, the benefit of using ultrasonication is that the crystal structure of the BC is not adversely affected and thus the beneficial wound-healing properties of the material can remain. Moreover, other mechanical methods of reducing BC size can be investigated, such as grinding or blending the BC into particles. The efficacy of such particles in wound healing needs to be also determined.

Blends of BC within different polymers, both synthetic and natural could prove to be a beneficial commodity in wound care. Composite materials with desired properties such as biocompatibility, biodegradability and anti-bacterial properties can be used to develop wound dressings that overcome the limitations of the production limitation of BC. There are many polymers systems yet to be trialled, even with the difficulty of processing BC, it can still be used to enhance the mechanical and biological properties for effective wound healing.

The remarkable properties of BC were only discovered in the mid-1980s, where before the applications of the it was only really limited to food production of nata-de-coco. Since then, there has been a steep incline in the number of research articles and patents relating to BC and various methods for extraction and processing.

A considerable challenge to overcome in BC technology is the unearthing of a suitable carbon source that is cheap and that does not compete with the production of food. Nevertheless, forming BC membranes into secondary fibres could maximise the use of the material in wound care applications and reduce the volume required to have its clinical effects. There are still many hurdles remaining for the wide use of BC in healthcare settings, but with the abundance of research

and patents, we could be on the verge of incorporating this very significant and valuable material in crucial advanced technology applications worldwide.

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Conflict of Interest

The authors declare no conflict of interest.

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Bacterial Cellulose Micro-Nano Fibres for Wound Healing Applications

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Abstract

Bacterial cellulose (BC) is cellulose produced by a few limited species of bacteria in given conditions. BC has many remarkable properties such as its high mechanical properties, water uptake ability and biocompatibility which makes it a very desirable material to be used for wound healing. Inherently due to these important properties, the material is very resistant to easy processing and thus difficult to produce into useful entities. Additionally, being rate limited by the dependency on bacterial production, high yield is difficult to obtain and thus secondary material processing is sought after. In this review, BC is explained in terms of synthesis, structure and properties. These beneficial properties are directly related to the material's great potential in wound healing where it has also been trialled commercially but ultimately failed due to processing issues. However, more recently there has been increased frequency in scientific work relating to BC processing into hybrid polymeric fibres using common laboratory fibre forming techniques such as electrospinning and pressurised gyration. This paper summarises current progress in BC fibre manufacturing, its downfalls and also gives a future perspective on how the landscape should change to allow BC to be utilised in wound care in the current environment.

Keywords: Bacterial Cellulose, wound healing, fibres, *Gluconacetobacter xylinum*, fibre production

1. Introduction

As early as in the 19th century A.J Brown, noted that a specific bacterium produced a solid membrane at the surface of his culture when grown in a carbohydrate-rich medium (Brown, 1886). Later studies demonstrated that the material of the membrane produced by these bacterial species were identical to the principle structural polysaccharide of plants, cellulose (Hibbert, 1930). In contrast to plant cellulose, the gelatinous membrane showed incredibly high strength, purity, porosity, a uniform fibre network and enhanced water holding ability (R. Chawla et al., 2009). The cellulose produced by the bacterial genera *Gluconacetobacter* (formerly *Acetobacter*) are commonly called bacterial cellulose (BC), which is in itself a biopolymer. Moreover, BC demonstrates the fascinating ability to enhance

wound healing recovery, revealing the potential to revolutionise the healthcare market (Sulaeva et al., 2015). The cost of wound care for any healthcare provider marks a significant portion of overall expenditure. In hospitals, more than 30% of the beds are occupied by patients having wounds, some of whom who do not require to stay in the hospital for their main disorders (Posnett et al., 2009). With the rise in global average life expectancy, chronic wounds have shown strong correlation with increasing age (Gould et al., 2015).

There is a growing pressure for the development of advanced wound care that has capacity to meet the soaring demands. Although there is an abundance of literature on BC and its applications, there is little on the processing of BC into biomaterials for wound healing, especially in fibrous structures (Carvalho et al., 2019; Picheth et al., 2017; Thomas, 2008). This review focuses on the structure and properties of BC, current progress on its processing for wound care applications and what is necessary to overcome in order to widely use this astonishing material in healthcare settings.

2. Bacterial Cellulose (BC) Synthesis

This cellulose is commonly referred to as “bacterial cellulose” or “microbial cellulose” which is found as a gelatinous membrane at the liquid-air interface of the culture medium (Kamide et al., 1990). BC is produced at certain culture conditions by a number of bacteria belonging to the genus: *Achromobacter, Aerobacter, Agrobacterium, Alcaligenes, Azotobacter, Gluconacetobacter, Rhizobium* and *Salmonella* (Rangaswamy et al., 2015). Yet, the gram negative *Gluconacetobacter xylinum,* has been primary focus in most BC related studies as the cellulose production is far greater in quantity and mass than the other strains, is of extraordinarily high purity and closely resembles that of algal and plant cellulose in its microfibrillar structure (Mikkelsen et al., 2014). Many strains of *G. xylinum* retain the ability to extracellularly produce cellulose in the form of flat, twisting ribbons. *G. xylinum* is an aerobic soil bacterium which belongs to a family of bacteria which are able to ferment carbohydrates into acetic acid (vinegar) (Peggy O'Neill and Cannon, 2000).

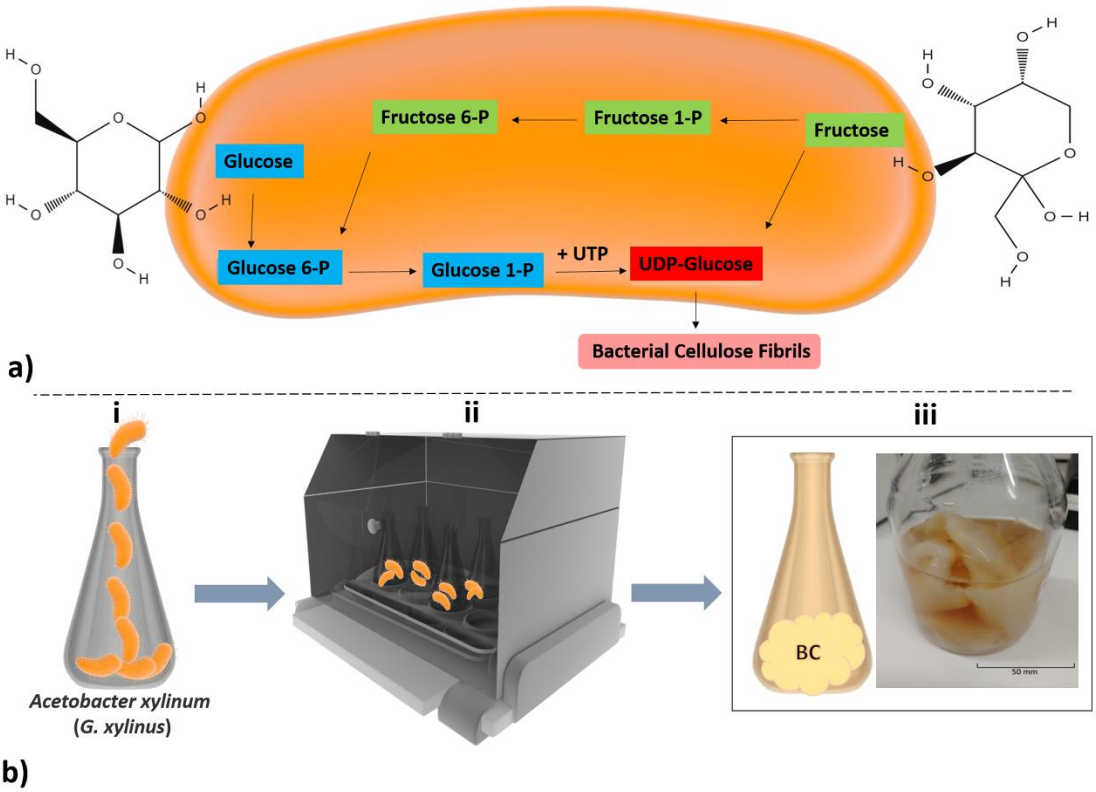

Figure 1: Schematic diagrams of: a) BC fibrils synthesis reaction from glucose and fructose pathways. b) Schematic representation of BC synthesis (i) *Acetobacter xylinum* (*G. xylinus*), (ii) *Acetobacter xylinum* (*G. xylinus*) incubation, (iii) Photograph of bacterial cellulose (BC) gelatinous membrane encased within a 200 mL glass vial and suspended in acetic acid.

The synthesis of cellulose in *G. xylinum* occurs in a multi-step biochemical pathway of reactions beginning with glucose, which is catalysed by multiple enzymes. Cellulose synthesis is considered to be the most crucial enzyme in the BC production process and is responsible to the catalysis of the step preceding the final cellulose production (Ross et al., 1990). The commonly accepted pathway for cellulose production in *G. xylinum* cultures can be summarised as (**Figure 1A**): Glucose (catalysed by glucokinase) → Glucose-6-Phosphate (catalysed by phosphoglucomutase) → Glucose-1-Phosphate (catalysed by UDP-glucose pyrophosphorylase) → UDP-Glucose (catalysed by cellulose synthase) → Cellulose (Klemm et al., 2001).

A single cell of *G. xylinum* has been shown to be able to polymerise up to 200,000 glucose molecules per second into ß-1,4-glucan chains (Hestrin and Schramm, 1954). These chains are extruded into the surrounding medium from the pole of the bacterial rod, which form a single ribbon-like bundle of microfibrils composed of single twisted strands (Ross et al., 1991). This ribbon elongates with the cell envelope at a rate of 2 µm per minute and remains associated during cell division, at the liquid-air interface the suspensions continue with their microfibrillar projections for several hours, giving rise to a cellulosic pellicle (Brown et al., 1976). The fibrils of the ribbons are in close association with the pores longitudinally positioned in the bacterial cell membrane, cellulose biogenesis in *G. xylinum* is one of the best proven examples of unidirectional growth of cellulose microfibrils.

(Zaar, 1979). A single cellulose fibril can be visualised as a cable where the lengthwise strands are D-glucose composed polymeric chains, each chain containing uniformly linked sugar monomers by ß-1,4 glycosidic bonds (Ross et al., 1991).

G. xylinum cultures are characterised as a thick glutinous cellulosic surface mat (**Figure 2)**. This gelatinous membrane (pellicle) is where the embedded cells have direct contact with the liquid/air interface (Schramm and Hestrin, 1954). *G. xylinum* grows and forms cellulose in a range of carbon sources which include glucose, fructose and glycerol (Jonas and Farah, 1998; Mikkelsen et al., 2009; Weinhouse and Benziman, 1974). The growth, metabolism and cellulose production of this bacterium is free from cellulase activity which would otherwise break down the cellulose, this provides a distinct advantage over plant cellulose by being metabolically inert and highly pure (Vandamme et al., 1998).

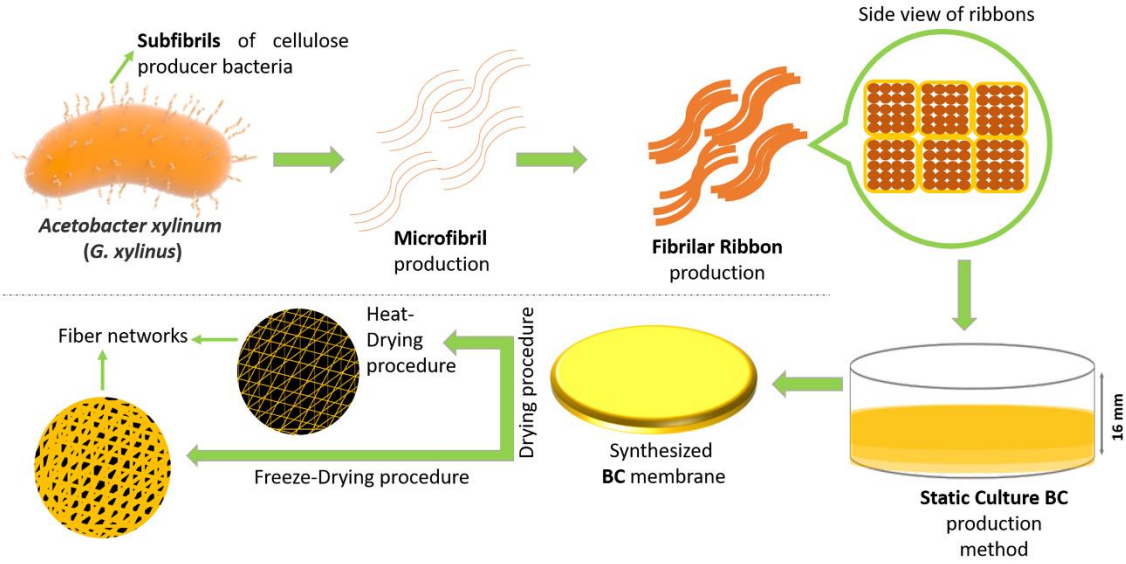

Figure 2: Diagrammatic representation of BC from microfibrils to fibre networks production, step by step in static conditions. Side view depiction of a thick BC gelatinous membrane mat which assumes shape of environment, shown here on a petri dish. The mat contains highly pure network of BC nanofibrils.

Several techniques exist for BC production that demonstrate different degrees of potential for economical and commercially viability as a BC fabrication method. The selection of the cultivation method stringently determines the cellulose microstructure and thus its mechanical and physical properties. Static culture methods (**Figure 2)** employ stationary culture in plastic trays or dishes and have shown to produce a thick and gelatinous BC membrane on the surface of the culture medium which compares with most BC produced and tested (Budhiono et al., 1999; Dudman, 1960). The BC pellicle in a static culture is visible at the surface of the liquid about 2 days from the beginning of the process (Schramm and Hestrin, 1954). An alternative approach to BC cultivation is incorporating an agitated culture such as jar fermenters, horizontal fermenters or internal loop airlift reactors (Kouda et al., 1997; Kouda et al., 1996). Agitated culture approaches can produce cellulose in fibrous suspension forms, pellets, spheres or irregular

masses (**Figure 1B**) (Chao et al., 2000; Naritomi et al., 1998a; Tsuchida and Yoshinaga, 1997).

Static culture systems have been widely investigated and their applications have seen successful commercial applications such as in food and in electronics (Bernardo et al., 1998; Yamanaka et al., 1989). Nevertheless, agitated culture methods are usually deemed more suitable for large scale production due to their higher potential production rates when considering total area of cultivation required. There are, however, many problems that are encountered with cellulose production in fermenters that utilise continuous aeration and agitation. The sporadic presence of non-cellulose producing mutants (*Cel*$^{-}$), leads to the decline in biopolymer production in agitated cultures (Jung et al., 2005; Ross et al., 1991). These mutants are a result of the inactivation of the gene coding for cellulose synthesis (Krystynowicz et al., 2002). In static conditions, cellulose-synthesising *Gluconacetobacter* cells (*Cel*$^{+}$) migrate towards the oxygen-rich medium air interface, where they produce the gelatinous membrane. The membrane limits access to oxygen into the lower depths of the culture and majority of the cells are found in the *Cel*$^{+}$ form. In agitated systems, the uniform aeration leads to preferential growth of bacterial cells instead of cellulose synthesis, in this case the culture is dominated with *Cel*$^{-}$ mutants (Krystynowicz et al., 2002). Furthermore, it was shown that static cultures of *G. xylinum* actually leads to higher yield levels than with swirled cultures, at a period of 2 days following incubation yield was 1.8 x higher in static cultures than with agitated and after 5 days yield was 2.8 x higher in static conditions (Schramm and Hestrin, 1954). Static systems can be less favourable for scale up operations due to the amount of free space required and could limit productivity rate.

Culture conditions can have a marked effect on cellulose production for many different strains of bacteria capable of producing BC (Rangaswamy et al., 2015). Factors such as inoculum density influence the microbial cellulose production, where increasing the concentration of the substance can lead to a reduction in yield, therefore there is an optimum density which needs to be considered. Additionally, there exists an ideal pH range in which both cell growth and cellulose production is the greatest. In tested conditions from pH 3-7, it was found that a pH of 6 led to maximum yield compared to the other pH values (Rangaswamy et al., 2015). Temperature furthermore effects cellulose production where favourable culture temperatures are around 28-30 °C and when temperatures exceed 40 °C, BC production was not observed. Carbon is the sole source of BC production and thus has a significant influence on the yield of BC and its final morphology. Carbon sources such as fructose, glucose, lactose, maltose, mannitol, mannose and sucrose can be utilised to produce BC from different bacteria, maximum yields are usually observed with using sucrose as the carbon source (Eslahi et al., 2020; Wang et al., 2019). Nitrogen is another essential component in cell growth and cellulose production for many bacterial strains, examples of nitrogen sources are: ammonium chloride, ammonium nitrate, ammonium sulphate and peptone. Optimal BC preparation for certain bacteria can result from the use of peptone as the source of nitrogen. On the other hand, cellulose formation from *G. xylinum* and glucose has been observed to be limited by the oxygen concentration of the culture, where negligible BC was produced with nitrogen and maximal amounts where produced with 100% oxygen (Schramm and Hestrin, 1954).

3. Structure of Bacterial Cellulose

Similar to that of plant cellulose, BC shares the same molecular formula $(C_6H_{10}O_5)_n$. The exopolysaccharide-produced BC differs from conventional cellulose in its physical and chemical features. The two cellulose types bear the same chemical similarity being ß-1,4-glucans, but differ in their degree of polymerisation (Yoshinaga et al., 1997). The degree of polymerisation for BC is considerably lower, having a typical polymerisation range between 2000-6000 compared to 13000-140000 of plant cellulose.

BC is composed of twisted ribbon-shaped fibrils approximately 50-100 nm in width and 3-8 nm in thickness (Astley et al., 2001; Brown et al., 1976; Yamanaka and Sugiyama, 2000; Zaar, 1977). It has been shown by X-ray diffraction (XRD), that the size of the microfibrils are associated with its crystallite size (Haase et al., 1974). These ultrafine ribbons have a length of 1-9 µm and form a densely arranged structure stabilised by comprehensive inter-and intra-hydrogen bonding (Bielecki et al., 2005; Esa et al., 2014). The average distance between junction points (pore size) of a typical BC membrane has been calculated to be 0.523 ± 0.273 µm, and the orientation of the segments as the average angle formed between the x-axis and the segments is 85.64 ± 0.56° (J Grande et al., 2008).

The macroscopic structure and morphology of BC fibres are strictly dependant on the cultivation techniques used to produce them (Watanabe et al., 1998). In a static culture, the bacterial cells produce cellulose mats at the surface of the nutrient broth where the interface between the liquid and the oxygen rich air exists. In these conditions, *G. xylinum* cells continuously extrude subfibrils of cellulose from their surface pores which in turn become crystallised into microfibrils, and are forced down deeper through the growth medium (Bielecki et al., 2005). As a result, the cellulose produced in static conditions result in leather-like pellicles which support the population of *G. xylinum* cells. These pellicles consist of overlapping and intertwined cellulose ribbons which form a grid of parallel but disorganised planes (Jonas and Farah, 1998). Comparatively with cellulose produced in agitated cultures, the adjacent strands of the cellulose mats branch and interconnect to a higher degree prevalent in static cultures. In agitated conditions, the increased branching is observable in the form of fibrous strands and irregular granules dispersed thoroughly through the culture broth (Vandamme et al., 1998). Furthermore, the agitated BC interconnect to form a grid-like pattern (Watanabe et al., 1998). The differences in morphology between cellulose produced by agitated and static conditions also contribute to differing levels of crystallinity, crystallite size and the content of cellulose I_α. The schematic BC microfibril model, physical properties and biomedical application areas are shown in **(Figure 3).**

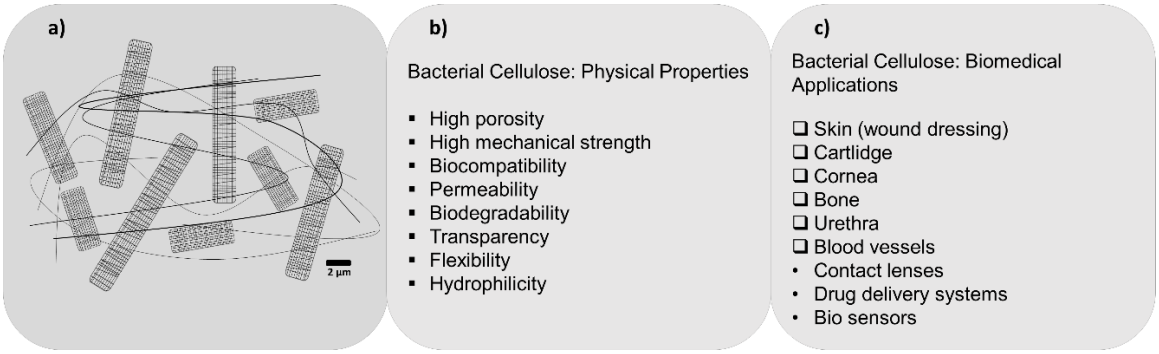

Figure 3: a) Schematic diagram of BC microfibrils, showing a unique structure that isn't commonly found in cellulose, b) Physical properties of BC (Hussain et al., 2019), c) Biomedical applications of BC (Gallegos et al., 2016; Portela et al., 2019).

Further differences between agitation produced BC and statically produced BC are obvious when viewed using a Scanning Electron Microscope (SEM). Statically produced BC have fibrils with a more extended morphology with fibrils stacked above one another in a crisscross pattern. Conversely, strands of agitation produced BC reveal an entangled and curved physiology (Johnson et al., 1989). Compared to plant cellulose, BC has a unique characteristic in its crystalline structure. Native cellulose consists of cellulose Iα and cellulose Iβ crystalline structures, where cellulose Iβ is the major component, approaching approximately 60% in composition. (VanderHart and Atalla, 1984; Yamamoto and Horii, 1993). Interestingly however, BC contains 60% cellulose Iα (Atalla and Vanderhart, 1984).

Another key difference between plant cellulose and BC lies in their morphological structures. In plant cellulose, several cellulose molecular chains assemble to form microfibrils. This assembly subsequently leads to the development of high-order bundles and clusters called fibril lamella and fibre cells (Shoda and Sugano, 2005). Plant cellulose forms a complex structure with impurities such as lignin and hemicellulose. Contrariwise, BC is secreted by *G. xylinus* cells fashioned into a ribbon-like structure composed of microfibril bundles. The fibre diameter of these ribbons are over a hundred times thinner than that of plant cellulose (Guhados et al., 2005). Due to the special ultrafine reticulated structure of BC, there are many unique characteristics that become apparent in their potential and current applications, these are discussed in the next section.

4. Properties of Bacterial Cellulose

BC has a wealth of useful properties that allow it to be used in a wide range of applications, especially in industry and healthcare. The properties are dependent on the structural features as mentioned previously. When the BC pellicle is chemically purified and dried on a flat substrate, a thin and translucent cellulose membrane is established. This membrane holds a plethora of unique properties due to its fine and continuous network of crystalline microfibrils, both in its dried and wet (never-dried) state (Shibazaki et al., 1993).

BC has been discovered to have the highest Young's modulus of any two-dimensional organic material, at a staggering stiffness value of 15 GPa. The

extraordinarily high stiffness arises from the strong interfibrillar binding in the network of its ultrafine fibrils and also owning to its high crystallinity (Yamanaka et al., 1989). The effect of sodium hypochlorite (NaClO) and sodium hydroxide (NaOH) on the stiffness of the BC was investigated, the Young's modulus of the BC sheets further increased to 23 GPa at a 0.5% concentration of NaClO and approached 30 GPa at a concentration of 5% NaOH (Nishi et al., 1990). Therefore, the mechanical properties of BC can be further improved with the treatment of alkaline or oxidative solutions, which can be beneficial in many industrial applications where greater stiffness is required. Post-processing of BC allows its mechanical properties to be tailored by exposing it to different chemical treatments, this is especially useful in applications where a highly specific stiffness is desired such as in tissue engineering and cellular wound healing (Chen et al., 2015; Wang et al., 2012).

BC shows further favourable mechanical properties with high tensile strength, afforded by its highly crystalline structure and fine diameter network of fibres which work together in unison with tensile loads. With a density of 1600 kg/m^3, BC microfibrils have an individual Young's modulus of 138 GPa and a tensile strength of more than 2 GPa (Dobre et al., 2010; Nishino et al., 1995). Aramid fibres, a class of heat-resistant and highly strong synthetic fibres used in body armour fabric and ballistic composites, show similar tensile strengths to that of BC, proving how much strength there is in its dense nanofibre network (Young et al., 1992). BC has shown good potential in material reinforcement in various composites which gives the newly formed composite greater mechanical properties (Gindl and Keckes, 2004; Yano et al., 2005).

Tissue engineering is a rapidly growing field which aims to restore, repair or maintain the function of various vital tissues and organs (Stock and Vacanti, 2001). Biomaterials have been widely used as tissue engineering scaffolds where an ideal material would successfully mimic the extracellular matrix and be able to guide the necessary cells towards effective tissue reformation. Being a natural polymer, BC proves to retain a high level of biocompatibility as shown by studies which show the *in vitro* and *in vivo* biocompatibility of BC. Especially, implantations of BC within rat models have successfully demonstrated biocompatibility with the absence of macroscopic indications of inflammation in response to the implant within the animal (Helenius et al., 2006). Absence of fibrotic encapsulations together with the absence of giant cells point towards good biocompatibility of the material in *in vivo* conditions. The results here are not surprising given that cellulose-based materials are generally considered biocompatible and thus invoke negligible inflammatory and foreign body responses (Miyamoto et al., 1989).

BC pellicles demonstrate a high level of chemical purity due to the absence of hemicellulose, lignin, pectin and other biogenic compounds (Song et al., 2009). Removal of hemicelluloses and lignin from cellulosic materials require difficult post processing which adds time and cost and would otherwise pose an economic burden in the manufacturing industry (Frederick et al., 2008). The energy requirement for the purification of BC is considerably lower than that of other cellulosic materials, allowing for a reduction in processing costs and chemically-intensive processes which can form hazardous waste products (Gea et al., 2011). Compared to plant and other cellulose sources, BC offers a more economical (in

terms of purification) and environmental source of cellulose which is unfortunately limited by its production rate.

Due to the nature of its ultrafine fibre network, BC has a very large surface area per unit mass, which gifts it the ability of having a very large water holding capacity. BC can hold up to 200 times its own dry mass in water, the majority of this liquid is not bound to the polymer and can be easily released via gentle pressing (Lin et al., 2009; Schrecker and Gostomski, 2005; Shezad et al., 2010). The excellent water holding capacity and water release rate of BC make it suitable as wound dressings. Capillary forces are responsible for holding the water in the cellulose pore structure where water is bound to the cellulose fibrils with hydrogen bonding (Gelin et al., 2007; Ul-Islam et al., 2012). Despite its high water holding ability, the actual BC fibres are very hydrophobic which permits it to be used in a wide range of civil and industrial applications (Feng et al., 2002; Marins et al., 2011; Yuyang et al., 2006).

XRD analysis on static-culture produced BC shows that this material has a crystallinity index of 50% (Krystynowicz et al., 2002). Cellulose produced by bacteria grown in agitated cultures have shown to acquire a reduced crystallinity compared to those produced in stationary cultures (Czaja et al., 2004). The movement and rotation in agitated cultures cause an external force of disturbance to the fibril crystallisation process, leading to lower crystallinity (Yan et al., 2008). Due to its high crystallinity however, BC has an incredibly low solubility and thus is limited in its processability (Hu et al., 2014). It is insoluble in most common solvents that are used in the manufacturing industry which limits its potential applications in these fields. A few solvents have been found to dissolve BC such as lithium chloride with N,N-dimethylacetamide, sodium hydroxide/urea aqueous solutions and some ionic liquids (Lu and Shen, 2011; Phisalaphong et al., 2008; Shen et al., 2010). These solvents however pose problems in terms of processing costs, health and safety issues due to toxicity, environmental devastation and can also negatively alter the properties of the BC (Aral and Vecchio-Sadus, 2008; Qin et al., 2014). On the other hand, the low solubility of BC can be advantageous in applications where the stability of the material in response to various gas and liquids is crucial, such as in air or water filtration systems (Kosmider and Scott, 2002).

Cellulose, being the most abundant natural homopolymer, shows excellent biodegradability from both plants based and bacterial sources. BC is completely biodegradable in a wide range of environmental conditions, which makes it a promising candidate in environmental protection, biomaterial and tissue engineering applications (Li et al., 2009; Wan et al., 2009). Another considerably attractive advantage of BC is its ability to be physically moulded into any form or size during synthesis (Bäckdahl et al., 2008). This mouldability does not come at the expense of causing any notable alteration to its physical properties. For example, BC grown in a petri dish will take up the shape and volume of the dish and will be formed into a circular gel-like pellicle. A summary of the properties of BC relating to wound healing can be found in **Table 1**.

Table 1: Table summarising the key properties of BC and its relevance to wound healing.

Property	Advantage	Benefits to Wound Healing	References
Biodegradability	Bandage for chronic wounds potentially doesn't need removing	Reduction of pain from bandage removal	(Hu and Catchmark, 2011; Laçin, 2014)
ECM Resembling Matrix	Biomimetic structure promotes prompt wound healing	Cells of the wound response can be guided to become more efficient	(Svensson et al., 2005; Wu et al., 2014)
Excellent Biocompatibility	Reduces complications with immune rejection	Risk of fibrotic scarring is lower	(Helenius et al., 2006; Torres et al., 2012)
High Stiffness	Great Durability	Allows bandage to withstand some trauma	(Lin et al., 2013; Nakayama et al., 2004)
High Tensile Strength	Resistance against tearing as a wound dressing	Provides mechanical protection against external trauma	(Naritomi et al., 1998b; Wan et al., 2009)
High Water Uptake Ability	Maintains moist environment and flow of wound exudate	Allows for a more efficient recovery process and management of osmotic environment of cells	(Lin et al., 2009; Schrecker and Gostomski, 2005; Ul-Islam et al., 2012)
Large Surface Area	Increased interactions with cells in the wound response	More efficient cellular interactions leading to a healthier recovery	(Iguchi et al., 2000; Nishi et al., 1990)

5. Wound Healing

The unique structural and mechanical properties of BC make it suitable for use in a variety of applications such as in food, electronics and medicine (Fontana et al., 1990; Jagannath et al., 2008; Shibazaki et al., 1993). However, out of all the applications, BC has revealed outstanding potential in wound healing and wound care products. The benefit of advanced wound care products and services that address infection and recovery times will function to revolutionise the healthcare industry, its impact would be remarkable for the entirety of the human population. As mentioned previously BC has valuable properties such as its high crystallinity, water holding and absorption capacity, low solubility in solvents and high tensile strength (**Figure 3B**). These features are all beneficial for skin repair materials.

A good wound repair material has the important characteristic to be able to absorb exudate during and after application and removal. Currently available wound care materials have traditionally showed good absorbance and permeability such as with gauzes which adhere to desiccated wound surfaces, but on removal can cause trauma and damage to the wound site (Boateng et al., 2008). When considering the properties of BC to current wound care materials, BC shows

incredible promise in overcoming the downfalls associated with current dressings. Consequently, BC membranes have been used as either wound dressings or skin substitutes. The membrane produced by the bacteria can be directly used from the culture by simply washing the pellicle with water. BC can also be processed further if need be to suit the exact wound healing application.

In the late 20th century, BC was first used as a temporary skin substitute and biological dressing under the trade name BioFill®, now known as Dermafill™ (Fontana et al., 1990). The product was intended to treat patients suffering from various skin wounds as a result of burns, dermabrasion, cuts and ulcers. Since then, many other BC based products have been commercially available for topological application for wound recovery. Studies show that the use of BC membrane-based dressings establish superiority to conventional materials in reducing wound pain, retaining exudate, accelerating and facilitating re-epithelialisation, reducing total healing times, diminishing infection rates and reducing visible scarring (Czaja et al., 2006; Czaja et al., 2007; Fontana et al., 1990). Moreover, due to the translucency of the BC dressing, it is remarkably simple and easy to inspect the wound, without interference or removal of the membrane from the patient.

During the wound healing process, correct moisture levels are required for efficient recovery times. Having a high-water holding ability, BC allows for the wound site to have the ideal moisture conditions. Furthermore, due to the network of its nanofibres, the membrane will prevent infection by creating a physical barrier that will prevent bacteria infiltrating into the wound site preventing the risk of infections (Kaewnopparat et al., 2008; Shezad et al., 2010). The heating of the skin in burn victims causes the breakdown of the semi-permeable membrane associated with the lipoprotein layer in the outermost layer of the skin (stratum corneum) (Jelenko et al., 1968). When the stratum corneum is destroyed, there is a substantial evaporative loss of water which is associated with a large degree of heat loss which can lead to hypermetabolism in burn patients (Lamke et al., 1977). The high-water absorptivity, water retention and vapour transmission features of BC creates an environment where the wound exudate is locked into the dressing whilst also preserving proper wound moisture during healing.

Owing to a multitude of hydroxyl groups, air-dried BC allows the for exceptional water vapour permeability which can be hugely beneficial in wound dressings (Fu et al., 2013). Using air-dried membranes allows for breathable dressings which permit the passage of water vapour through the material. Studies show that an ideal moisture content of a wound environment is one of the most important factors of successful wound healing (Fleck and Simman, 2010). Experimental values of controlled water vapour tests on wound re-epithelialisation and contraction enhancement show that in the case of a dressing with a water vapour transmission rate of 2028 ± 237.8 g/m^2. 24h was found to be in the optimal timescale for healing. (Xu et al., 2016).

A necessity for wound dressings is its competence in maintaining structural integrity between the time period of application and removal, especially when applied near joint areas where movement can cause failure of the dressings. The tensile strength of a BC membrane has been experimentally calculated to be approximately 15 MPa with 32% elongation at break, the addition of chitosan can increase the Young's modulus (Lin et al., 2013). The tensile strength of BC

membranes is also dependant of culture conditions and post treatment which can be found to as high as 260 MPa (Kim et al., 2011; Yano et al., 2008). The elongation at break of 32% for the BC membrane reveals a high degree of toughness. These properties allow BC to be extremely suited in a wide range of wound dressings for different wound sites. For example, BC is both mechanically strong and flexible and can thus be produced and be given to patients with knee wounds where their movement will not be restricted and the dressing will not fail.

Cytotoxicity and cell attachment testing on BC membranes have shown that BC maintains high fibroblast viability which is highly desired in a dressing material as cell toxicity would be a major concern for any material that comes in contact with an open wound (Moreira et al., 2009). BC additionally accommodates high level of cell attachment due to its ultrafine network of nanofibers, this feature is especially useful in the progression of wound healing where enhanced cell attachment would play a role in healing acceleration (Diegelmann and Evans, 2004). Furthermore, the ultrafine network presents a high surface area to volume ratio that has potential in cell seeding which can facilitate faster wound regeneration.

The bio-absorbability of BC allows enhanced restoration of the targeted tissue in a wound environment. Bioabsorbable BC has been developed and tested in pH conditions that are commonly found in wound environments (Hu and Catchmark, 2011). It was shown that by incorporating BC with different cellulases, that the degradation rate of the material could be controlled. This permits modified BC to be able to degrade through a function of a predetermined and configurable time.

BC has shown similarity to the human carotid artery in its stress-strain response curve (Bäckdahl et al., 2006). The resemblance to soft tissue could be due to the comparable architecture of the carotid artery and BC, but this finding also suggests that BC can be formed to be biomimetic towards tissue and skin. Numerous publications that BC is also similar to skin, making it suitable as a skin substitute material or a temporary wound treatment dressing (Ciechańska, 2004; Fu et al., 2013; Lee and Park, 2017). An ideal wound dressing system would present similarity to the autograft skin in structure and in functionality (Jones et al., 2002). By mimicking native soft tissue, wound care materials made of BC could prove to improve patient compliance.

Given its highly nano-porous structure, BC allows for the incorporation of pharmaceuticals and antibiotics into a wound, whilst simultaneously serving as an effective physical barrier against potential infections with its filter-like mesh of microfibrils. Porous fibres for the delivery of active pharmaceutical ingredients is not a new concept, drugs can be easily incorporated into the BC dressing to be released at a controlled or delayed release rate (van de Witte et al., 1993).

When BC grows in its native conditions, it takes the form of the surrounding environment such as the petri dish. The membrane remains highly mouldable even after extraction from the growth medium. Wounds come in different shapes and sizes and can occur at any part of the body and therefore should not be thought of as a flat surface. The mouldability of BC allows it to be placed on any wound irrespective of where it may be on the patient. BC-based wound dressings can be made to be extremely conformable to the exterior or wounds and allow great levels of comfort that is not experienced by standard gauzes.

6. Bacterial Cellulose Processing (fibres)

There has been an abundance of work focusing on the improvement of static culture methods for producing BC (Çakar et al., 2014; De Wulf et al., 1996; Vandamme et al., 1998). From an industrial point of view however, the fact remains that these culture systems are inefficient as they are labour intensive and have a long turnaround time. Johnson & Johnson, a major pharmaceutical company, attempted the commercialisation of BC as early as in the 1980s. The company supported a pioneering series of investigations into the application of BC for different types of wounds, but details of any clinical trials have never been published, and many companies have failed to introduce a commercial wound healing product which incorporates the benefits of BC due to the many difficulties associated with the efficiency of large-scale fermentation (Ring et al., 1986a, b).

Commercial production of BC was again investigated in the 1990s by a number of large Japanese companies and governmental organisations aiming to efficiently mass produce BC (United and Congress, 1993). The $45 million effort from these companies resulted in many patents and publications, however there was no indication of commercial success. The 1990's was also the decade when fundamental studies on BC biosynthesis was carried out in Poland. The government-backed initiative lead to successful clinical trials continuing through to the new millennium (Czaja et al., 2006). The study also led to the discovery of an efficient strain of *Gluconacetobacter,* which is able to produce cellulose in nutrient mediums which were more economical (Krystynowicz, 1997). Therefore, there was a shift in focus to unearthing strains of *Gluconacetobacter* which would result in higher yields and production rates of BC. The discovery of more efficient bacterial strains allows for advancement into fermentation scale up with promise of commercialisation.

The major obstacle preventing commercialisation is the efficiency of the current production technologies. Manufacturers of BC based artificial skin have been varying concentration of carbon sources, surface/volume ratios of the cultures, and duration of fermentation in the effort to scale production (Czaja et al., 2006). Unlike other bacterial polysaccharides, BC cannot feasibly be synthesised economically in large stirred-tank fermentation systems. Agitated microbial cultures have been shown to have a reduction in cellulose yield and a loss of attractive properties such as crystallinity.

Until very recently, a different approach to BC manufacturing has been on the rise with numerous publications from both academia and industry. The endeavour to form BC into a secondary fibrous form via highly controlled fibre forming techniques has seen a rise. Fibre forming techniques such as electrospinning have been utilised to create ultrafine fibres with BC that can be used in a wide range of potential applications such as drug delivery, tissue engineering and wound healing (Abeer Muhammad et al., 2014; Mohd Amin et al., 2012; Svensson et al., 2005). The benefit of being able to process BC into fibres are vast. The ability to produce continuous nano- and micro-fibres from BC allows for the fabrication of bandages from small amounts of raw material. Furthermore, this allows for the tailor ability of fibre morphology and also allows for potential industrial scale up of BC manufacturing which requires less raw or pure BC.

6.1. Electrospinning

Electrospinning is an electrohydrodynamic technology in which a polymer solution is fed through a needle that is connected to a high voltage power supply (Luo et al., 2012). The solution becomes charged as it flows through the needle and the electrical stresses overcome the surface tension of the polymer solution (Deitzel et al., 2001). The droplets emerging from the tip of the needle converge into a conical shape (Taylor cone) as a result of the balance between various forces, and a polymer jet is ejected from the apex of this cone (Kim and Reneker, 1999). It is this jet that leads to the production mechanism as the solvent subsequently evaporates and in its stead leaves dried, uniform fibres (Feng, 2002). The technology is summarised by (**Figure 4**).

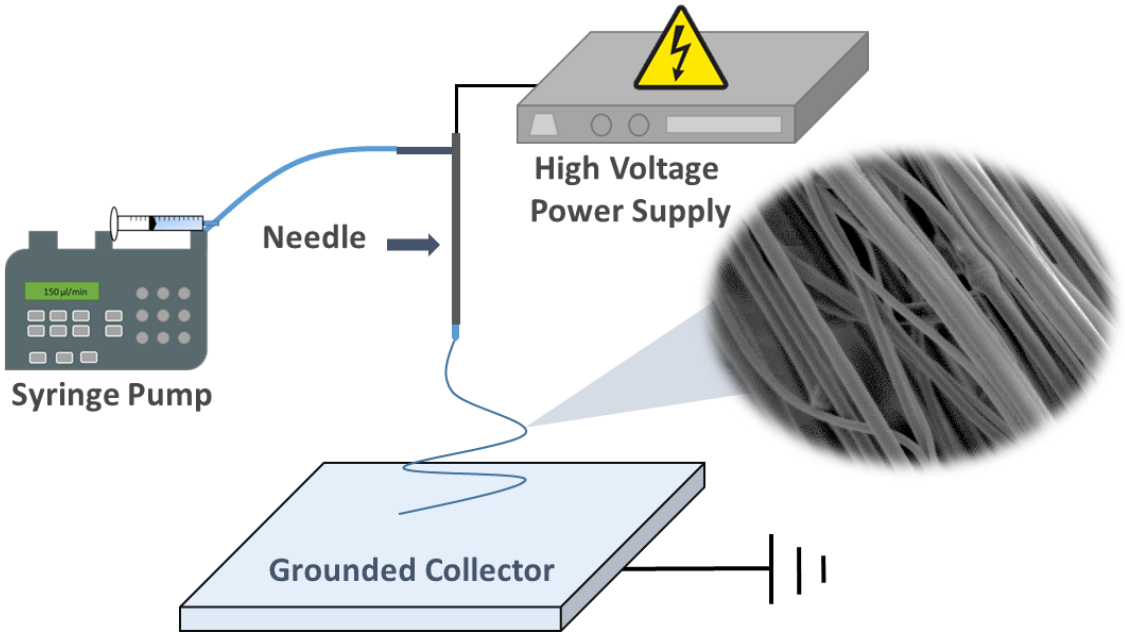

Figure 4: Schematic representation of the electrospinning setup showing a syringe pump where polymer solution is fed through the needle, upon contact with a high voltage electric field, a Taylor cone appears, and fine fibres are formed produced as a result.

Being one of the more established laboratory fibre forming techniques, much attention has gone into forming fibres via this facile technique. BC nano whiskers have been used to improve the mechanical properties of other fibres which are produced by other polymers. The improvement of mechanical properties mainly depends on the extent of BC nano whiskers dispersion in the fibres within the matrix. These whiskers are high aspect ratio (length to diameter ratio) cellulose crystal suspensions, extracted from the cellulose source and reveal a needle like structure under SEM (Bercea and Navard, 2000). They are identified as whiskers due to their elongated shape and their high crystallinity achievement, by creating mixtures of these crystal suspensions with polymer lattices, there is a drastic enhancement of mechanical properties at even a low weight fractions (Favier et al., 1997). BC whiskers can also be obtained by acid hydrolysis of the BC microfibrils, forming highly crystalline rod-like particles (Dufresne, 2000).

Blends of BC and Poly(ethylene oxide) (PEO), a water soluble polymer have undergone electrospinning with aqueous BC solutions of 5 wt% (Park et al., 2007). The solution was able to form fibres such as the PEO would, the BC whiskers-reinforced fibres showed a significant increase in Young's modulus, percentage extension at break and maximum stress. Furthermore, ethylene vinyl alcohol (EVOH) fibres were also spun with electrospinning, XRD studies showed that the BC whiskers had a highly crystalline structure (73.1% crystallinity index) compared to untreated BC membranes (Martínez-Sanz et al., 2011). There is an abundance of polymers used in biomedical and tissue engineering that suffer from poor mechanical properties, therefore, electrospinning of BC has shown to have great potential in composite material reinforcement (Gindl and Keckes, 2004; Pommet et al., 2008; Wan et al., 2009).

More recently, improvements in the portability of electrospinning devices have allowed for point-of-need spinning of fibrous constructs with great potential in wound healing applications (Sofokleous et al., 2013). The ability to directly spray an active patch onto a wounded patient allows for the control of fibre morphology, patch thickness, material choice, easy transport and storage of nanofibrous products and gives complete control over wound coverage and thickness. Polycaprolactone (PCL) was used as a carrier polymer along with 8 differing ratios of BC to generate BC-PCL composite nanofibres which could be exploited in use as emergency point-of-need wound care using a novel electrohydrodynamic gun (Aydogdu, M. O. et al., 2018). BC was processed into fibres after being suspended in dimethylformamide (DMF) and subjected to ultrasonication to form a gel-like solution that could be mixed with the PCL polymer solution. BC shows only slight solubility in DMF, but the sonication process reduces the particle size of the BC membrane to improve solubility.

From the electrohydrodynamic gun study on BC, it was found that the increase in BC content from 5 to 10 wt% resulted in an increased frequency of beads in the fibres (Aydogdu, Mehmet Onur et al., 2018). However, it was also observed that the bead count could be reduced by increasing the carrier polymer concentration. Other experimental studies show that the main factors which contribute to bead formation in electrospinning are to do with solution properties such as: low molecular weight, low concentration, low viscosity, high surface tension and low charge density (Fong et al., 1999). The solution properties of the BC-PCL solutions where experimentally measured, it was found that the increase of BC content from 5 to 10 wt% actually increased viscosity and electrical conductivity but only slightly increased the surface tension of the solution. The increased presence of beads in this case may be due to the rise in surface tension seen from the addition of BC, other than the other measured solution properties.

An important property of BC is it's biocompatibility and ability to mediate cellular interactions similarly to that of native tissue in numerous instances (Bäckdahl et al., 2006; Torres et al., 2012). The produced BC-PCL fibres where tested with Saos-2-human osteosarcoma cell line which had osteoblastic characteristics (Rodan et al., 1987). In an MTT assay after 72 hours, all BC-PCL fibrous samples showed cell viability in excess of 75%. It was found that by increasing the PCL concentration, the cell viability increased, possibly due to the increase in fibre diameter favoured by the cells. In the case for 5 and 15% PCL, cell viability increased with increasing BC content, however due to the cell viability of PCL

alone being very high, it is difficult to determine whether any increase in cell viability was due to an increase in BC content. Nonetheless, it can be concluded that a BC-PCL composite system is very capable of retaining an acceptable level of cell viability.

The cellular interaction with the BC-PCL scaffolds were observed by SEM. Cells appeared to cover the scaffold and fill the spaces in the nanofibre matrix. Here were two dominant cell morphologies that could be determined from the micrographs, the cells along the axial length of the fibres depicted an elongated morphology whilst globule-shaped cells where seen at the intersections of the fibres. The presence of the elongated cells indicated that cytoskeletal rearrangement may have taken place which has been previously reported to activate nearby receptors which affects gene expression (Curtis and Wilkinson, 1997). The ability for a material to absorb water is an important factor in a wound dressing, a high swelling ratio permits exudate absorption and the efficient exchange of nutrients and waste (Martin, 1997). All BC-PCL samples showed a high level of water uptake in swelling tests whilst the sample with the highest concentration of BC and polymer showing the highest swelling percentage.

Nerve tissue engineering is a popular topic in biomedicine due to the limited regeneration capacity of native nerves. A study into the production of nanofibrous scaffolds for enhancing peripheral nervous system neural tissue regeneration and neurite outgrowth was carried out using a BC-PCL polymer mix (Altun et al., 2019). When a gap larger than 3 cm between peripheral nerves occurs, axon regrowth is extremely difficult, nerve tissue engineering thus provide scaffolds that aid this crucial regeneration (Monaco et al., 2017). Here a concentration of 5% (w/w) BC was dissolved in a 50:50 solvent ratio of chloroform and DMF, dissolution required ultrasonic agitation of 5 hours over a period of 15 days. The dissolution process was captured optically every 3 days: days 1-3 showed no disintegration of the BC, days 4-6 showed slight disintegration, days 7-9 illustrated decomposition of the BC particles, at days 10-12 the dissolution process continued where whisker-like structures where observed, day 15 showed good dissolution (**Figure 5**). Mechanical strength is important in nerve tissue engineering as the constructs must be able to withstand the forces and motion of everyday interaction and movement where nerves will stretch and contract. The addition of BC into the fibrous scaffold doubles the tensile strength from 14.6 MPa to 29.3 MPa. The average diameter of the produces fibres for the PCL scaffolds was 527 nm and for the BC-PCL scaffolds there was a range of 70-120 nm.

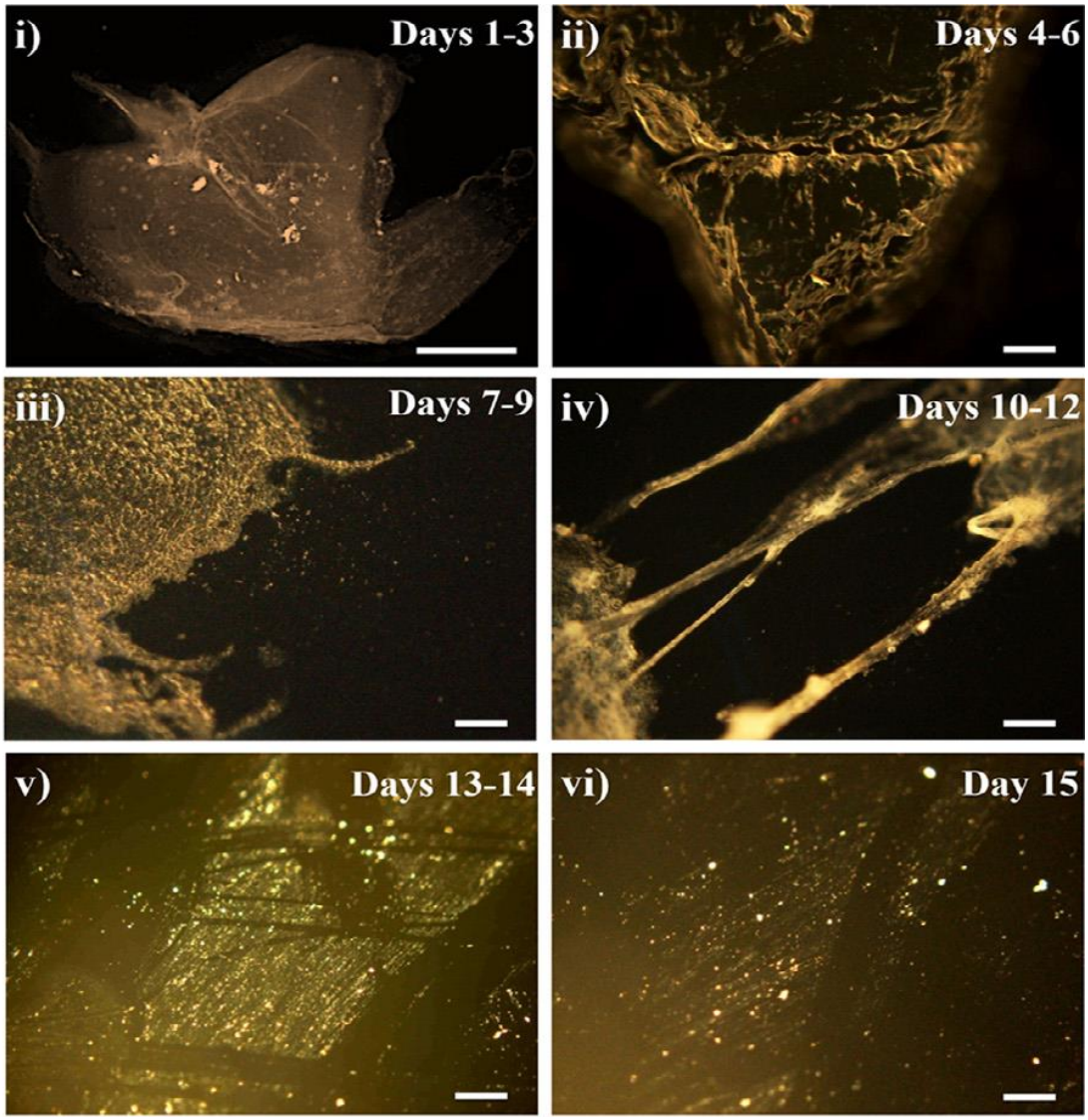

Figure 5: BC dissolution process is illustrated using optical microscope images: (i) Days 1–3, (ii) Days 4–6, (iii) Days 7–9, (iv) Days 10–12, (v) Days 13–14 and (vi) Day 15. Scale bar = 1 mm (Altun et al., 2019).

The hybridisation of fibre scaffolds with hydrogels improves mechanical durability and alters its biocompatibility and functionality (Kouhi et al., 2019). A concurrent electrospinning/electrospraying technique was utilised to produce fibrous hydrogel of keratin/ tragacanth gum-conjugated BC hydrogel (Azarniya et al., 2019). The setup was centred around a rotating mechanical mandrel where two separate electrohydrodynamic setups could deposit onto it, on one side was an electrospinning needle and on the other was an electrospraying needle. The benefit of this arrangement is that hydrogel particles can be uniformly embedded into the fibre network without having an effect on its porosity or diameter distribution. The hybrid product would act as a temporary skin substitute, in order to cope with the mechanical durability demands, BC was incorporated into the fibrous mats at different concentrations. In this work a concentration of 1,3 and 5 wt% BC was prepared in a solution with keratin and PEO where acetic acid was used as the solvent. The produced fibrous mats without BC had an average fibre diameter of 243 ± 57 nm. With the addition of BC, it was noticed that there were fibre breakdowns and a higher number of inter-fibre bonds present which may be

the result of BC affecting the solvent evaporation rate. The formation of fibre branches when BC was added can be explained by the theory that the surface of a conductive fluid jet can undergo statistic equilibrium undulations via the combined effects of surface tension and electric Maxwell stresses (Yarin et al., 2005). Remarkably, the average fibre diameter was reduced to 150 ± 43 nm when BC was added at 1% and subsequent higher conditions did not yield much change in the fibre diameter.

Hydrophobicity is an important characteristic to consider for materials in wound healing and in tissue engineering as it can affect biocompatibility of protein adsorption and cellular interaction with the material (Pertile et al., 2010). The keratin-based nanofibers produced without BC were hydrophobic and had a water contact angle of 126°. The addition of BC saw the hydrophobicity to significantly reduce and at 1 wt% BC, the water contact angle was 83°. This enhanced hydrophobicity of the fibres and is due to the hydrophobic nature of BC via its highly porous nonwoven network of nanofibrils. The incorporation of BC into the fibres also shows a significant enhancement in mechanical strength. At only 1% BC concentration and compared to keratin-PEO fibres, there is an increase from 7.1 MPa to 13.3 MPa in the tensile strength,123 MPa to 250 MPa in the elastic modulus and reduction in the elongation at break from about 15% to 10%. The enhanced mechanical durability of the BC-reinforced fibres is probably afforded by the reorientation of the BC fibrils and the entanglements between the keratin-PEO fibres (Astley et al., 2003). Furthermore, the interfacial cohesion between the BC and the keratin-PEO fibres in addition to the reduction in fibre diameter from the inclusion of BC can also be responsible for the improved mechanical properties (Wan et al., 2009). The study also carried out *in vitro* cell studies with the fibres, it was found that keratin-BC fibrous composites had an acceptable level of cytocompatibility as assessed through MTT assays where there was over 90% cell viability in L929 fibroblast cells (Azarniya et al., 2019).

6.2. Pressurised Gyration

Pressurised gyration is a hybrid fibre forming technique which combines solution blow spinning with centrifugal spinning to form low diameter fibres with a rapid production rate and can be used to generate bandage-like fibrous mats (Ahmed et al., 2019; Heseltine et al., 2018; Mahalingam and Edirisinghe, 2013). The setup consists of an aluminium vessel with multiple small apertures on its exterior which is connected to a high-speed motor and a gas inlet. The vessel rotates at high speeds and gas is infused simultaneously into the vessel which drives the polymer solution out through the orifices forming a polymer jet (Ahmed et al., 2018). The polymer jet gives rise to fibre production much like electrospinning as the solvent evaporates. This technique not only allows for very high throughput of production, but also allows you to control final fibre morphology by varying the rotation speed and the magnitude of applied gas pressure (Alenezi et al., 2019). Orientation of fibre bundles to generate mats of wound dressings can be manufactured in this way.

BC fibres blended with poly(methyl methacrylate) (PMMA) at several different ratios have been successfully formed with pressurised gyration to produce biocompatible fibrous scaffolds (**Figure 6**) (Altun et al., 2018a). 5 and 10 wt% of BC solutions were made in a 50:50 wt:wt ratio in DMF and tetrahydrofuran (THF).

The BC was subjected to ultrasonication for an hour in order to form a gel that could be spun using pressurised gyration. The ratio of BC:PMMA was altered and physical properties were determined along with further tests including SEM imaging, fourier-transform infrared spectroscopy (FT-IR) and cell proliferation studies. Solution viscosity and surface tension was discovered to have increased with elevating BC-PMMA wt ratios, similar with electrospinning, these parameters fundamentally alter fibre formation in pressurised gyration. SEM imaging showed greater particle count on the fibres with higher ratios of BC-PMMA, indicating that these particles were caused by the higher BC content. The FT-IR spectra on the BC-PMMA fibres confirmed presence of BC on the fibres as the profiles were consistent with that of pure BC and PMMA.

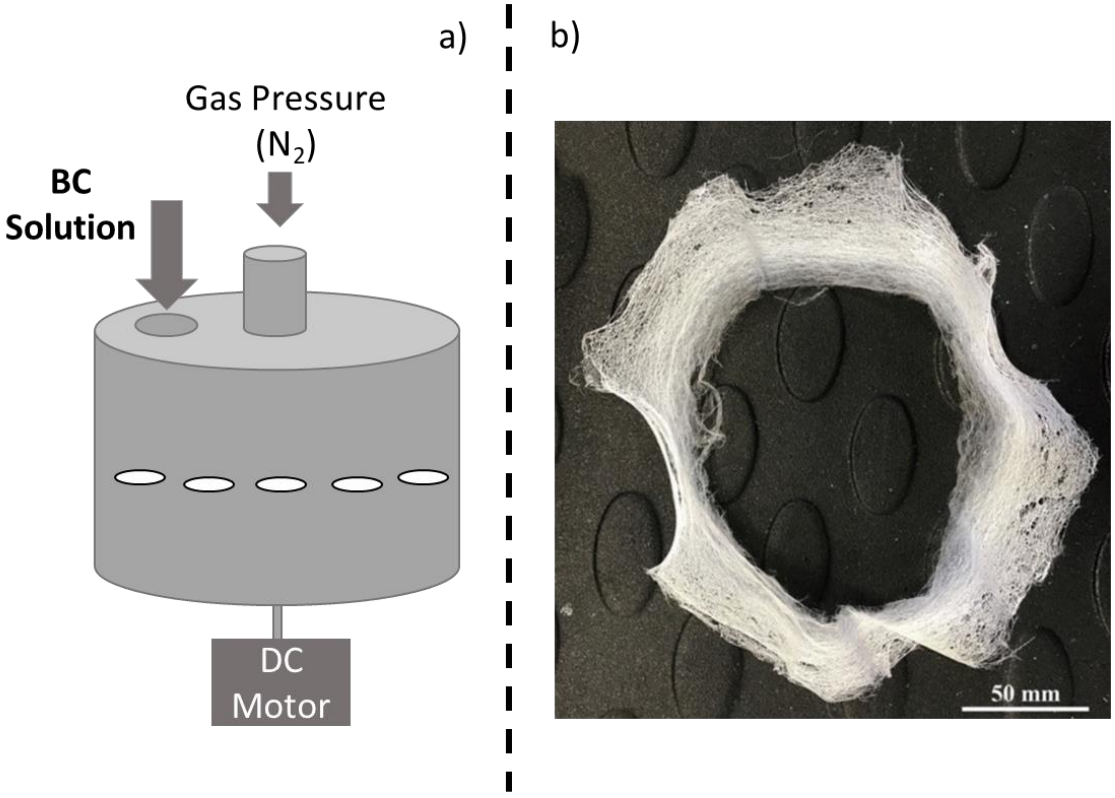

Figure 6: Schematic representation of a) pressurised gyration setup, b) Photograph of the bandage-like fibrous mat produced from the 5:50 (wt ratio) BC:PMMA blend.

Having applications in wound healing the scaffold must be biocompatible, non-toxic and must allow for adequate cell attachment, migration, proliferation and differentiation (Sachlos and Czernuszka, 2003). BC-PMMA scaffolds produced by pressurised gyration where investigated and found to be biocompatible with no indication of toxicity to the tested Saos-2 cell line. Adding BC to the BC-PMMA fibres increased cell viability compared to just solely using PMMA fibres. BC-PMMA scaffolds with 5 wt% BC were considered appropriate for wounds dressing applications because they retained cell viability of over 85%. The produced scaffold demonstrated cell spreading and proliferation of DAPI stained cells, the scaffolds showed enhanced metabolic activity compared to the control (**Figure 7**). MTT assays demonstrated that the scaffolds of 5 wt% had improved metabolic

activity and proliferation of the seeded cells compared to the 10 wt% BC. Furthermore, preliminary mechanical tests on the scaffolds revealed that the BC-PMMA fibres had lower stiffness and higher ductility, the tensile strength of 5:50 BC-PMMA was 2.6 times greater than PMMA fibres produced by electrospinning.

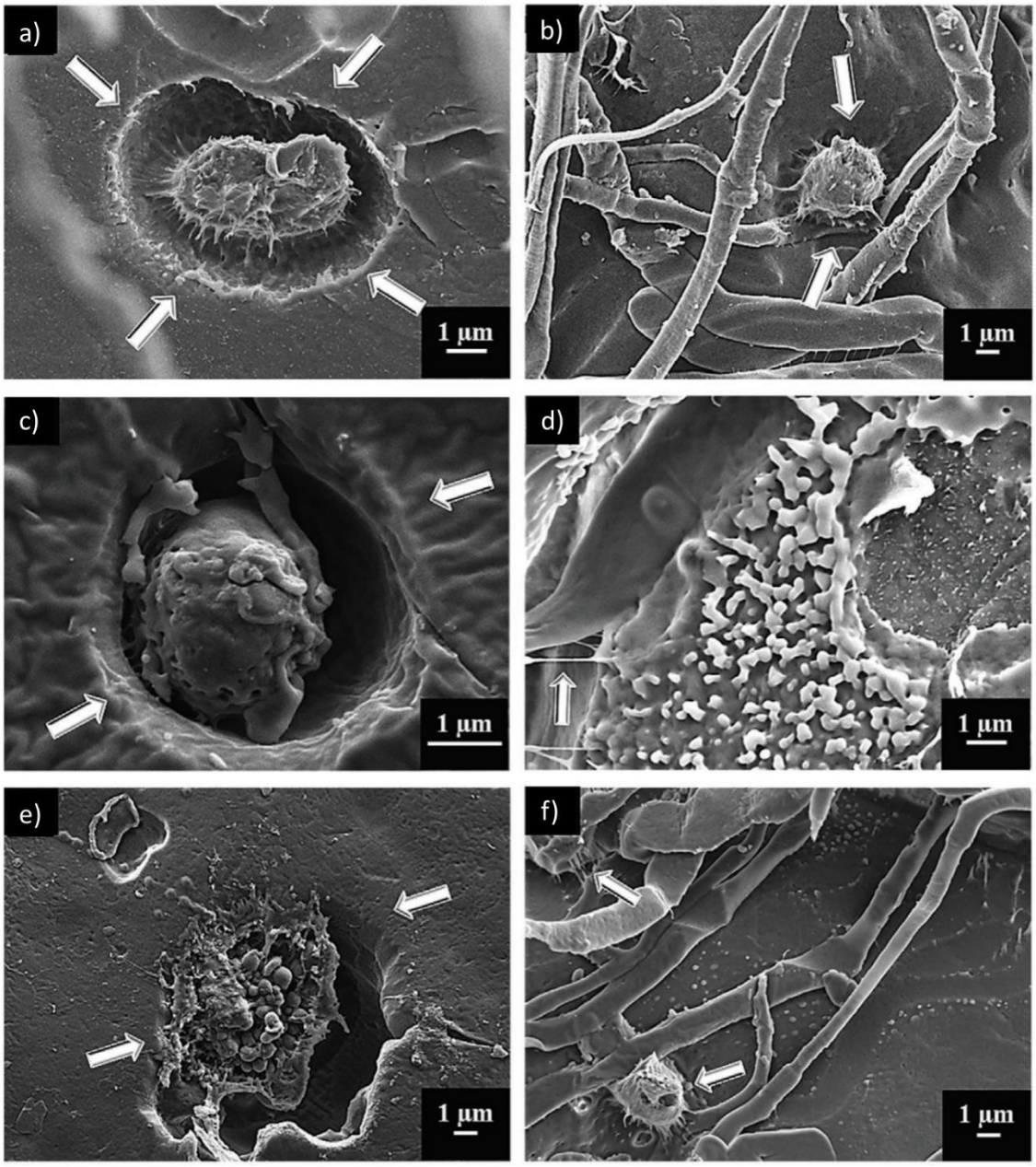

Figure 7: Scanning electron microscopy images of the BC:PMMA scaffold samples 72 hours after incubation with Saos-2 cell line with ratios of: a) 5:30, b) 10:20, c) 5:40, d) 10:30, e) 5:50, and f) 10:40. Arrows indicate embedded cells and their extension (Altun et al., 2018a).

Bandage-like polymeric structures were also produced using pressurised gyration using BC and PMMA blends with the addition of metallic antimicrobial nanoparticles (Altun et al., 2018b). In this study, BC was incorporated into a polymer solution of PMMA using sonication in a 50:50 solvent mixture of DMF and THF. Additionally, two types of nanoparticle mixtures were also added; one using Cu-Ag-Zn/CuO and the other including Cu-Ag-Tungsten carbide. The study

showed that BC-PMMA bandage-like fibres could be produced at a high yield with pressurised gyration and that these fibres can have antimicrobial nanoparticles incorporated for improved mechanical properties, higher water uptake ability and lower cell cytotoxicity.

An investigation into the maximal loading of BC in binary and ternary blends of fibres was carried out with an emphasis on production yield and mechanical properties by (Aydogdu et al., 2019). Poly(lactic acid) (PLA) and PCL fibres were created with and without blends of BC, eventually an optimised composite of PCL-PLA-BC was also created. For pure PLA fibres, there was a 92% yield, and the addition of BC into the polymer matrix caused a deterioration of yield down to 54% at only 10 wt% BC. It was observed that a huge fall in yield occurs as a result of higher BC loadings, as attested to by many other articles (Altun et al., 2018b; Aydogdu et al., 2019; Azarniya et al., 2019). Pure PCL fibres had a yield of 87% and saw a drop to 61% yield when loaded by 10 wt% BC. PLA and PCL composites were also produced and tested to compare the ternary behaviour of the different polymer systems. The 90:10 PLA-PCL blend had a very high yield of 97%, which also showed that these polymers worked very well as composites.

A BC concentration of 30 wt% was deemed the highest concentration whilst maintaining an acceptable level of yield (> 30%) and mechanical integrity. The BC in the polymeric solution also caused an increased frequency of beads within the fibres. As expected, the addition of BC to the solutions lead to an increase in viscosity and thus caused thicker fibres to be formed in the presence of BC.

With an increasing concentration of BC in PLA binary systems, the ultimate tensile increases with each 10% increment. PLA alone has a tensile strength of 2.3 MPa, at 10 wt% BC concentration the tensile strength is 3.8 MPa, 20 wt% it's at 5.4 MPa and at 30 wt% it is 6.5 MPa. At 40 wt% BC concentration, the PLA fibres lose mechanical integrity and the tensile strength drops to 2.3 MPa as the BC content increases. This drop in tensile strength corresponds with the reduced fibre count and yield with high BC levels which impairs the integrity of the bandages. The results for the stiffness of the PLA-BC binary system follows the same trend. The stiffness of PLA increases from 10 wt% to 30 wt% of added BC, it then falls sharply at 40 wt% and continues to drop.

The mechanical behaviour of the PLA-BC binary polymer system follows a similar trend with the PLA-BC polymeric fibres. With 100% PCL, the tensile strength is around 2.3 MPa, the addition of 10 wt% BC creates an increase in tensile strength to about 2.7 MPa. PCL proves to be a superior carrier of BC compared to PLA when comparing tensile strength as 50 wt % BC shows the highest value at around 6.7 MPa. At a 100% concentration of PCL, the Young's modulus is around 23 MPa, the addition of BC at 10 wt % causes an increase of stiffness to about 27 MPa and at a 40 wt % concentration of BC the stiffness drops to ~ 12 MPa.

This study then focused on the production of PCL and PLA fibres with BC loading, ultimately to design an optimised ternary polymeric system with a mixture of PCL, PLA and BC. The optimised ternary sample consisted of 70 wt% mixture of PLA and PCL and 30 wt% BC, it had a higher tensile strength than both PCL and PLA at around 9 MPa and had a high stiffness of around 19.6 MPa. It showed that BC can be used in binary and ternary polymeric systems to produce fibres that can benefit from the mechanical characteristics of multiple polymers.

6.3. Bacterial Cellulose Solutions

Due to the large number of inter- and intra- molecular hydrogen bonds, BC is very difficult to process into solution, which is a necessity in order to generate fibres using major methods such as electrospinning. BC is an especially insoluble material and does not dissolve in common organic solvents such as acetone, chloroform and DCM. Experimental results show that BC has partial solubility in 8.5 wt% aqueous sodium hydroxide (NaOH) solution (Łaskiewicz, 1998). Even then, temperatures of -5°C are required, only about 20 wt% of the cellulose is dissolved and the degree of polymerisation of the BC source must be low too. The solubility of BC in NaOH solution can however be further increased when 1 wt% urea is added. Even then, BC is not completely soluble in these conditions, and the use of such acids and chemicals can lead to toxic production environments and hazardous industrial waste.

High molecular weight BC was discovered to be soluble in a binary solvent system of lithium chloride/N,N-dimethylacetamide (LiCl/DMAc) (Shen et al., 2010). It was also found that the type of BC membrane and how it was formed had a large effect on its solubility with these solvents. BC samples with large grains in their microstructure were more prone to form large gels during the swelling stage of dissolution which hindered additional diffusion of the solvent into the fibres. The samples that showed good solubility were those that were in powdered form, having much higher surface area to volume ratio. There are several activation procedures that can improve the initial solubility of cellulose and BC including treatment with liquid ammonia, freeze drying and swelling in water followed by solvent exchange in dimethylacetamide (Morgenstern and Berger, 1993; Rohrling et al., 2002). These activation steps are thought to induce inter- and intra-crystallite swelling, increase accessibility and break of hydrogen bonds. Temperature was also found to have a marked effect on dissolution where temperatures below 45°C caused difficulty in dissolution and activation temperatures over 60°C showed greater dissolution.

BC with a high degree of polymerisation (6500) was dissolved in 1-n-butyl-3-methylimidazolium where temperatures of 80°C and 12 hours of mechanical stirring were required (Schlufter et al., 2006). The dissolution by 1-n-butyl-3-methylimidazolium was found not to significantly degrade the polymer chains. The ionic liquid, 1-allyl-3-methyl-imidazolium chloride was also used to dissolve BC but a transition from cellulose I to the cellulose II allomorph was observed with the resulting electrospun fibres (Chen et al., 2010).

Although solubility of BC has been observed with some ionic liquids, the case remains that these solutions would pose an obstacle in the mass production of BC fibres and other derivative wound care materials. Firstly, the acute toxicity of these liquids is a great concern at both the factory level and through run-off. For example, the toxicity caused by 1-butyl-3-methylimidazolium chloride was investigated in zebrafish and it was found to cause oxidative damage as well as DNA damage (Zhang et al., 2017). Furthermore, the economics of such solvent systems, binary and otherwise, increase the costs to the end consumer with higher processing expenditures and prolonged manufacturing times. High temperature

processing of BC increases energy input during manufacturing which is both environmentally and economically detrimental.

7. Future Developments and Conclusions

The secondary processing of BC has proven to be difficult. Due to its nature, large scale production of BC in wound care materials is not feasible. Therefore, by reprocessing the BC into secondary fibres and blends, there can be a more commercially feasible methods of mass-producing for the healthcare market. The answer may lie in fibre forming techniques such as electrospinning and pressurised gyration, these methods allow for the tailoring of the fibre structure to best suit for wound healing applications.

However, the solubility of BC has played a major obstacle in forming spinnable solutions. Work needs to be done to discover solvents that can dissolve the BC membrane in a non-toxic and economical manner, as well as to not remove the fundamental properties of high utilisation value. Spinnable solutions can then be processed into fibres, added to blends containing other natural polymers which can have antibacterial and pro-wound healing effects.

An alternative approach into forming BC solutions can be to use mechanical force, whereby the BC membrane is broken into smaller particles or fibrils which may improve its solubility in several solvents. Such an approach has been used to spin BC-PMMA scaffolds as discussed previously where high frequency ultrasound has been used to form a gel-like spinnable solution within a carrier polymer. As discussed earlier, the benefit of using ultrasonication is that the crystal structure of the BC is not adversely affected and thus the beneficial wound-healing properties of the material can remain. Moreover, other mechanical methods of reducing BC size can be investigated, such as grinding or blending the BC into particles. The efficacy of such particles in wound healing needs to be also determined.

Blends of BC within different polymers, both synthetic and natural could prove to be a beneficial commodity in wound care. Composite materials with desired properties such as biocompatibility, biodegradability and anti-bacterial properties can be used to develop wound dressings that overcome the limitations of the production limitation of BC. There are many polymers systems yet to be trialled, even with the difficulty of processing BC, it can still be used to enhance the mechanical and biological properties for effective wound healing.

The remarkable properties of BC were only discovered in the mid-1980s, where before the applications of the it was only really limited to food production of nata-de-coco. Since then, there has been a steep incline in the number of research articles and patents relating to BC and various methods for extraction and processing.

A considerable challenge to overcome in BC technology is the unearthing of a suitable carbon source that is cheap and that does not compete with the production of food. Nevertheless, forming BC membranes into secondary fibres could maximise the use of the material in wound care applications and reduce the volume required to have its clinical effects. There are still many hurdles remaining for the wide use of BC in healthcare settings, but with the abundance of research

and patents, we could be on the verge of incorporating this very significant and valuable material in crucial advanced technology applications worldwide.

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Conflict of Interest

The authors declare no conflict of interest.

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Figure 1

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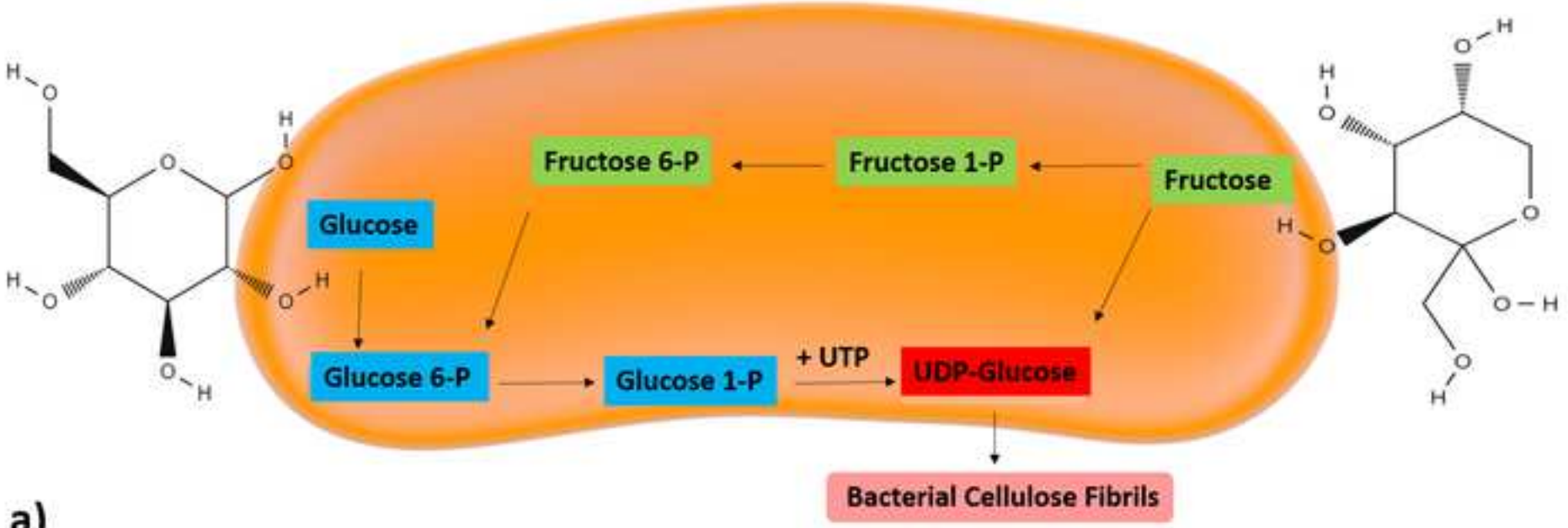
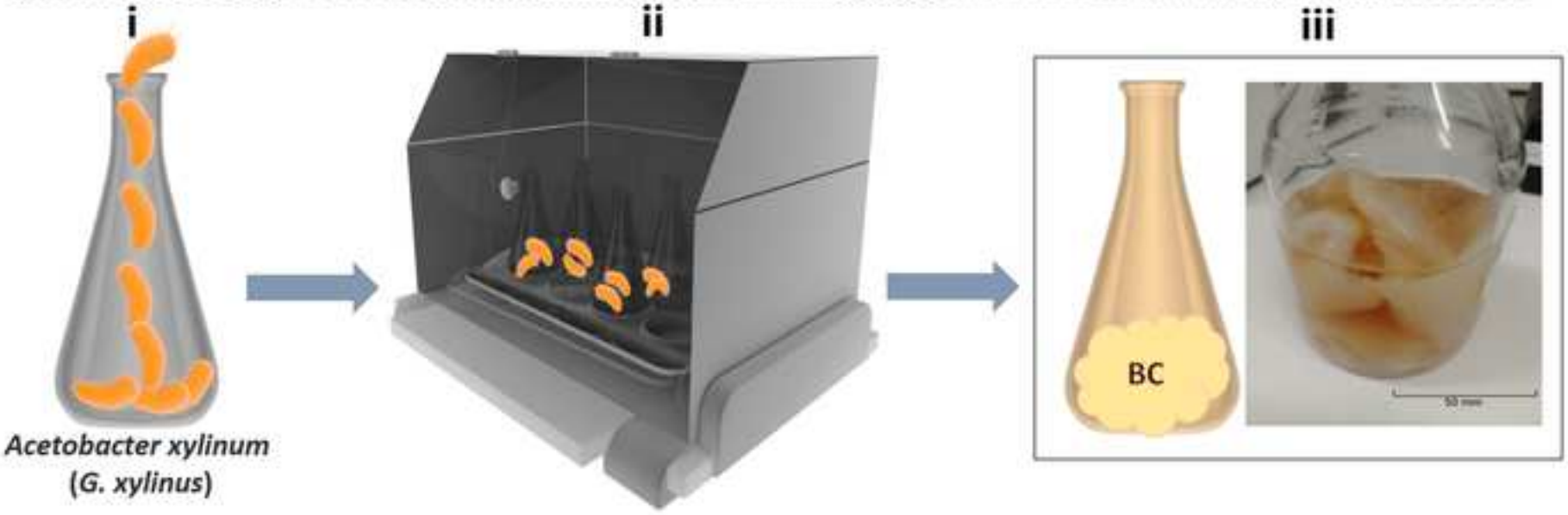


a)



b)

Figure 2

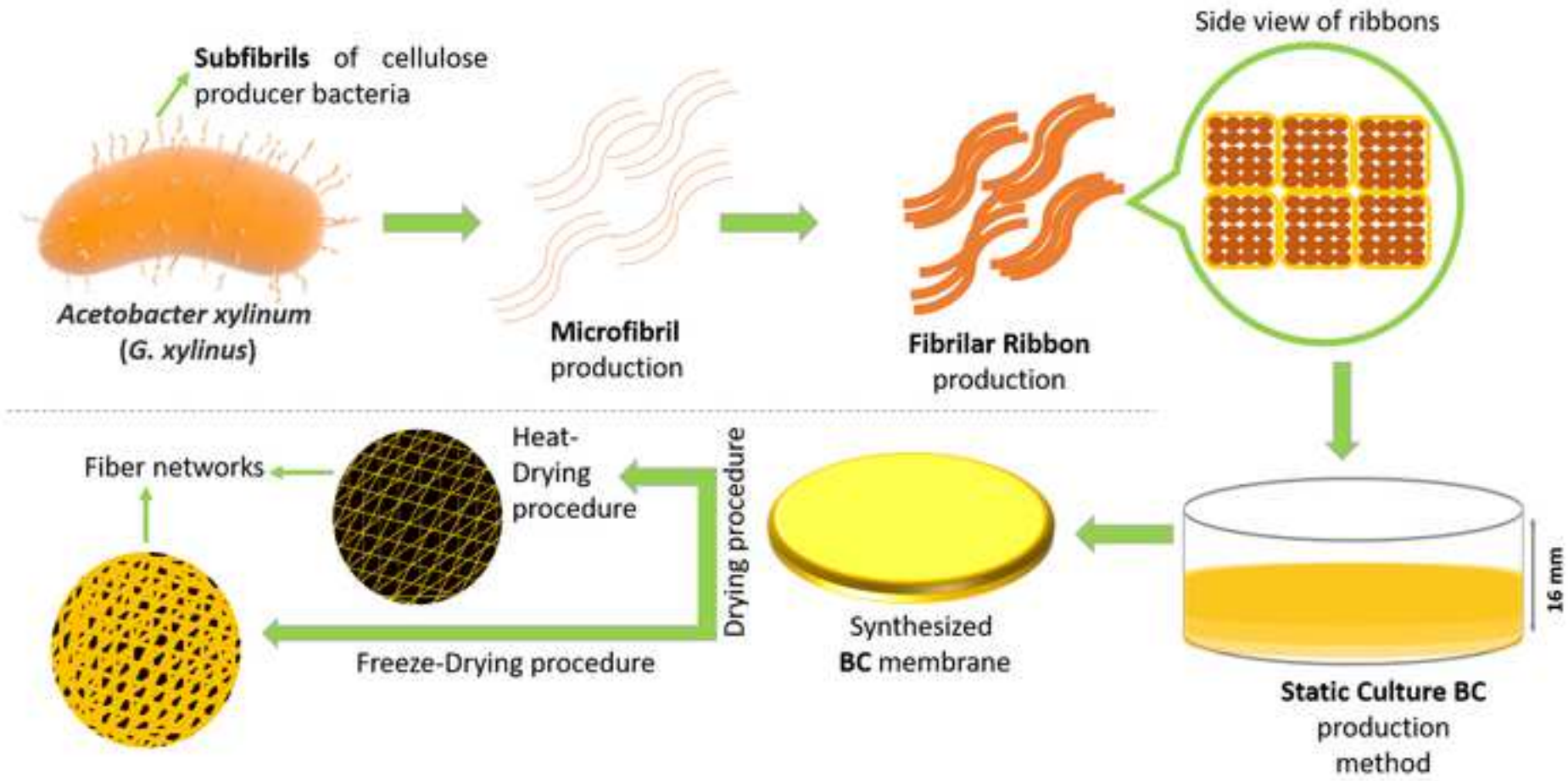
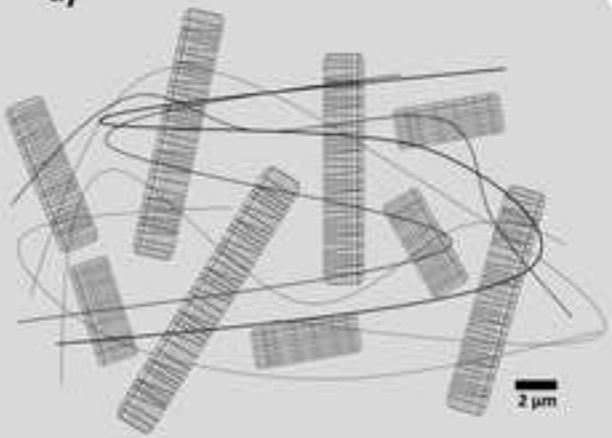


Figure 3

a)



b)

Bacterial Cellulose: Physical Properties

- High porosity
- High mechanical strength
- Biocompatibility
- Permeability
- Biodegradability
- Transparency
- Flexibility
- Hydrophilicity

c)

Bacterial Cellulose: Biomedical Applications

- ❑ Skin (wound dressing)
- ❑ Cartlidge
- ❑ Cornea
- ❑ Bone
- ❑ Urethra
- ❑ Blood vessels
- Contact lenses
- Drug delivery systems
- Bio sensors

Figure 4

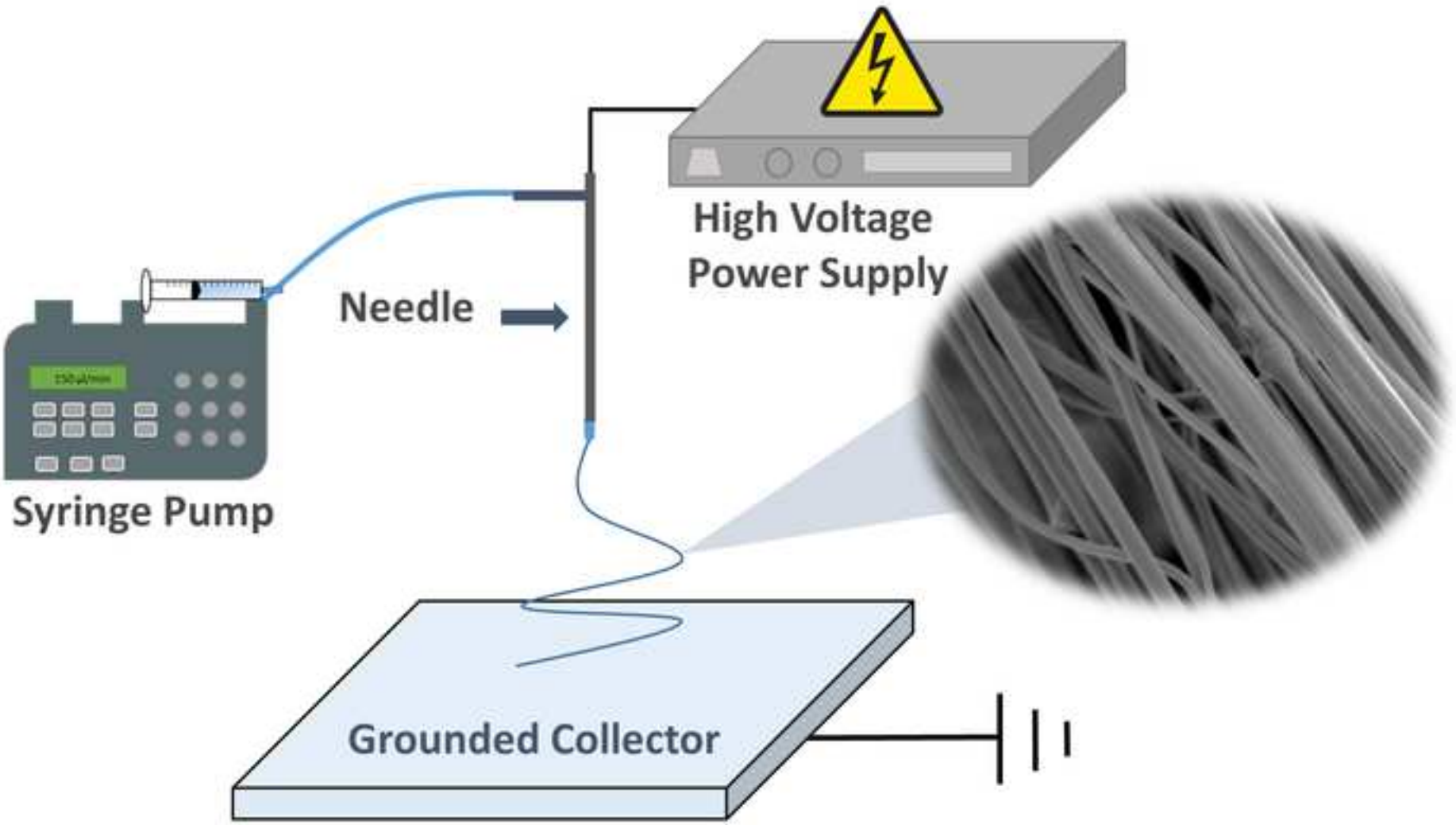


Figure 5

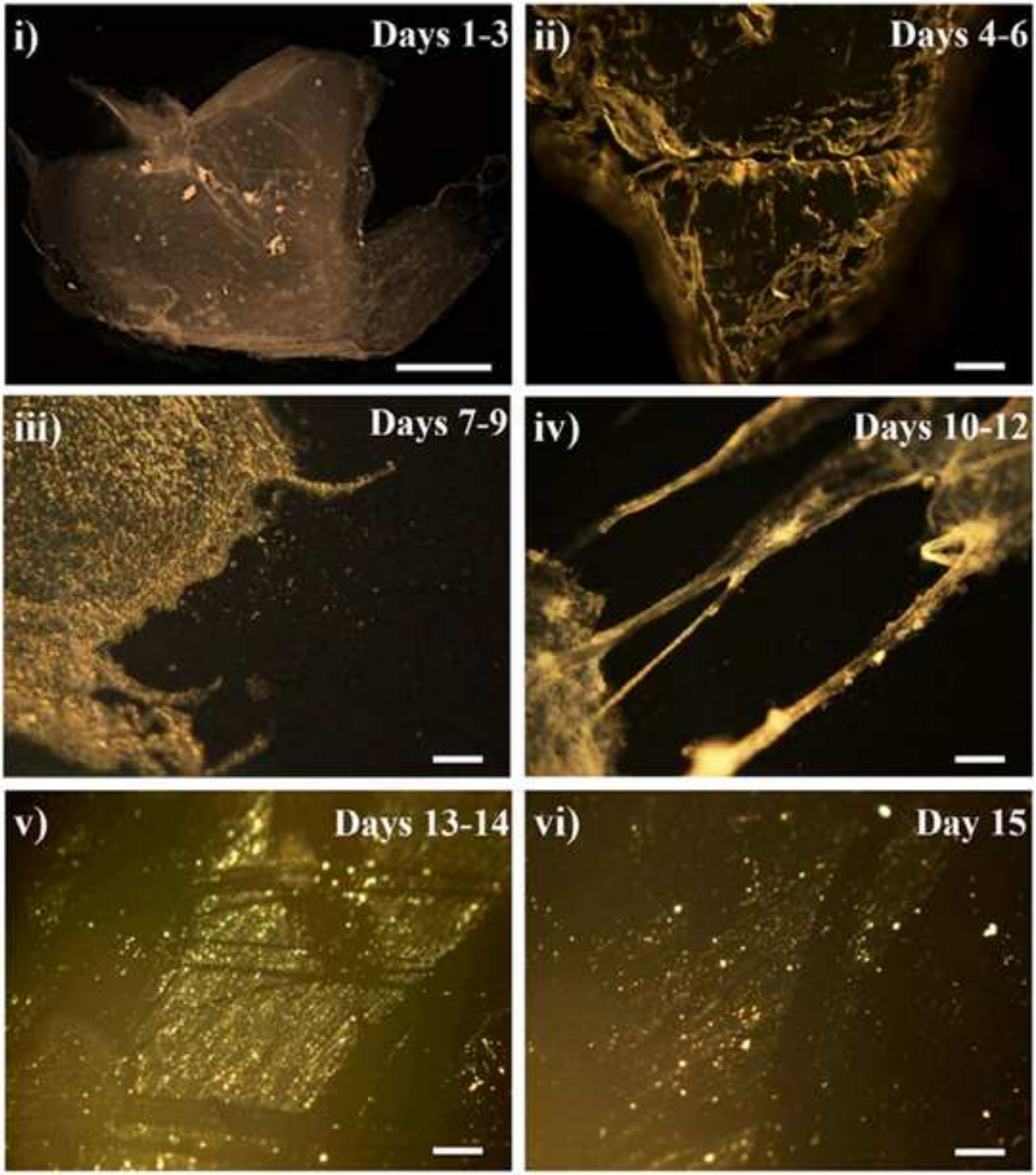


Figure 6

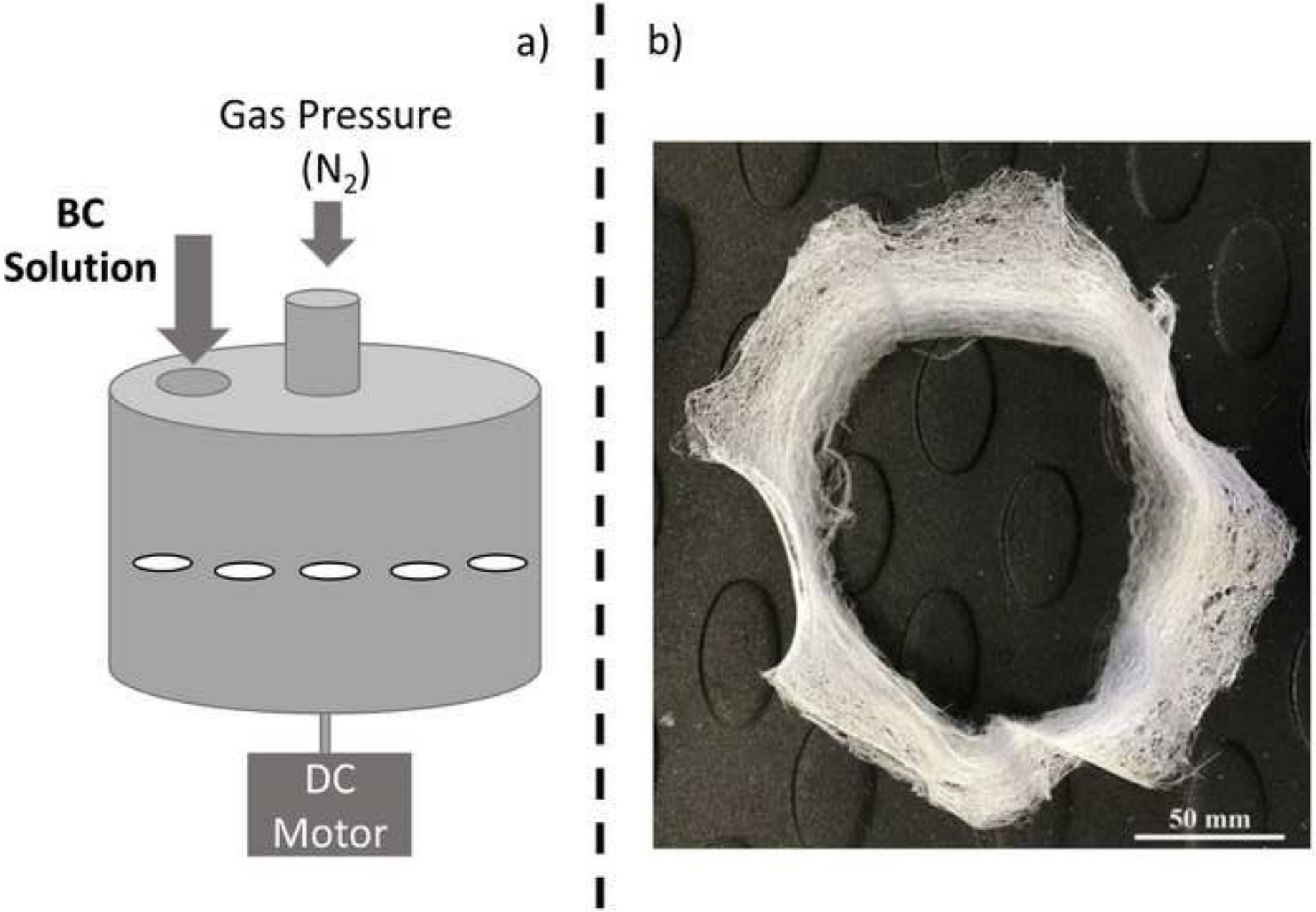


Figure 7

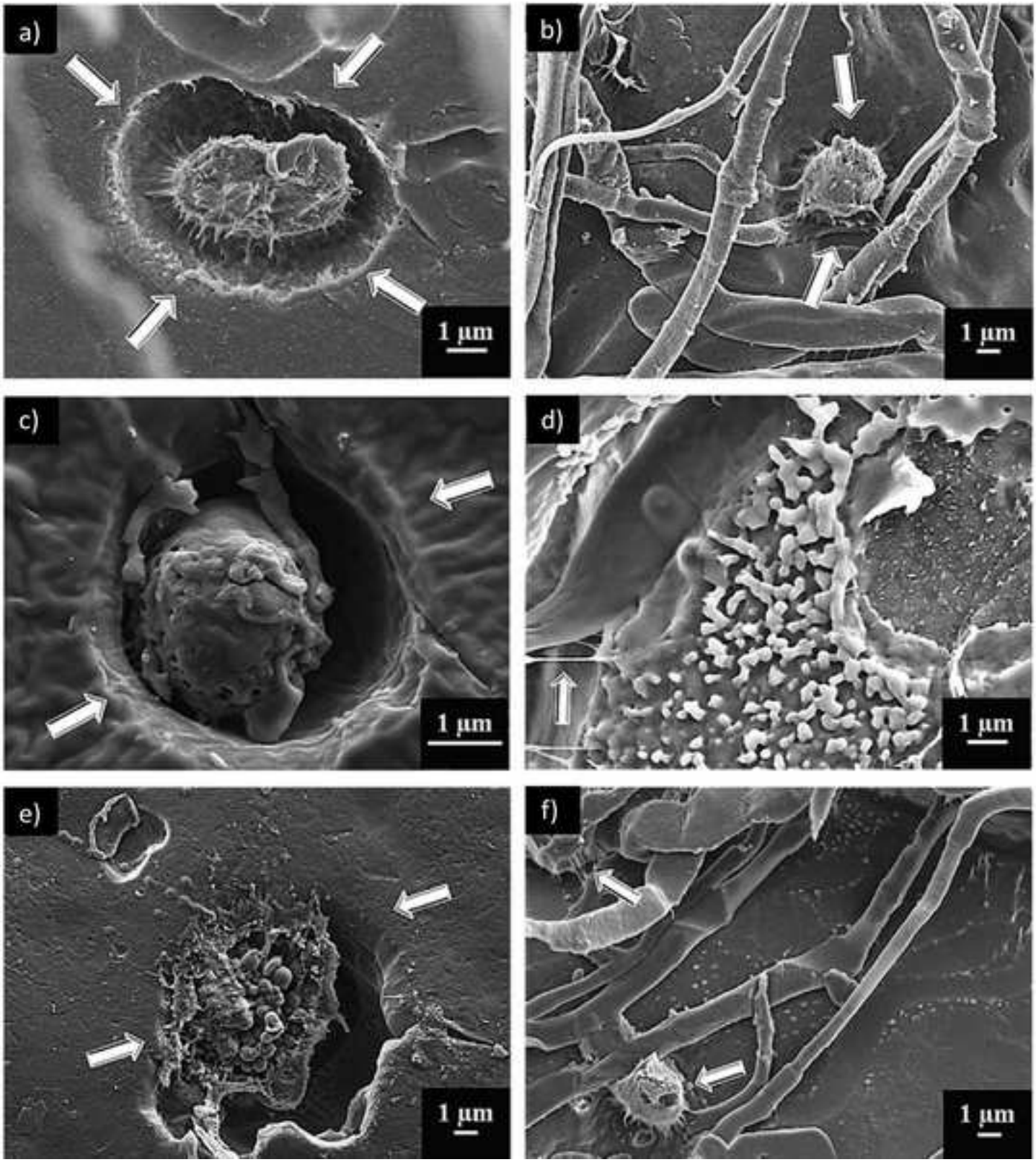


Table 1: Table summarising the key properties of BC and its relevance to wound healing.

Property	Advantage	Benefits to Wound Healing	References
Biodegradability	Bandage for chronic wounds potentially doesn't need removing	Reduction of pain from bandage removal	(Hu and Catchmark, 2011; Laçin, 2014)
ECM Resembling Matrix	Biomimetic structure promotes prompt wound healing	Cells of the wound response can be guided to become more efficient	(Svensson et al., 2005; Wu et al., 2014)
Excellent Biocompatibility	Reduces complications with immune rejection	Risk of fibrotic scarring is lower	(Helenius et al., 2006; Torres et al., 2012)
High Stiffness	Great Durability	Allows bandage to withstand some trauma	(Lin et al., 2013; Nakayama et al., 2004)
High Tensile Strength	Resistance against tearing as a wound dressing	Provides mechanical protection against external trauma	(Naritomi et al., 1998b; Wan et al., 2009)
High Water Uptake Ability	Maintains moist environment and flow of wound exudate	Allows for a more efficient recovery process and management of osmotic environment of cells	(Lin et al., 2009; Schrecker and Gostomski, 2005; Ul-Islam et al., 2012)
Large Surface Area	Increased interactions with cells in the wound response	More efficient cellular interactions leading to a healthier recovery	(Iguchi et al., 2000; Nishi et al., 1990)