FABRICATION AND EXAMINATION OF MAGNETIC BACTERIAL CELLULOSE AS A MATERIAL FOR BIOMEDICAL ENGINEERING

by

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ABSTRACT

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Bacterial cellulose (BC) is one of the most abundant biopolymers in nature. Several attributes such as facilitative modification, high pore size/porosity, able to mass produce as well as biocompatibility renders BC a promising candidate for many fronts of biomedical engineering. One consideration for BC as a biomedical material over conventional plant cellulose is the ease in which BC can be obtained to provide high purity cellulose. In comparison to collagen, another biopolymer with considerable prospects for biomedical engineering, BC possesses a higher degree of elasticity, water retention, and lower degradability. The high affinity towards water and high porosity provide BC with great flexibility as a medium for drug release as well as protein and cell binding with appropriate modifications. The transparency of thin layer bacterial cellulose could be appropriate as components of certain specialized optical technology. Its high holding capacity due to the porosity provide BC as a potential drug loading material. Due to the mechanical properties and slow degradability, BC have been heavily utilized on tissue engineering such as osteo and dermal regeneration. Well established research topic in BC as wound dressings encourage the possibility of more commercialization of BC in wound healing products.

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CHAPTER 1: INTRODUCTION

1.1. Overview of Biosynthesis and Applications of Bacterial Cellulose

Bacterial cellulose (BC) is reported to have tremendous mechanical and chemical properties including high water holding capacity, tensile strength, and modulus of elasticity while being biocompatible [1]. To relieve some of the environmental strain of utilizing wood derived cellulose, substantial developments have turned to the possibility of utilizing bacterial derived cellulose. A significant benefit of bacterial cellulose over plant-based cellulose is the lack of contaminants such as lignin and pectin [2]. While the scale of bacterial cellulose production remains small, BC has far-reaching applications to various industries. Here we will focus primarily on how BC has significant value for medical products and biomedical applications. Throughout this review, we will offer a comprehensive look at the properties of BC and examine the recent advances in its modifications as well as provide a survey of the latest applications of bacterial cellulose in the area of biomedical materials. To begin understanding about BC and how this biomaterial can be engineered for medical applications, we must first consider how it forms from microbial cultures.

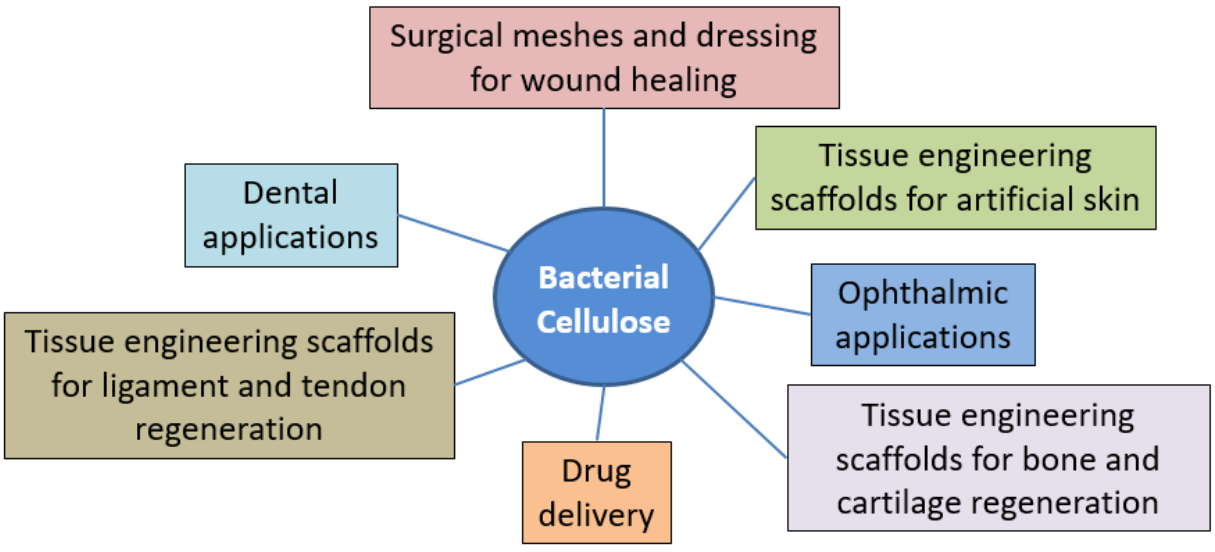

Figure 1 Biomedical applications of bacterial cellulose [69].

A number of bacterial have been reported as producers of extracellular cellulose including species from Gluconacetobacter, Agrobacterium, Aerobacter, Achromobacter, Azotobacter, Rhizobium, Sarcina, Salmonella and Escherichia [3]. Certain species of Gluconacetobacter been found to be particularly efficient in their biological synthesis of cellulose including strains of G. hansenii [1]. Biological synthesis of the interconnected cellulose that surrounds these cells (where this cellulose network is referred to as a pellicle) necessarily requires several genes whose products carry out the formation of the nanofibrous cellulose and its secretion [4]. While the entire set of biosynthetic genes necessary to produce bacterial cellulose were previously not confirmed [3], recently it has been shown that a key set of genes could be transformed into other bacteria to confer production of cellulose pellicles [1]. The genes include those within the bcs (bacterial cellulose synthesis) operon, specifically bcsA, bcsB, bcsC, and bcsD, as well as the genes cmcax and ccpAx. The roles of the respective genes have been reported, where bcsA yields the catalytic subunit of cellulose synthase while bcsB produces the regulatory subunit of the enzymes that binds to cyclic di-guanylic acid (cyclic di-GMP) [5]. The cellulose synthase activity of the bcsA subunit can thus be allosterically regulated by cyclic di-GMP control of the bcsB switch. Production of bcsC is suggested to result in the formation of membrane channels for cellulose secretion while bcsD is believed to play a role in forming the cellulose into crystalline fibrils [1]. Along with this are the downstream cmcax gene which encode for endo-beta-1,4glucanase that is secreted into the extracellular space and is believed to influence the assembly of cellulose ribbons when there is failure in arrangement by cleaving tangled chains of cellulose [6]. The ccpAx product has proven to also be important in locating the bcs complex to the cell membrane and interacting with the bcsD subunits. Of course to provide the UDP-glucose necessary for the cellulose synthase to begin this process, the cell must have the common enzymes of glucose kinase to generate glucose-6-

phosphate from glucose, phosphoglucomutase to isomerize glucose-6-phosphate to glucose-1-phospate, and UDP-glucose pyrophosphorylase to form UDP-glucose from UTP and glucose-1-phosphate.

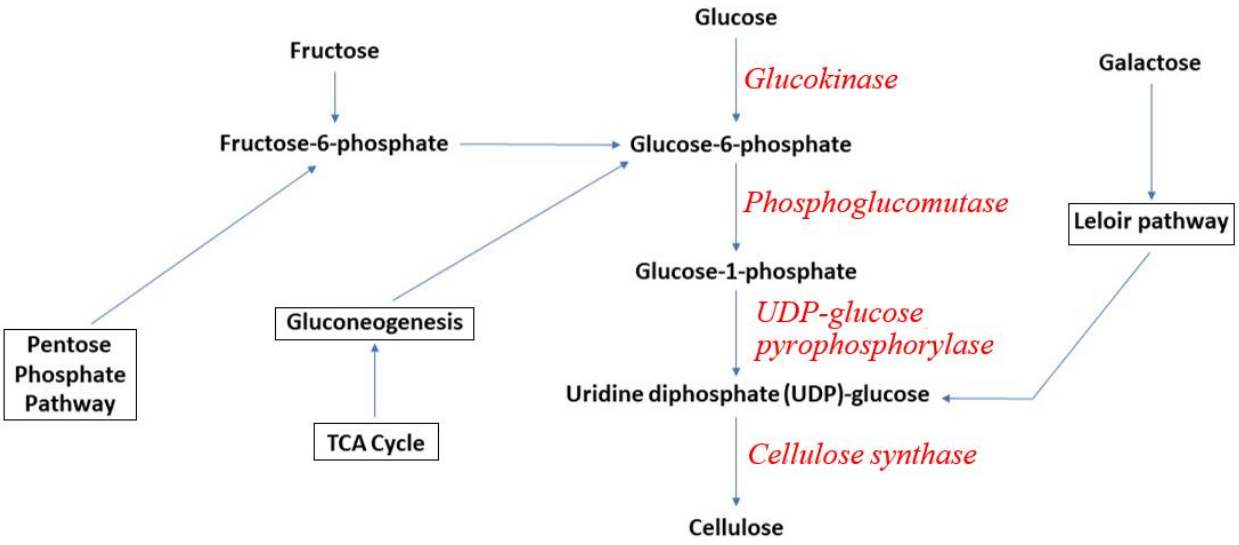

Figure 2 Bacterial cellulose biosynthesis pathway with metabolite denoted in black, metabolic pathways as black boxes, and enzymes involved in the respective reactions denoted in red [69].

Apart from the specialized enzymatic machinery needed to produce the bacterial cellulose into ribbons, the cell must also have the necessary means to generate the UDP-glucose from various carbon sources that are available in its environment. Figure 2 depicts a simplified biosynthesis pathway for bacterial cellulose in Acetobacter xylinum [7-10]. Different carbon sources may enter from the bacterial cellulose biosynthesis pathway in different ways as glucose, fructose, and galactose [11]. Disaccharides like sucrose, lactose, or maltose and more complex sugars will of course first be converted to their respective monosaccharides via enzymatic hydrolysis, which are then fed directly into the cellulose biosynthesis pathway [8, 11]. Different species of cellulose producing bacteria have been indicated to have preferential carbon sources, and the carbon source

will itself affect the rate and yield of cellulose [11, 12]. The rate of production in looking at the cellulose synthases function to polymerize UDP-glucose into β linked chains is also inherently linked to the regulation of this enzyme. As has been previously shown, cyclic-di-GMP is an allosteric activator of cellulose synthase implying that absence of the cyclic-di-GMP leads to inactive cellulose synthase [8]. Indeed cyclic-di-GMP reversibly binds to cyclic-di-GMP binding protein (a membrane protein) and becomes unavailable; hence, controlling the equilibrium between bound and unbound cyclic-di-GMP which may be done via the intracellular potassium concentration may serve to push for enhanced bacterial cellulose production [8].

As with most biomaterials of interest, it is not only the production of the cellulose but its hierarchical structure that lends itself to the resulting properties. Examination of the cell-directed assembly of cellulose has shown highly crystalline cellulose networks to result from secretion of the cellulose through the membrane embedded enzyme complexes discussed above. In contrast, it has been shown that transformation of only the bcsABC genes results in a non-crystalline material with no utility for manufacturing as a biomaterial [13]. The organized self-assembly has been characterized in recent works revealing that van der Waals forces first facilitate the crystallization of cellulose chains into mini-sheets and hydrogen bonding of the mini-sheets into mini-crystals that emerge from the membrane bound pore complex as a single terminal complex (TC) subunit that are precisely spaced as to allow formation of crystalline cellulose I microfibrils [14]. As illustrated in Figure 3, further organization of the microfibrils into bundles of microfibrils by sufficiently close proximity of neighboring TC subunits into a functional row of TCs and finally formation of ribbons have been shown that there is a significant hierarchical cellulose assembly process which is largely controlled by the bcsD driven arrangement of linear TC arrays and their

orientation longitudinal to the axis of the cell [14]. The highly organized nature of this resulting 3D bacterial cellulose network affords its superior strength to and stability for which it continues to find applications where limitations in its industrial use are predominantly attributed to its relatively low yield and higher cost than plant cellulose.

Production of the bacterial cellulose necessarily requires a culture environment, whether static or agitated bioreactor, for the cellulose producing strain of bacteria to be used along with considerations of the growth media (specifically the source of carbohydrates and other nutrients as well as the acidity). In general, the production rate of BC is directly dependent on the oxygen transfer coefficient of the culture where typically continuous cultures outperform batch cultures [3]. Because this aerobic process occurs predominantly at the interface between the air and the medium for static cultures, the rate of BC production is relatively low in static batch cultures despite their yield of very uniform sheets of BC. Agitated fed-batch cultures with glucose supply and control over dissolved oxygen content have shown relative enhancement in yield up to 15g/L [15]. The degree of agitation of cultures has benefits in generating homogenous distributions and enhancement of oxygenation but can also have drawback in requiring energy consumption and where issues of bacterial cellulose produced through agitation have weaker mechanical properties and are produced in small granules as opposed to larger pellicles depending on the degree of agitation [16]. To reduce costs, air lift reactors have been found to require only 1/6th the energy of a stirred tank reactor for a given equivalent amount of BC production [3], where the use of air lift reactors become more beneficial as the degree of culture viscosity increases.

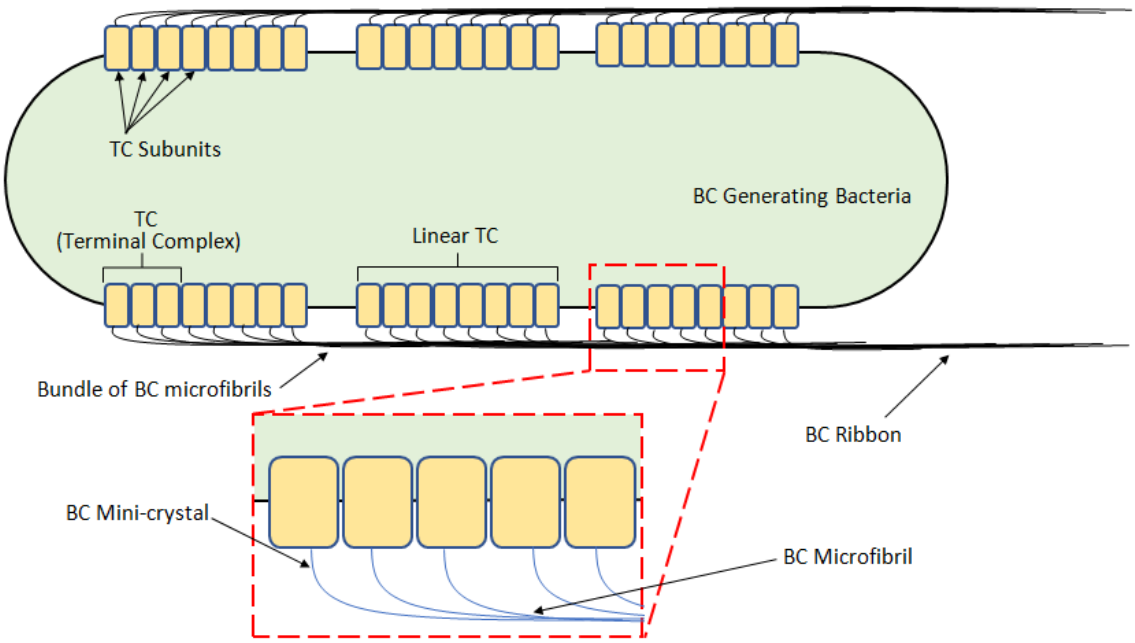

Figure 3 Hierarchical organization of bacterial cellulose (BC) formation into ribbons during biosynthesis of pellicles.

Aside from the culture method, the quantity of BC production is highly dependent on the composition of the media. Among the most common lab scale media is the Hestrin-Schramm formulation which has been reported to provide higher production than the Yamanaka media formulation [17]. In comparison to Yamanaka formulations and Zhou formulations, the pH stability of the Hestrin-Schramm formulation was the most stable to buffering the gluconic acid by-product formation resulting from bacterial cellulose production [18]. Because a decrease in the pH of the medium reduces the production of BC, studies have explored the use of additives including lignosulfonate to successfully inhibit gluconic acid oligomer formation. While lab scale media formulations are relatively expensive, to make bacterial cellulose economically feasible to a range of applications a significant amount of research has gone into identification of low-price culture medium carbon sources [18]. For example, fruit juices have been examined for BC production by the strain G. persimmonis, and have shown muskmelon juice to provide over 8

grams of bacterial cellulose production per liter [19]. Agricultural and industrial wastes have also been actively researched with cotton-based textile waste fabrics proving to be an interesting source of carbon feedstock after enzymatic treatment to produce 10.8 grams of BC per liter of culture [20]. On par with this production level was the use of carbon-rich drainage water from rice wine production, resulting in BC production at yields of 10.38g/L [21]. Surpassing this, the use of confectionery industry waste water as flour-rich hydrolysates allowed production of BC at 13g/L [22]. It is worth noting that while the composition of the BC produced by these different means discussed above remains the same, the effects of their extent of polymerization, mechanical properties, water holding capacity, and degree of crystallinity are highly depending on the carbon sources as well as the production technique. Herein we'll discuss more about the properties of bacterial cellulose as related to biomedical applications, how these properties and capabilities of bacterial cellulose have been altered and expanded in recent studies, and the biomedical products to which bacterial cellulose has been successfully implemented.

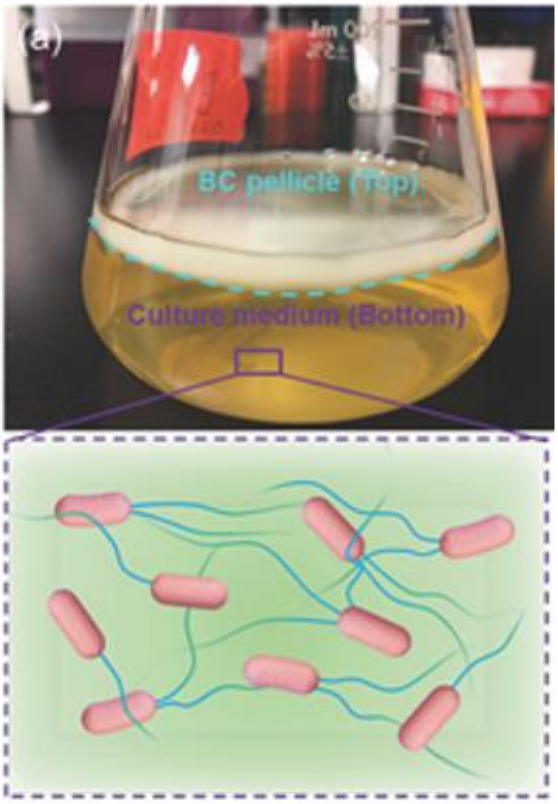

Figure 4 Bacterial pellicle formed at the air liquid interface of a static batch culture. Adapted with permission from Wang et al. [23]

1.2. Properties of Bacterial Cellulose

1.2.1 Water Content

One of the most important properties of BC is its ability to intake and retain water [69]. This makes BC a suitable material for biomedical applications, as the extracellular matrix of most living tissues largely consist of water [24]. BC can hold from 60 to 700 times its dry weight in water, depending on how it's manufactured or modified [25]. Most of this water is chemically bound to the BC fibrils, and is not free. Specifically, 10% of this water that is chemically bound exists in the form of hydration shells around the cellulose microfibrils comprising the BC structure [26]. The abundant hydrogen bonds that are present in between the cellulose microfibrils are what helps

grants BC with its water retention capacity. There are several ways to modify BC in order to effect its swelling and water retention capacities. Chemical modification is the most common way, with the use of composites including silk fibroin being one such method [27]. Silk fibroin has been successfully proven to substantially improve the swelling properties of BC [4] as have incorporation of other component into BC including chitosan and montmorillonite [27, 28]. These approaches largely make use of altering the pore size, pore volume and surface area of the BC, which in turn affects its water retention and intake.

1.2.2 Mechanical Properties

BC is also known to have good mechanical properties, which makes it a good material for tissue engineering [69]. In its hydrated form, it has a Young's modulus of 10 MPa and a stress test value (at failure) of 1 MPa [29]. In contrast, sheets prepared from dried bacterial cellulose have been reported to have a Young's modulus of over 15 GPa along the plane of the sheet and tensile strengths of 260 MPa [30]. By altering the water content of the BC, it is thereby possible to greatly affect the membrane stiffness, namely where decreasing water content will lead to increasing membrane stiffness [31]. Other results have shown BC in its hydrated form to have a similar Young's modulus of 11 MPa with percent elongation at breaking point of 35%; while, the dry BC exhibited a Young's modulus of 1.3 GPa, and an almost zero percent elongation at break [32]. Aside from hydration state, researchers have found several other ways to modulate the mechanical properties of BC for instance through the use of composites. One such example is to the use of cross-linked BC/collagen mixtures, which showed an increase in tensile strength by 57.9% over BC alone [33]. By incorporating a layer of hydroxyapatite on the BC via biomimetic mineralization, research have even created a biomaterial with mechanical properties of interest for bone tissue

scaffolds [34]. In another method, the incorporation of paraffin beads during growth of the bacterial cellulose served as a porogen which after removal of the paraffin resulted in an interconnected porous network that would mimic the mechanical properties of extracellular matrix and this resulting BC scaffold promoted substantial regeneration of the human auricle [35]. Yet a different approach is to generate a composite in situ by co-culture of G. hansenii and E. coli that produce a mannose-rich exopolysaccharide which incorporates into the BC microfibril network. The Young's modulus for such BC increased from 2.6 GPa to 4.8 GPa by incorporating the co-cultured process; while the stress at breaking point increased 80 MPa as compared to 45 MPa for the monocultured BC [36].

1.2.3 Structure

BC is a biopolymer that is composed of ultrafine nanofibers, which give it a natural pellicle (hydrogel-like) structure [37]. Specifically, it is composed of linear strands of ultrafine nanofibers, which assemble to form microfibrils that generate tight bundles giving rise to compact ribbon-like structures that form an interwoven network providing the pellicle [38]. The structure is comprised of β-(1,4)-linked D-glucose residues, with strong hydrogen bonds between the adjacent cellulose nanofibers [39, 40]. This structure allows BC to have numerous hydrogen bonds, which is allows it to retain water and also form its hierarchical structure through inter and intra-molecular with neighboring hydroxyl groups [31]. Because this nanofibrous network resembles aspects of the extracellular matrix of some tissues. This structure in combination with the ability of BC to be easily modified in situ and ex situ opens the potential for this material serving as an important biomaterial for wound healing and tissue regeneration applications. One such example is the use of silver nanoparticles (Ag-NPs), which form a robust BC-AgNP hybrid with excellent

antimicrobial properties, making it ideal for wound healing [37]. Another way is to utilize polyethylene glycol (PEG) to create scaffolds with improved viscoelasticity [41]. There are also methods that can be used to template the BC to alter its surface as well as overall architecture. Agarose film scaffolds with honey-comb patterned grooves can be used to guide G. xylinus to produce honey-comb patterned BC [42]. Polydimethylsiloxane (PDMS) substrates can be used to orientate BC fibers by controlling the ridged morphology of the PDMS [43]. It is also possible to create microporous BC scaffolds, via the use of paraffin wax microspheres, to create ideal scaffolds (pore size: 300-500 μm) for bone regeneration [44], among other purposes.

1.2.4 Porosity

Pore size is critical when developing scaffolds for tissue engineering [69]. Depending on the type of tissue and the function of the scaffold, the pore size will vary anywhere from 100 nm (e.g. extracellular matrix) to 100 μm (e.g. neovascularization) [45]. Figure 5 shows an example of the structure and porosity of bacterial cellulose. A good example of the effect of pore size was shown when researchers constructed a microporous BC scaffold with good interconnectivity of 300-500 μm size pores resulting in improved cell penetration and seeding within the scaffold [44]. Other techniques that can be used to create porous BC scaffolds include freeze-drying BC-hydrocolloid mixtures, laser patterning, and 3D-printing [46-48].

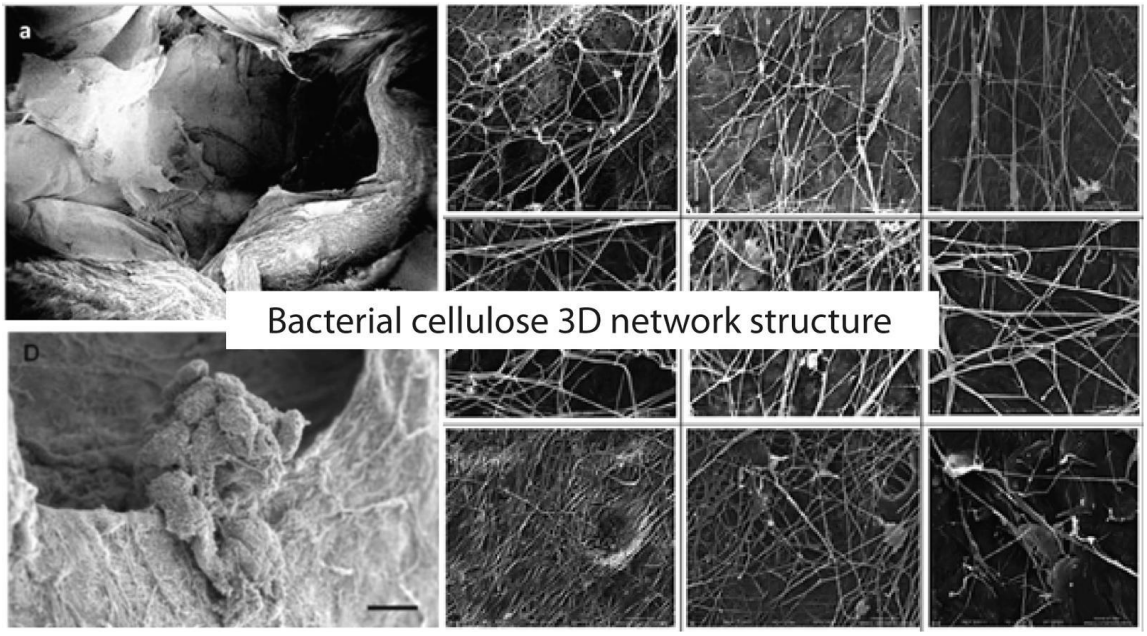

Figure 5 Three-dimensional network structure of bacterial cellulose showing fiber and pore structures. Adapted with permissions from Halib et al. [38]

1.2.5 Biocompatibility

Because bacteria like Komagataeibacter xylinus and G. hansenii, are used to produce bacterial cellulose in laboratories and industry, the lipopolysaccharide component of their outer membrane presents an endotoxin, which if not removed, would cause biocompatibility issues [69][49]. One way to remove endotoxins and improve biocompatibility is to wash the bacterial cellulose (BC) with sodium hydroxide solution. Avila et. al carried out such a set of experiments where they washed BC hydrogels with sodium hydroxide, thereby reducing the endotoxin content from 2390 EU/ml to 0.1 EU/ml, thereby improving the biocompatibility considerably [50]. In conjunction with this, an additional approach to modify the BC to even further improve biocompatibility is via in situ carboxymethylation [51]. Such an approach can improve the biocompatibility by reducing the inflammatory response but even as a sterilized material BC has been shown to have good hemocompatibility and cytocompatibility [52].

1.2.6 Biodegradability

Bacterial cellulose (BC) is highly resistant to degradation and can withstand high thermal, mechanical and chemical stress [69][53]. This interesting property could inherently limit the in vivo use of this material in certain biomedical applications, as an ideal implantable tissue scaffold should degrade while it facilitates tissue growth. Nonetheless, cellulolytic enzymes can be used to degrade the material and this is often employed as are a number of other chemical processing strategies for native BC. One such group has shown that incorporating graphene oxide/hydroxyapatite with BC could help to create an osteoconductive scaffolds that have claimed to improve the biodegradability [54]. A second group used sodium periodate and hyaluronic acid along with BC to synthesize a scaffolding material with improved degradability for bone tissue engineering applications [55]. In general the in vivo use degradation approaches for bacterial cellulose are limited. BC composites with chitin have nonetheless been made which are enzymatically cleavable by metabolic engineering [56], and controlled oxidation of BC sheets that have been previously gamma irradiated offer a material that is more bioresorbable [57]. In contrast, because microorganisms in the environment can readily cleave the beta glycosidic bonds of bacterial cellulose, BC has the ability to undergo relatively rapid biodegradation making it an attractive polymer with a low environmental footprint.

Overview of research project

The overall goal of the project described here is to optimize the production of bacterial cellulose encapsulated with magnetite nanoparticles and examine the feasibility of these materials for use in biomedical applications including as possible controlled release biomaterials. The overall design and fabrication mechanism is presented below.

Specific aims:

Aim 1 – Optimize Production of Bacterial Cellulose

- ❖ Incubation conditions
- ❖ Media conditions

Aim 2 – Incorporating Magnetite Nanoparticles in Bacterial Cellulose

- ❖ Controlling In Situ Synthesis
- ❖ Characterization of Magnetite Nanoparticle Embedded Bacterial Cellulose

Aim 3 – Investigating Feasibility of Magnetite Embedded BC for Biomedical Applications

- ❖ Evaluate ability to load pellicles with cargo for controlled release applications
- ❖ Test the infrared absorption capability of the material for localized heating

The novelty of this project encompasses the development of bacterial cellulose biomaterials that are able to be functionalized in situ with magnetite nanoparticles. These magnetic nanoparticles are predicted to provide a functional benefit in allowing for external modulation of the biomaterial.

CHAPTER 2: EXPERIMENTAL METHODS

2.1 Bacterial Strain and Cultural Condition: Gluconoacetobacter hansenii ATCC 53582 isolated from kombucha was grown at 25˚C in sterilized Hestrix-Schramm medium (per liter) consist of 20 g D-dextrose (glucose), 10 g peptone, 10 g yeast extract, 1.35 g sodium phosphate, 0.65 g citric acid.

2.2 Bacterial Cellulose Production: Bacterial Cellulose production in 125 ml Erlenmeyer Flask containing 40 mL of Hestrix Schramm media (pH 5.0)supplemented with 20 g/L dextrose, 10 g/L peptone, 10 g/L yeast extract, 1.35 g/L, with sodium phosphate and 0.65 g/L citric acid as media buffer over cultivation time of 14 days.

2.3 Bacterial Cellulose Drying: Harvesting the bacterial cellulose pellicles after 14 days of incubation, lays the pellicle over the plastic weighing trays for 2~3 days of air-drying at 25 ˚C. It is recommended to apply thin layer of lubricant such as vegetable oil to prevent adhesion between BC pellicles and weighing trays. After the air-drying process, the BC dried sheet were harvested and stack together for mechanical tensile testing.

2.4 Sample preparation and mechanical testing: Stack 6-8 dried BC sheets together, sketch the outline of the testing sample such as the dog bone shape, which is recommended for majority of mechanical tensile testing. The dimension of the sample are 25 mm ×40 mm, with the end height of 5 mm and the neck part in 10 mm in width.

2.5 Glycerol influence on production over bacterial cellulose: Glycerol is another source for Gluconoacetobacter incubation for the purpose of bacterial cellulose synthesis. The advantage of utilizing glycerol for media is glycerol is more readily available, glycerol extract typically extracted from biodiesel production as part of the process [53][54] render it more economically viable as bacteria cellulose substrate. Compares to glucose, glycerol consumes relatively less substrates per carbon unit and marginally higher bacterial cellulose productivity, with 47.96% of glycerol consumed for BC production in contrast 19.05% in glucose as well as resulting in cleaner BC product [53][54].

2.6 Magnetic Bacterial Cellulose Fabrication: We prepare the 2:1 molar ratio of iron (III) chloride hexahydrate (0.54 g) and iron (II) chloride tetrahydrate (0.198 g) with 20 ml deoxygenated water as well as a magnetic stir bar in a two-neck round bottom flask. To acquire deoxygenated water, simply bubbling the water with Nitrogen gas. Seal up the round bottom flask with rubber stoppers and create small opening by inserting syringe needles before heating up the round bottle flask with hot plate heated silicon oil bath. Start stirring the iron chloride solution in 350 rpm while the iron chloride solution heating up to 80˚C. After 5 minutes of stirring, double the stirring speed up to 700 rpm and injecting 5 mL of 10%~15% ammonium hydroxide in 5 minutes duration. The color of the solution should turn from brownish yellow to ink black once in contact with ammonium hydroxide. After the ammonium injection, continue heating and stirring the synthesized magnetite solution in 15 minutes. Remove the heat source and mechanical stirring after 15 minutes and separate magnetite precipitates and magnetic bacterial cellulose from supernatant with strong magnet. Removes the supernatant with disposable glass pipette and pour in 100 mL deionized water to clean up the residual ammonium hydroxide. After the initial cleaning

process, transfer them into 50 mL plastic tubes and perform sonication [55]. For certain experiments such as analyzing heat sensitivity, lyophilized the magnetic bacterial cellulose in tissue cultural plates

2.7 Liposome diffusion procedure: prepare 10 mL of 1mg/mL PCDA-EDEA solution in two 12.5 mL tubes each and suspend both magnetic bacterial cellulose (MBC) and Bacterial Cellulose (BC) into the respective tubes. Then perform serial dilution in daily basis: After each day, remove the MBC and BC, suspend them in 9 mL of deionized as both MBC and BC theorized to absorbed 1mL of liquid from previous solution. After the serial dilution, subject PCDA solutions and their dilutes to spectrophotometry to determine the concentration of PCDA from 450 nm wavelength absorption.

2.8 Heat Conversion Analysis Procedure: Utilize 1064 nm laser as the heat source as higher frequency light can be deliver in short distance with little loss in energy. Expose each magnetic bacterial cellulose pellicles in different molar concentration in magnetite with laser for 10 seconds duration. Right after the removal of the power source, record the temperature of the pellicles with temperature probes. To better monitor the heat sensitivity magnetite from laser exposure, we are setting 3 trials of delivering 1 W/cm2, 2 W/cm2 and 3 W/cm2 of laser.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Bacterial Cellulose Production

As carbon substrates, glycerol may inhibit the growth of bacterial cellulose when mixed with glucose in Hestrix-Schramm medium due to glycerol disrupts solute equilibrium: As higher concentration of glycerol occurs in HS medium, the solute concentration in media exceeds to that of the cell, thus suppresses cell proliferation due to dehydration [58]. Thus, to further exemplify the extent glycerol can influence the overall growth of bacterial cellulose, growing bacterial cellulose in a controlled environment such as tissue culture plates with successive concentration of glycerol in Hestrix-Schramm for the purpose of determine the optimal growth method of bacterial cellulose.

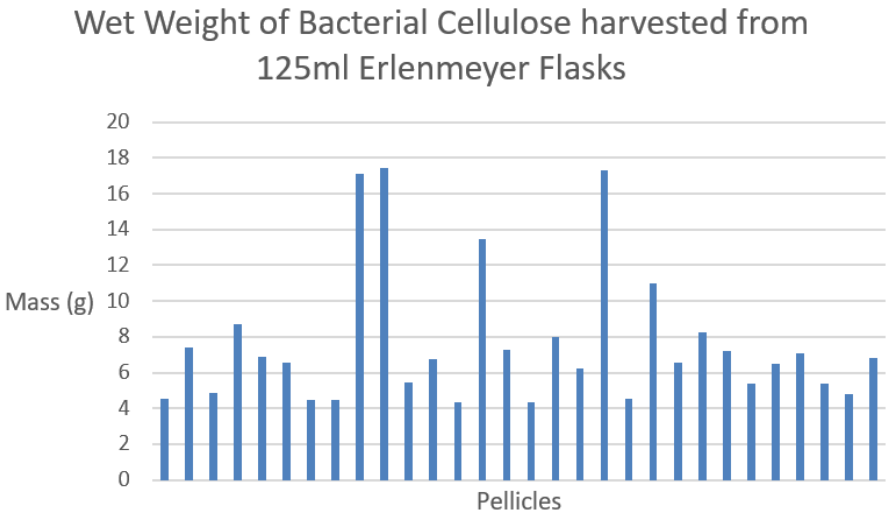

Figure 6 Initial weighing of bacterial cellulose pellicles

Figure 7 Air-dried bacterial cellulose pellicles harvested from the plastic weighing trays

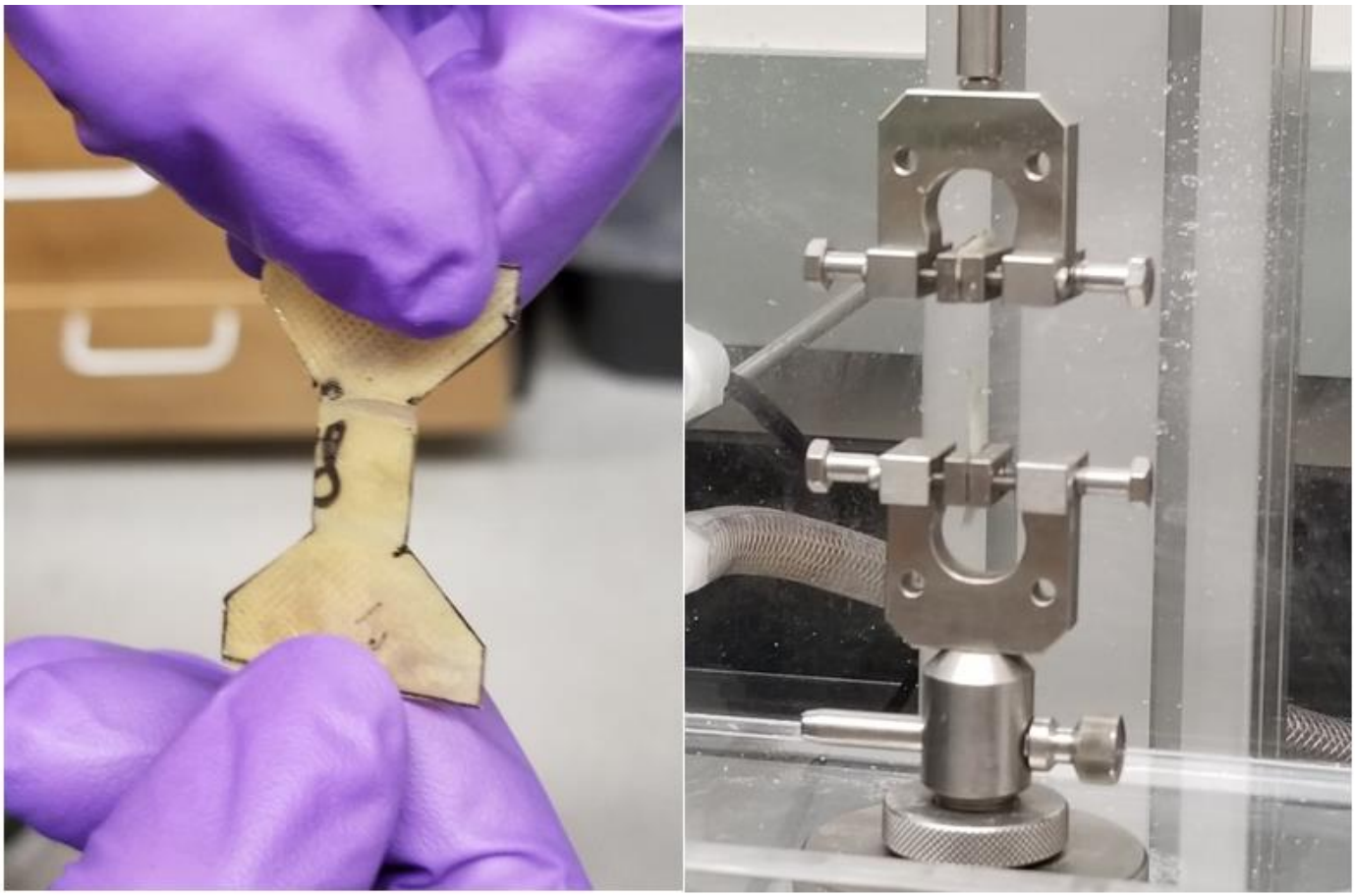

Figure 8 Bacterial cellulose mechanical testing sample and illustration of tensile testing.

Despite the layering of bacterial cellulose samples, the exact dimension of the cross section that subject to uniaxial stress has yet quantified. Therefore, the specific mechanical stress for dried BC sample is unknown

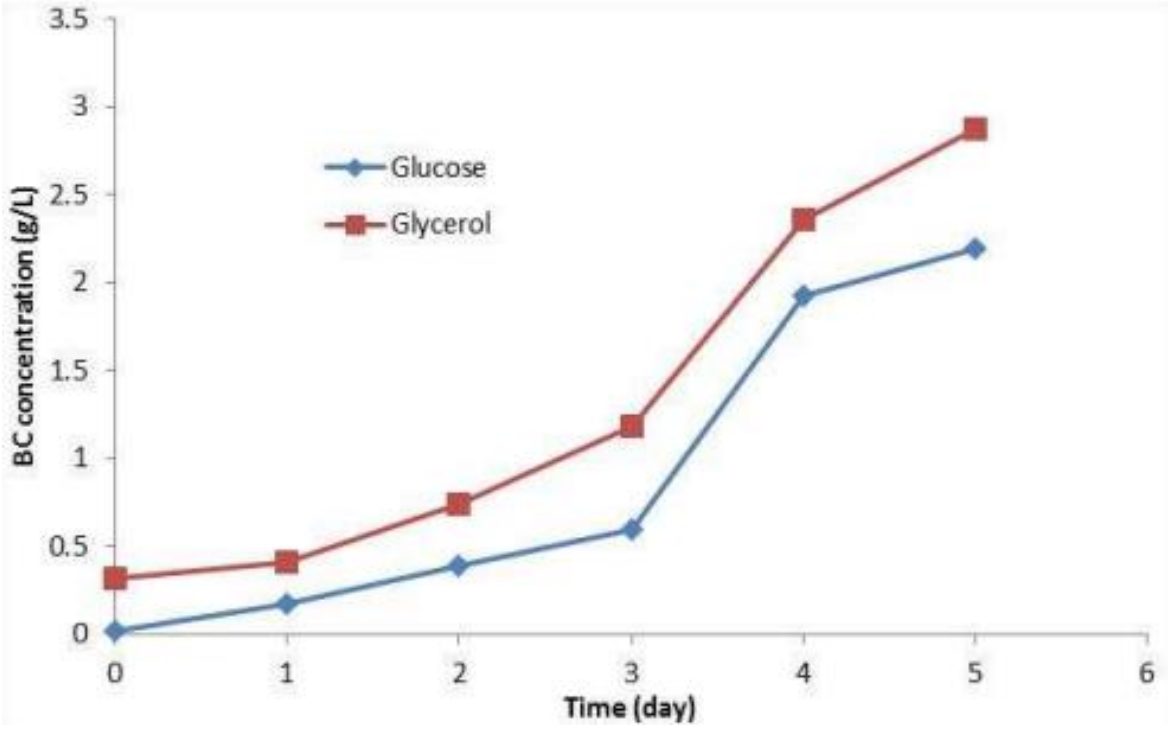

Figure 9: Profile for BC production when growing G. xylinus DSM46604 on 50 g/L glucose or 20 g/L glycerol in a 3-L bioreactor [59]

Based on initial results of bacterial cellulose growth profile in between 2% up to 46% glycerol concentration in HS media, observable BC formation can be seen from 2% to 10% glycerol concentration, reflect the nature which the increase of glycerol concentration reduce the growth rate of bacterial cellulose. Therefore, for the subsequent exploration in optimal glycerol ratio, the replicating results narrowed to 2% to 10% glycerol concentration.

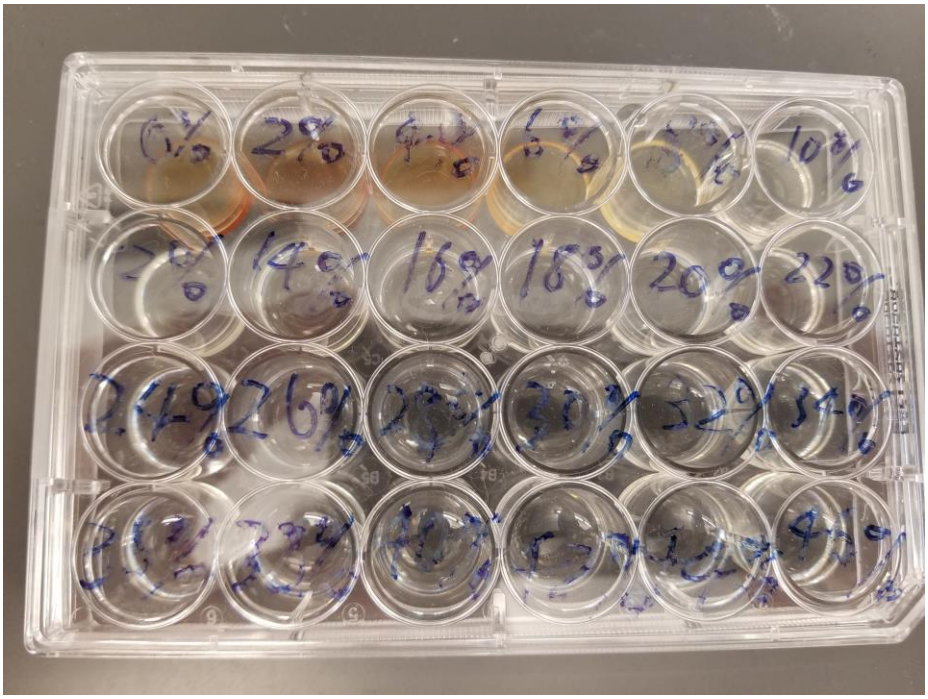

Figure 10. BC incubation outcome with successive volume percentage increase in glycerol after 3 months

Glycerol-HS media culturing procedure: prepare the respective percentage of glycerol-HS mix by adding different ratio of glycerol in volume as well as Hestrix-Schramm media. Suspend 1 mL of prepared media with different glycerol concentration on 24-well tissue cultural plate with 10 tissue cultural plates in total prepared. In a 6-day interval, harvest BC pellicles from one tissue cultural plate and later lyophilized in 1 day for better results to determine the mass of bacterial cellulose by weighing pellicles with analytical balance.

From test results shown in Figure 11. and prior studies, the moderate glycerol volume concentration (6%) provide the maximum yield in bacterial cellulose whereas too little or too much of glycerol concentration in HS media leave substandard yield. The optimal ratio appears consistent with previous findings [58]. To explain the phenomenon of delay and decrease in

Bacterial Cellulose yield by further induction of glycerol concentration, one aspect to consider would be the inhibitory effect of glycerol metabolism over the efficiency of glucose metabolism: Mitochondrial sn-glycerol 3-phosphate dehydrogenase (mGPDH), an essential enzyme for metabolic regulation of glycerol, is known to limit mitochondrial production of H_2O_2 [62]. May indicate the limiting participation of glucose over bacterial cellulose biogenesis since the production of mitochondrial H_2O_2 often interpreted as active glucose metabolism; In matters of equilibrium, glycerol often disrupt the water balance based on the principle of osmosis: As the culture media composed of less water content than the bacterial cell, the cell will dehydrate, thus reduces their viability and duration in bacterial cellulose synthesis [58].

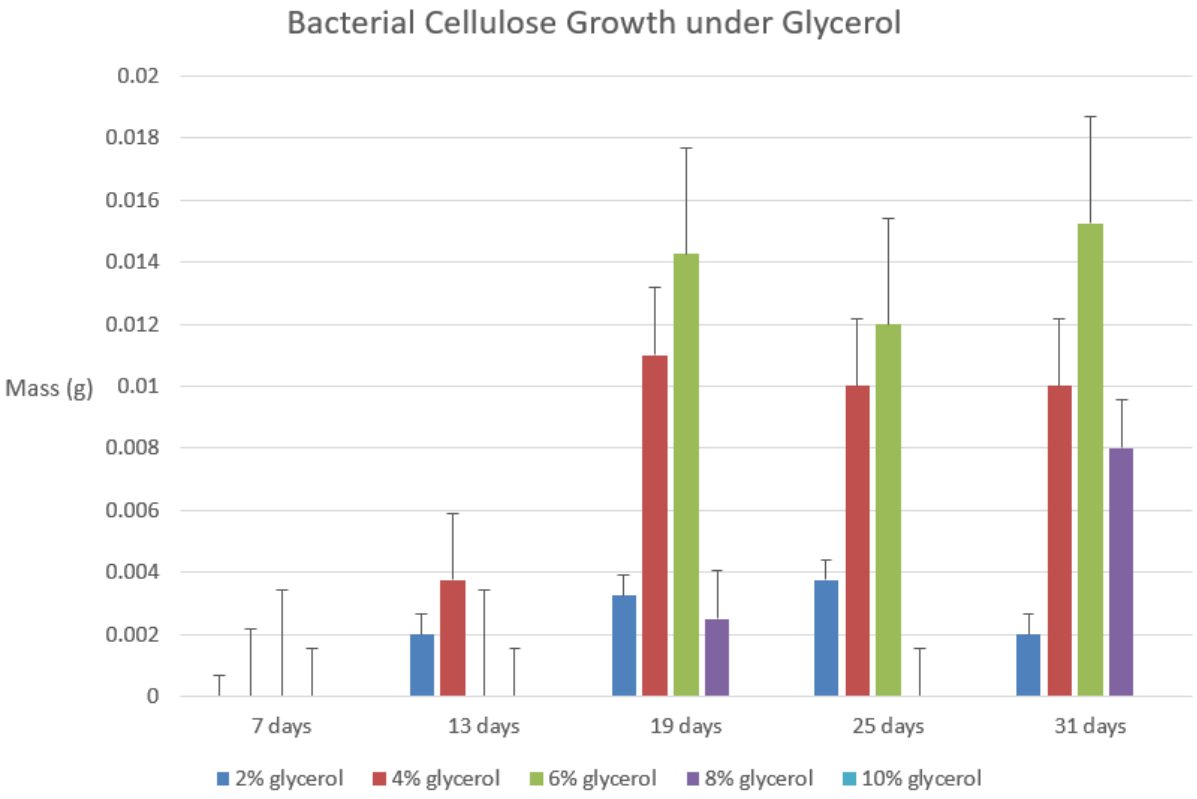

Figure 11. Bacterial cellulose production as a function of glycerol content and growth time.

3.2 Magnetic Nanoparticle Bacterial Cellulose Synthesis and Characterization

Magnetic Nanoparticles gained more interests in biomedical engineering such as drug delivery, biosensing and tissue engineering. As a novel method to introduce hyperthermia, magnetic nanoparticles proven to be optimal candidates due to its heat sensitivity [64]. Magnetic nanoparticles as cancer treatment, reserved as alternative of radiotherapy: Traditional radiotherapy often introduces ionizing radiations which terminates tumor cells proliferation by DNA disruption, often leaves collateral damage on peripheral cells on the tumor sites. For magnetic nanoparticles induced hyperthermia, the healthy cells adjacent to cancer are less vulnerable from the treatment as they are less sensitive to the enzymatic degradation during hyperthermia compares to the cancer cells [65]. Magnetic nanoparticles as method to introduce hyperthermia, possess adequate sensitivity as well as efficient heat conversion rate, from Rashad et al. As energy potential of electromagnetic field multiplied, the magnetic fluids induce heat proportional to the multiplication of voltage in alternating magnetic field [66].

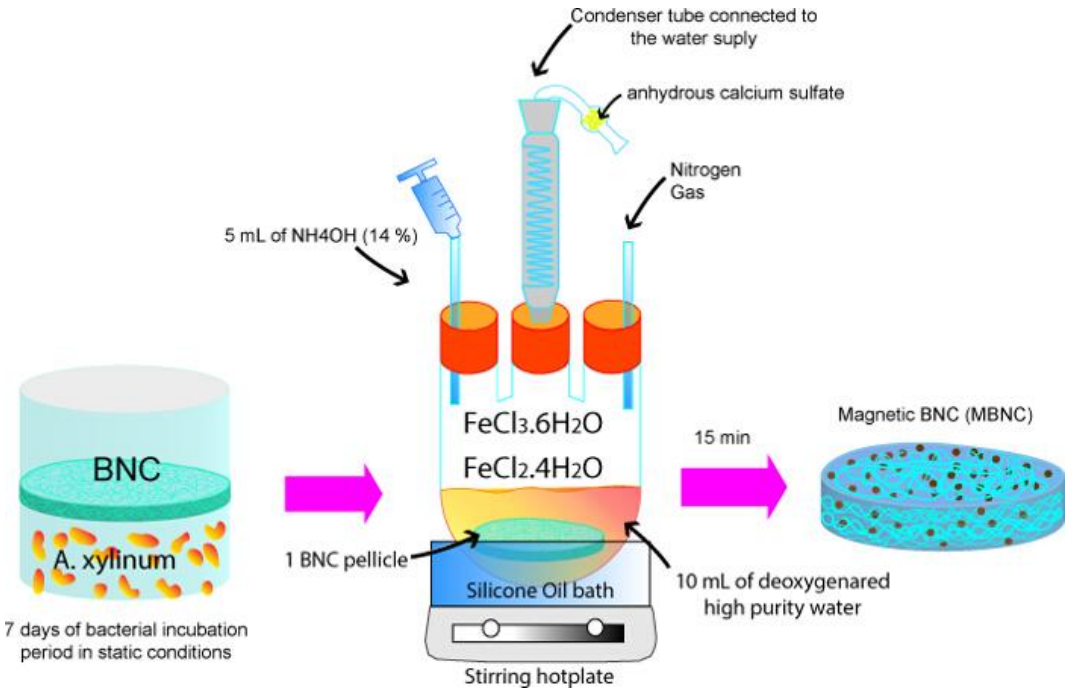

Figure 12 Magnetic bacterial cellulose fabrication method [148]

Magnetic Bacterial Cellulose are also known in the utilization of drug delivery and known to be used in fabricating blood vessel graft [55]. It may possess certain attributes that renders it better at immobilizing or trapping other biomolecules. To expand on the understanding of its immobilization effectiveness. We are utilizing pentacosadiynoic- 2,2'-(ethylene dioxy)-bis-(ethylamine) (PCDA-EDEA) as the targeted "cargo" for the study. PCDA-EDEA as liposome is high modifiable and able to mimic some form of protein structure and reactions. And there are applications such as utilizing PCDA to fabricate vesicles for delivering genetic information [63].

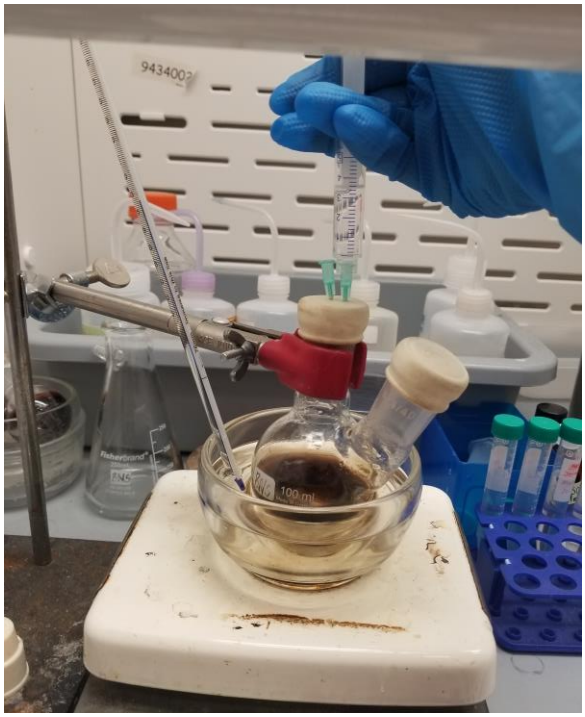

Figure 13 Image of magnetic bacterial cellulose synthesis setup

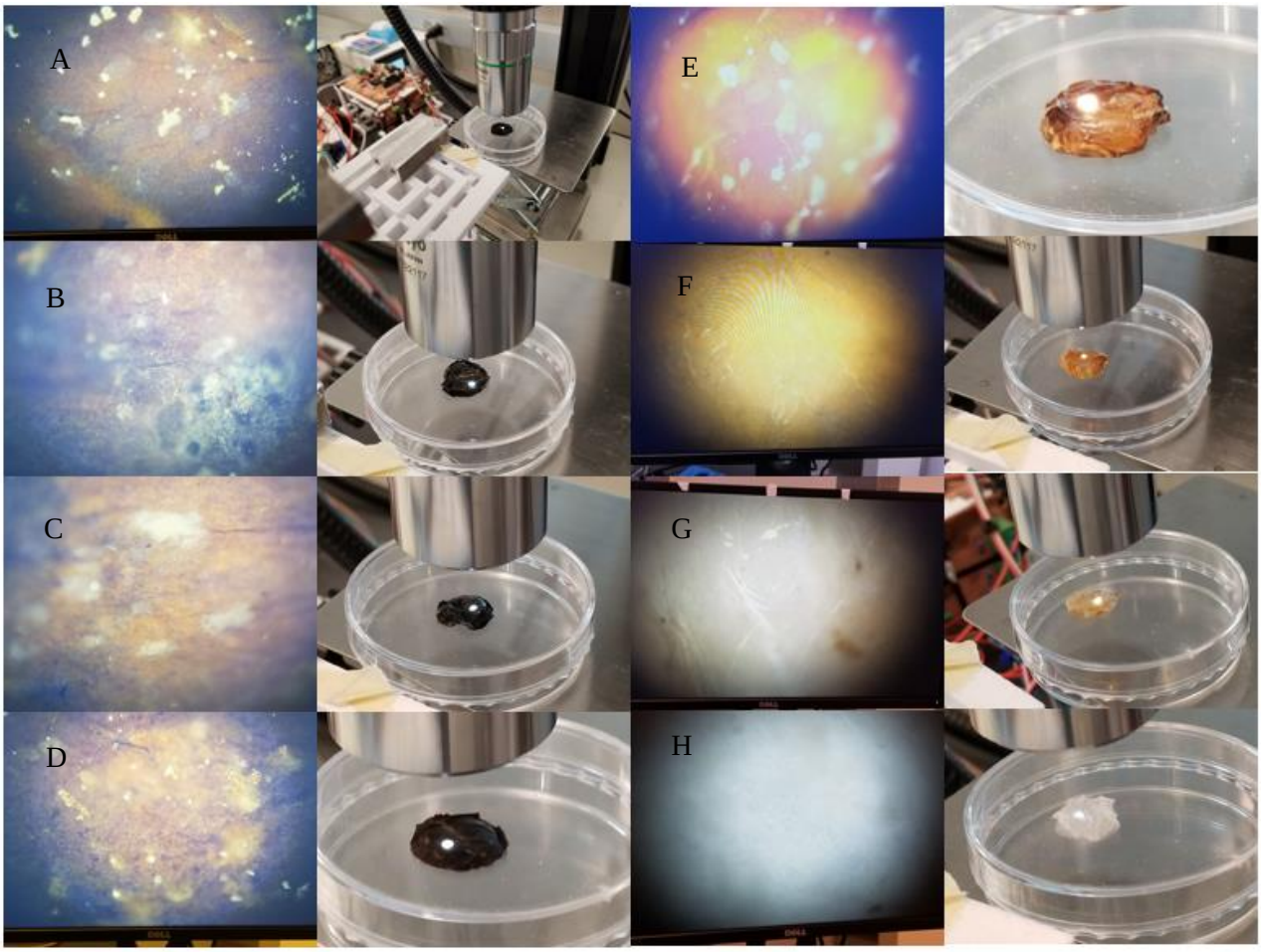

Figure 14 Magnetic Bacterial Cellulose with different magnetite concentration (A: 0.1 mol, B: 0.08 mol, C: 0.06 mol, D: 0.04 mol, E: 0.02 mol, F: 0.01 mol, G: 0.005 mol, H: Control.) under microscope

3.3 Examining Feasibility of Magnetic Bacterial Cellulose for Biomedical Applications

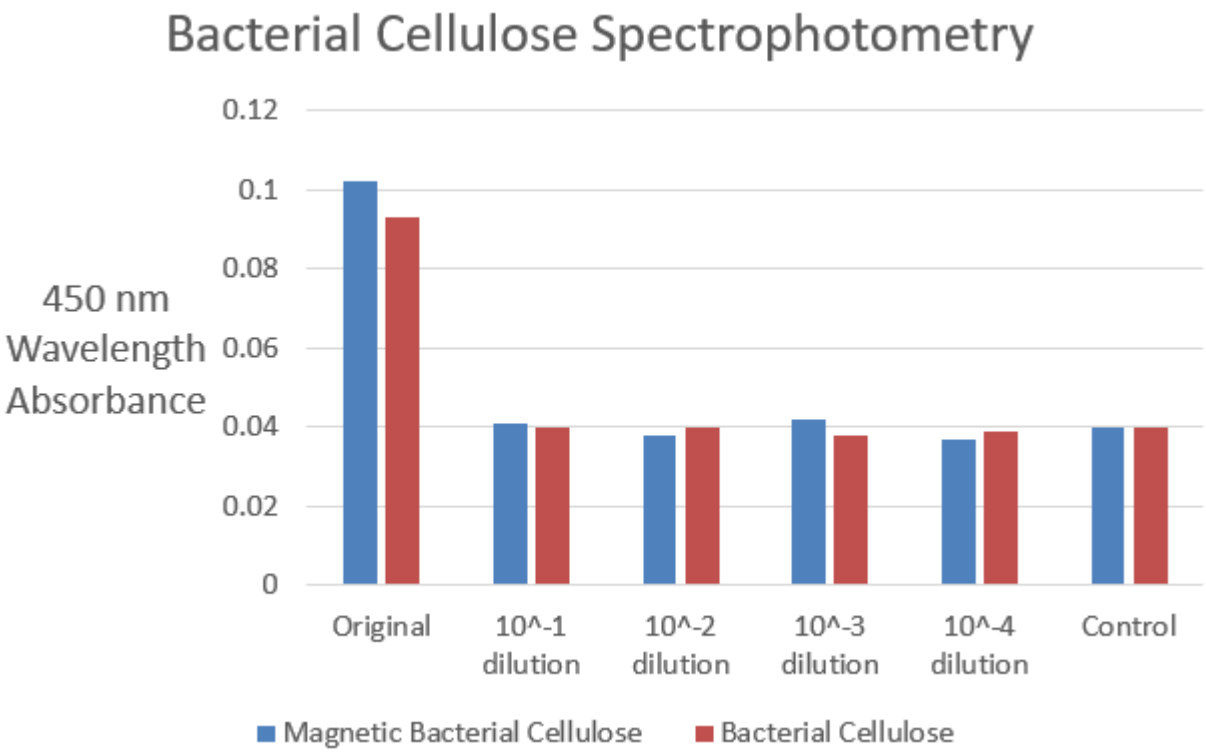

Figure 15 Liposome concentration from the serial dilution.

From the analysis, magnetic bacterial cellulose ability of immobilizing Liposome such as PCDA-EDEA proves to be inconsequential compared to the bacterial cellulose. This could be explained by the supposed high porosity of bacterial cellulose despite the incorporation of magnetic nanoparticles. In addition, magnetite being an effective method in drug delivery, provide little biochemical reaction with cell surface in the form of adhesion. The purpose why magnetite incorporated as blood vessel construct is for the prevention of abnormal cells or biomolecules to build up on the vessel walls [61].

Magnetic nanoparticles often utilized mainly for hyperthermia in clinical treatments. The hyperthermia induced by magnetic hysteresis is safely conducted for its regulation of abnormal

cell proliferation without the consequence of cell necrosis. In consequence, magnetic nanoparticles often repurposed for inducing tumor cell apoptosis. To expand on what is the optimal magnetic nanoparticles for hypothermia. We have conducted the heat conversion analysis on magnetic bacterial cellulose with different concentration and different power input.

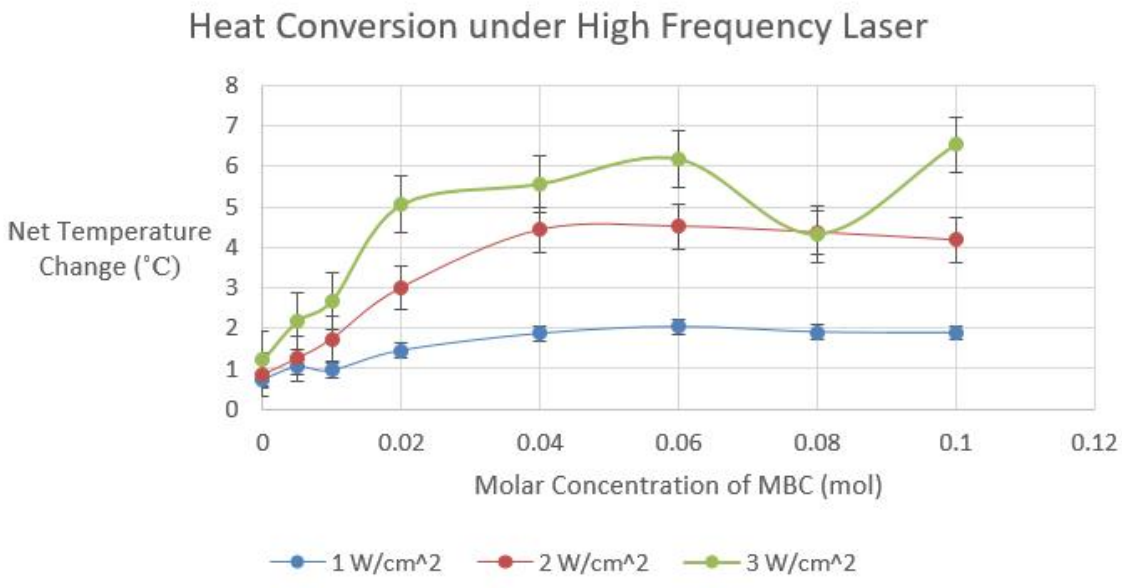

Figure 16 Heat Conversion analysis procedure

From the analysis, the heat sensitivity of Magnetite is apparent as each unit of power density increases, the net temperature increases for about 2~3 ˚C. The overall heat conversion profile is also consistent with the nature of magnetic hysteresis as increase in dipole moment from external sources, the temperature increase of magnetic nanoparticles were in logarithmic trajectory. Therefore, with lower energy input for desired hyperthermia reaction, 0.04 molar concentration of Magnetite in Bacterial Cellulose is sufficient. The configuration and the size of its cluster for magnetic nanoparticles can also contribute to the magnetism and efficiency in hyperthermia: The Thermal energy generation from Magnetic Hysteresis is due to vibration and resistance while nanoparticles realign to create dipole moment under the influence from the external electromagnetic field. To maximize the vibration and geometric resistivity, there are structures such as cubes or clusters of irregularities of nanoparticles possess considerably higher rate of absorption [68].

CHAPTER 4: CONCLUSIONS, LIMITATIONS, AND FUTURE WORK

BC manufacturing happens at a smaller scale than conventional plant cellulose but holds higher interest from a medical and ecological perspective due to providing a non-toxic, biocompatible, fully biodegradable, and renewable material source [69]. While significant advances have been made in BC processing, identifying low cost substrates, and development of efficient BC producing bacterial strains, there remains to be commercial scale bioprocessing of BC that is economically feasible to compete with traditional cellulose for low cost applications as opposed to higher value biomedical applications. Room for future investigations to lower the costs of BC continue to exist in finding culturing conditions that may utilize waste materials for feedstock. For overcoming areas of difficulty for clinical progress, future work in providing better quality control over the porosity and consistency throughout the material, where the culture environment creates non-uniformities not only batch to batch but even for internal vs external regions of the same batch. The issue of requiring consistency for biomedical applications can be complicated from the property of bacterial cellulose to be an easily tunable material and can inherently be affected by production processes intended for enhancing yield or reducing costs. In addition to the challenges of establishment of consistent manufacturing for bringing BC based biomedical products to market, there is also the important aspects of assuring non-inferiority over current technologies. The considerable efforts to improve its processing ability for construction of tailored devices and improving its antimicrobial properties have similarly pushed forward the feasibility of new and exciting biomedical products. A promising future for new biomedical technologies based on BC will certainly stem from their adaptability in bestowing new functions through their easy of modification and capability of formulating composites. Perhaps with this natural fibrous network

capable of facilitating cell adhesion, we will continue to see improvements in synthetic composites to provide antimicrobial properties and further manipulate the porosity to satisfy control of cell uptake while providing a barrier with high water content for wound healing and skin regeneration applications. While commercial products based on BC are already available in these areas, additional progress in providing encapsulation of drugs are expected to expand its use to practical transdermal and drug delivery applications. It is anticipated that as continued interest in the genetic engineering of these BC producing organisms finds ways for incorporating additional monosaccharides as we have recently seen, we will continue to find new biomedical application areas than originally imagined.

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