Tampereen yliopisto

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BACTERIAL CELLULOSE AS A SURGICAL SUTURE MATERIAL

Bachelor's thesis
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ABSTRACT

Ella Meriläinen: Bacterial Cellulose as a Surgical Suture Material
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Surgical sutures are used to approximate tissues together which enables the wound closure and healing. An ideal suture should have a good balance between the suture diameter and tensile strength and the surgeons should be able to handle the suture easily while making the stitches.

Bacterial cellulose (BC) is cellulose that is produced extracellularly by some bacteria. Although the BC and plant-based cellulose (PC) have the same molecular formula, their material properties are different. BC is an attractive biomaterial in the medical engineering field because it is very pure, has good biocompatibility, and it does not normally degrade in the body environment. The properties of BC can be changed for example via chemical modifications or changing the culture conditions. BC is a very versatile material which makes it an attractive material for surgical suture research.

This bachelor's thesis aimed to study if BC can be used as surgical suture material and how this type of sutures could be produced. The goal was also to find out if the research results of the studies about the usage of PC in surgical sutures can be applied with BC. The research was done by using literature sources, like existing research and patent documents.

The findings of this thesis were able to answer all research questions. BC has been used as a surgical suture material in several studies with promising results but currently, there are no commercialized BC-based sutures in the market. Multiple ways to produce BC sutures were found, including wet-spinning, hydrogel strip cutting and cultivation form-based methods. Studies with PC were assumed to be appliable with BC in the cases where the PC was not used as a natural plant fibre structure. For example, studies with PC-based hydrogel sutures could be appliable with BC due to the similarities of their chemical structures.

Keywords: Bacterial cellulose, Surgical sutures, Plant cellulose

TIIVISTELMÄ

Ella Meriläinen: Bakteeriselluloosan käyttö kirurgisena ommelmateriaalina
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Kirurgisten ommellankojen avulla potilaaseen voidaan tehdä ompeleita, jotta parantuvat kudokset pysyvät lähellä toisiaan. Ommellanka mahdollistaa siis haavan sulkeutumisen ja parantumisen. Ihanteellisen ommellangan halkaisijan ja vetolujuuden suuruuden pitäisi olla tasapainossa toistensa kanssa. Kirurgin pitäisi myös pystyä käsittelemään ommellankaa vaivattomasti ommellessaan potilaan haavaa kiinni.

Bakteeriselluloosa (BS) on selluloosaa, jota tietyt bakteerilajit pystyvät tuottamaan solujen ulkopuolelle. Vaikka BS:lla ja kasviperäisellä selluloosalla (KS) on sama kemiallinen kaava, ovat näiden materiaalien ominaisuudet kuitenkin keskenään erilaiset. BS on houkutteleva biomateriaali lääketieteellisen tekniikan alalla, sillä se on erittäin puhdasta, sen bioyhteensopivuus on hyvä ja se ei normaalisti ole biohajoavaa fysiologisessa ympäristössä. BS:n ominaisuuksia voidaan muuttaa esimerkiksi muokkaamalla sitä kemiallisesti tai vaihtamalla sitä tuottavien bakteerien viljelyolosuhteita. BS on hyvin monipuolinen materiaali, mikä tekee siitä houkuttelevan tutkimuskohteen kirurgisten ommellankojen materiaalina.

Tämän kandidaatintyön tavoitteena oli tutkia, voidaanko BS:aa käyttää kirurgisena ommellankamateriaalina ja miten tällaista ommellankaa voitaisiin valmistaa. Tavoitteena oli myös selvittää, voidaanko tutkimuksia KS:n käytöstä kirurgisena ommellankana soveltaa BS:n kanssa. Tutkimus tehtiin käyttäen kirjallisuuslähteitä, kuten olemassa olevia tutkimuksia sekä patenttidokumentteja.

Tämä kandidaatintyö vastasi kaikkiin tutkimuskysymyksiin. BS:aa on käytetty ommellankamateriaalina monissa tutkimuksissa lupaavin tuloksin, mutta BS-ommellankaa ei ole vielä saatavilla kaupallisilla markkinoilla. BS-pohjaisia ommellankoja voidaan tutkimusten mukaan tuottaa monilla eri tavoilla, kuten esimerkiksi märkäkehruulla, leikkaamalla kapeita suikaleita hydrogeelistä tai viljelemällä BS:aa tuottavia bakteereita tietyn muotoisissa viljelyastioissa. KS:n käyttöä kirurgisena ommellankana tutkivien tutkimusten otaksuttiin olevan sovellettavissa myös bakteeriperäiselle selluloosalle esimerkiksi niissä tapauksissa, joissa käytetty KS ei ollut kasvien luonnossa tuottaman kasvikuidun muodossa. Esimerkiksi tutkimukset, joissa KS:aa käytettiin selluloosahydrogeelinä voisivat olla sovellettavissa myös BS:n kanssa näiden hydrogeelien kemiallisten rakenteiden samankaltaisuuden vuoksi.

Avainsanat: bakteeriselluloosa, kirurginen ommellanka, kasviselluloosa

CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

BC	Bacterial cellulose
PC	Plant cellulose
USP	United States Pharmacopeia
SSIs	Suture site infections
FDA	The U.S. Food & Drug Administration
EU	European Union
NaOH	Sodium hydroxide
GK	Glucokinase
PGM	Phosphoglucomutase
UDPGlc	UDP-glucose
CS	Cellulose synthase
RC	Regenerated chitin
BCNCs	Bacterial cellulose nanocrystals
PBS	Phosphate buffered saline
BHF	Biomimetic hydrogel fibre
NFC	Native cellulose nanofibers
hASC	Human adipose mesenchymal stem cells

1. INTRODUCTION

Surgical sutures are used to obtain closure and healing of the wound by approximating tissues together. Throughout history, different metals like gold, silver, and steel wires, in addition to natural materials like the animal gut, horsehair, silk, and plant fibres like linen and cotton were used to make sutures. Nowadays, some of these materials are still used in surgical operations, but also different synthetic biomaterials have established their place in the suture material's field. [1]

Bacterial cellulose (BC) is cellulose that is produced by bacteria [2]. BC can be produced by Gram-negative bacteria genera like *Gluconacetobacter*, *Acetobacter*, *Agrobacterium*, *Achromobacter, Aerobacter, Azobacter, Rhizobium, Pseudomonas, Salmonella, and Alcaligenes* [3], but also some gram-positive bacteria like *Sarcina ventriculi* can synthesize BC [4]. Bacteria produce BC extracellularly and depending on the culture method, the BC film is formed either at the air-liquid interface of the culture or dispersed pellicles in the culture medium [2].

Bacterial cellulose has the same molecular form $(C_6H_{10}O_5)_n$ than the cellulose obtained from plants, but their physical and chemical properties are different. For example, BC's purity rate and crystallinity index are higher, and the diameter of the fibres are thinner when compared to the plant cellulose (PC). [5] This is why not all the studies made with PC are straightforwardly appliable to BC.

BC is an attractive biomaterial that is widely used and studied in the biomedical field. In addition to its many favourable features, the production of BC is easy and safe in a laboratory environment [3]. In the laboratory, BC cultivation time is around one week [5], which makes the BC obtainment significantly faster than with PC that is obtained for example from trees. BC is an environment-friendly choice of material because it is renewable, abundant, and degradable in nature [6]. In addition, BC does not require expensive purification processes which might include harsh and pollutant treatments [3].

Because of the sutures' critical role in wound closure and the increasing number of surgical procedures, the demands of surgical sutures will increase in the future [1]. According to some reports, the global surgical suture market could reach $6,4 billion in the year 2024 [7].

The purpose of this thesis was to study if the BC can be used as a material for surgical sutures. In addition, the goal was to find out if the results of the studies about PC usage in surgical sutures can be applied also with BC. Because of BC's excellent biomedical features and the great demand for surgical sutures, the topic of this thesis is very relevant.

In this thesis, the usage of BC as a surgical suture material is studied through literature. In chapter 2, the properties of ideal surgical sutures are presented, the different types of sutures are being compared with each other and the classification and the regulation of sutures are briefly studied. The BC's production and biosynthesis as well as its properties and application areas are discussed in chapter 3, in addition to the comparison between BC and PC. In chapter 4, the usage of BC as a suture material is presented through existing research and patents and the applicability of PC suture results is discussed briefly. Finally, the results of this thesis are presented in the conclusions chapter.

2. SURGICAL SUTURES

Surgical sutures are used to obtain closure and healing of the wound by approximating tissues together. [1] The ideal surgical suture is easy to handle, and its knot-tying properties should be secured [8]. It is easy to understand why these properties are so important: the surgeon needs to trust their instruments while operating living human tissue. If the suture thread is not easy to handle it can hamper the surgeons work and concentration and so make the medical procedure more dangerous. With good knot-tying properties, the surgeons can trust that the knots made in the suture will hold and the approximated tissues will stay together. The ideal suture must also produce as minimal tissue drag as possible [9]. The approximation of tissues should not cause any excess tension and in the process and the ischemia and tissue injury should be minimized [8].

In addition to these properties, the ideal suture should also have a good balance between its diameter and tensile strength [8]. Tensile strength is describing the material ability to resist a force that is pulling it apart [10]. The need to balance between the diameter and the tensile strength arises from the fact that when the diameter of the suture is decreasing, also its tensile strength decreases [11]. Sufficient tensile strength is important in order to prevent unwanted suture breakdown or rupture.

The range of available suture materials is wide, but still, no single type of suture material fits all surgical and medical requirements. The material choice is depending on multiple factors regarding the wound itself, the required material properties, and the handling properties of the suture. The answers to the questions concerning the wound like how many layers of tissue must be approximated, what kind of tension there is across the wound, how deep do the sutures need to be placed, are their oedema present and when do the sutures are planned to be removed, are affecting to the choice of suture materials. Also, the properties of the suture material itself, including sufficient strength, suitable flexibility, and the lack of irritating or infectious substances guide the material choice. In addition, the suture materials handling properties, like the ease in knot placement and the high knot security can make one suture material favoured over another. [1] Lastly, also the surgeon’s area of specialization, their experience in wound closure during clinical training and their professional experience in the operating room can affect the surgeon's preference of the suture material [11].

2.1 Absorbable and nonabsorbable surgical sutures

Sutures can be categorised based on their degradation properties. Absorbable sutures degrade in the tissue environment and lose their tensile strength within 60 days. [11] If the suture is absorbable, it is made of biodegradable material. With suture applications, biodegradability describes the material's ability to degrade through biological processes in tissues of living organisms. The material is degraded into base substances like water, carbon dioxide and some basic elements. [12] Nonabsorbable sutures can maintain their tensile strength for longer than 60 days period. Absorbable sutures can further be divided into natural and synthetic absorbable sutures. [11]

Natural absorbable sutures are usually made from mammalian collagen [8] that is derived for example from the intestines of sheep and cows [13]. The obtained threads of intestines, also called catgut, undergo many complex preparation processes before they can be used as suture material. If the catgut is treated with chromium salt solution, the degradation time of the suture can be prolonged. This type of catgut suture is called a chromic gut. [14] The diameter of the sutures extracted from animal sources can be uneven and their usage carries a risk of infections. In addition, their degradation rate can be difficult to control. [13]

During the digestion of the natural absorbable sutures, their strands are broken down by body enzymes [11]. Synthetic absorbable sutures are more uniform in their size and diameter than natural ones, and their degradation rate is easier to control [13]. Their degradation process happens through hydrolysis. Hydrolysis is a chemical reaction in which the suture's polymer chain is broken down by the water when it slowly penetrates the suture material filaments. After the implantation, hydrolysis causes lesser tissue reactions than enzymatic degradation. [11] Polyglycolide acid, also known as PGA, is one of the most used materials for synthetic absorbable sutures [15].

Nonabsorbable sutures do not undergo enzymatic degradation or hydrolysis when implanted in the body. They remain in the body and are eventually encapsulated by fibroblasts. Nonabsorbable sutures must be removed after the wound healing if they are used for skin closure. Nonabsorbable sutures can be used both in exterior skin closure and in the approximation of tissues in the body cavity but also in the situations, where the patient has previously had unwanted reactions with absorbable sutures or when attaching the prostheses, like defibrillators, pacemakers, or drug delivery machines.

Silk and the surgical stainless steel 316L are examples of natural nonabsorbable sutures [8], whereas nylon, polypropylene and polybutester sutures are characterised as nonab-

sorbable, synthetic, monofilament sutures. Nonabsorbable, synthetic, multifilament materials include nylon and polyester. [16] When the new synthetic sutures are being developed, their properties are usually compared to the silk suture due to their superior handling properties. [11]

Nonabsorbable sutures are classified by the United States Pharmacopeia (USP) in a way described in table 1. [11]

Table 1: Classification of nonabsorbable sutures, adapted from [11]

Class number	Class content
Class I	Monofilament silk or synthetic fibres that are twisted or braided together.
Class II	Cotton or linen fibres, or coated fibres (natural or synthetic), where the coating increases the thickness of the suture without increasing its strength.
Class III	Metal wire, mono- or multifilament

In Table 2 below, different suture types are compared with each other based on their origin, absorbability, and mechanical properties. Mechanical properties are evaluated by comparing suture types' failure load, failure stress and initial modulus both when the suture is straight and when it is knotted. Failure load is used to describe the load value where the material failure, like deformation or breakage, occurs whereas failure stress tells the amount of stress material can take without failing [17]. The initial modulus, also known as Young's modulus, is used to describe a material's ability to maintain its original length under a one-directional tension [18].

Table 2: The comparison of surgical sutures by their origin, degradability, and the ability to resist different kinds of stress, based on [17]

Origin	Absorbability	Suture type	Failure load (N)	Failure Stress (MPa)	Initial modulus (MPa)	Knot-pulling failure load (N)	Knot-pulling failure stress (MPa)	Knot-pulling initial modulus (MPa)
N	N	Silk	20.7	1136.3	8701.0	14.4	682.2	5499.5
N	A	Plain gut	28.9	412.9	706.8	17.3	234.9	634.3
S	N	Nylon	30.9	656.5	1163.9	11.7	243.5	700.9
S	N	Polypropylene	23.2	493.1	745.6	18.9	394.8	672.0
S	N	Polybutester	25.7	595.3	945.3	15.4	353.1	592.6
S	A	Polyglactin 910	38.4	1377.0	4464.0	23.6	1091.8	4664.6
S	A	Polyglyconate	51.6	726.3	393.3	34.2	481.1	217.7

The values in table 2 above were measured with 3-0 sized sutures and the measurement procedures of USP was followed [17]. Because this procedure was followed, these results are comparable to the values in Table 4 in chapter 2.3. The sizing system of sutures is described in the same chapter in Table 3. To ensure the comparability of these different sutures with each other, the values of this table are based on only one study, performed by Naleway et al. [17]. In literature, mechanical properties of different suture materials are widely reported, but the comparison of the results of these studies is challenging. This results from the variability of suture diameter, origin of material, test room environment and measurement values and units. For example, silk can be obtained from various species of silkworms [19] as well as some spider species [20], the values of knot-pull tensile strength vary between the different knot tying methods [21] and the preferred ways and units to state the values related to tensile properties of suture can vary from tensile loads and newtons (N) [22] to tensile strength and megapascals (MPa) [21].

2.2 Other ways to characterise surgical sutures

The sutures can be characterised by their surface smoothness. Barbed sutures have little barbs on their surface that can grip the tissue approximated. When the barbs have

been added so that they are linked to the suture core, the barbed sutures maintain their tensile strength better when compared to the smooth ones. The barbed sutures eliminate the need for surgical knots and thus removes the probability of knot-related complications. [8] They also reduce the needed operation time and improve the tissue connection. In addition, with barbed sutures, the distributed tension across the wound is divided more evenly. By reducing the ischemia, they also promote better wound-healing compared to the smooth sutures. [23]

Sutures can also be categorised based on the number of strands they are made of. When the suture is made of one single strand, it is called a monofilament suture. Multifilament sutures are sutures made of multiple strands of material that is twisted or braided together. [11] Monofilament sutures cause lower resistance when the suture is drawn through the tissue. They also tie down well and are less likely to accommodate microorganisms. Because of those characteristics, monofilament sutures are preferred in vascular and microvascular surgery [8]. However, monofilament sutures must be handled with great care because their structure is prone to breaking. A weaker spot in the suture caused by the crushing or crimping of the strand can lead to the suture breaking. [11]

Multifilament sutures tensile strength, flexibility and pliability are higher than the monofilament one's [11]. By coating the multifilament suture, the resistance caused by the passage of tissue and the likelihood of the potential infections can be decreased [8]. Multifilament sutures with coatings perform well in intestinal surgery [11]. When compared to monofilament sutures, multifilament ones may be more reactive in the body: if the sutures are used subcutaneous or intradermally, the body can be prone to form a small sinus or abscess around the suture [8].

Some sutures can also have antibacterial properties. Sutures, like other foreign materials implanted in the body, can be prone to bacterial adherence that can later lead to colonization and biofilm formation. [24] Suture site infections (SSIs) increases the number of morbidity and mortality and they are especially dangerous in high-risk patients. It has been shown that microbes attach more easily to the braided suture structures than to the monofilament one. Braided sutures have a complex three-dimensional architecture and a larger surface area can entrap microbes within the suture and thus increase the risk of infection. [25] Antibacterial properties can be pursued by coating the suture with antibiotics or other antibacterial substances that decrease or prevent bacterial adhesion [24]. Triclosan, an antiseptic broad-spectrum compound, is one example of such coating material [25]. Because the number of antibiotic-resistant bacterial strains is increasing, also naturally antibacterial substances are attracting interest. For example, Reinbold et. al

studied the efficiency of totarol as a naturally antibacterial coating material with promising results. [24]

2.3 Classifications and regulations of surgical sutures

The U.S. Food & Drug Administration's (FDA) classification of medical devices is based on the risks that the device might cause to the patient or the user. FDA has three risk classes, Class I, II and III. The risk is lowest in Class I and highest in Class III. Also, both the intended use and the indication for use affects the classification of the device. The indications for use can be marked to the device labelling, thus guiding the application area of the device. [26] For example, the intended use of surgical suture is to approximate tissues together. If the label of the suture has a more specific suggestion like "surgical suture for aorta rapture approximation", the risks of this one surgical suture greatly increase because the indication of use guides the surgeons to use this device in very difficult procedure and it has life-sustain functions. According to the FDA's Medical Device Product Classification database, the sutures are classified in class II or class III [27].

The classification of surgical sutures is similar in European Union (EU) region, where the medical devices are divided into 4 classes, Class I, IIa, IIb and III. Sutures belong to Class IIa, IIb or III and the class of the device is determined by the intended purpose of the device. The classification of the medical device can be estimated by using the rules presented in the European Commission's document guiding the classification of medical devices. In the document, the medical devices are compared by for example their invasiveness, duration of use and application area. Sutures are considered surgically invasive devices (they penetrate inside the body with the aid or in the context of surgical operation) and most of them are implantable devices (they are introduced into the human body). If the duration of use is less than 30 days, the device is a short-term device, and if the usage exceeds that time, it is considered a long-term device. For example, if the suture is used to approximate tissues of the gut, according to the document it belongs in Class IIb. However, if the suture is absorbable, the class changes to Class III. [28]

In addition to the regulation offices of countries and unions, also some other organisations are affecting the requirements of different medical instruments. United States Pharmacopeia (USP) is one example of this kind of organisation. USP is a scientific non-profit organisation that focuses on trust-building towards medical applications [29] and the standards they develop and publish are recognised by the FDA [30].

The suture sizes are usually express with the USP system. [8] The excerpt of the USP number and their corresponding diameter in millimetres are presented in the two left

columns of Table 3. To minimize the tissue trauma and to leave behind as little foreign material as possible, the surgeons should choose the suture with the smallest size that can reach the wanted requirements. As stated earlier, when the diameter of the suture is decreasing, also its tensile strength decreases, so the right balance between these two aspects must be found. [11]

The sufficient knot-pull tensile strength values of different types of surgical sutures according to the USP are presented in Table 3 below. The knot-pull tensile strength describes knotted suture's breaking strength when the suture is under tensile force [31]. Knot-Pull Tensile Strength (N),

Table 3: Sutures' sizes and their corresponding minimum value of required tensile strength measured on average minimum except for the synthetic sutures that are measured by minimum.

		Absorbable Sutures		**Nonabsorbable Sutures**		
		Collagen [32]	**Synthetic [32]**	**Class I* [33]**	**Class II* [33]**	**Class III* [33]**
Size in USP	**Size in mm**	**Knot-Pull Tensile strength (N)**				
2	0.500–0.599	44.2	62.3	24.5	24.9	57.8**
1	0.400–0.499	37.3	49.8	26.7	17.8	46.7**
0	0.350–0.399	27.2	38.2	21.2	14.2	33.3**
2-0	0.300–0.339	19.6	26.3	14.1	10.0	17.6
3-0	0.200–0.249	12.2	17.4	9.41	6.47	13.3
4-0	0.150–0.199	7.55	9.32	5.88	4.51	8.04

*) Class I, II and III are describing the classes presented in Table 1

**) The tensile strength is measured by straight pull, not knot-pull

When the new suture's tensile strength is tested, the test should be repeated at least with 10 strands of suture [32]. Tensile strength tests must be performed as described in the USP's Tensile strength sections, under Surgical Suture [34]. The knot-pull tensile strength is tested while using the surgeon's knot.

As seen from Table 4 above, the knot-pull tensile strength values are demanded from absorbable synthetic sutures. Nonabsorbable class II sutures have the lowest required knot-pull tensile strength values.

Throughout the development process of new surgical suture, it is important to be aware of the registration process and the demands of properties set by authorities or organisations like FDA, EU and USP. For example, a great tensile strength of a material in one application does not ensure that the material has sufficient tensile properties when the diameter of the material bulk is reduced to fit the demands of the surgical suture. In this case, the suitability of material has to be re-evaluated, or some reinforcement materials or changes in producing methods need to be considered. These factors in addition to the properties of the ideal surgical suture described earlier are important to keep in mind when the new suture materials are being considered.

3. BACTERIAL CELLULOSE

Cellulose is one of the most common biopolymers on Earth [2], by the production of $10^{11} - 10^{12}$ tons in nature per year [35]. It is the most prevalent component of the plant cell walls [4] where it acts as a reinforcing material of plant structures [2]. Cotton, hemp, and wood are the sources where plant-based cellulose (PC) is usually obtained. Plants are not the only organisms that can produce cellulose: some micro-organisms, including bacteria, fungi, and algae, can also synthesise cellulose. [4] The type of cellulose that is produced by various kinds of bacteria species is called bacterial cellulose (BC) [2].

The molecular form of bacterial cellulose is the same as the plant cellulose: $(C_6H_{10}O_5)_n$ [5]. Cellulose's chemical structure consists of (1→4)-D-anhydroglycopyranose chains. These chains are bounded to each other via β-glycosidic linkages. [3] The repeating units of cellulose consist of three hydroxyl groups. Cellulose's physical characteristics are greatly resulted from the hydrogen bond that held cellulose structure together. Van der Waals and intramolecular forces are both present when the cellulose structure is formed, and this has a great effect on the formation of cellulose fibrils by facilitating the aggregation of cellulose chains. [36]

One way to classify cellulose is by its ability to dissolve in a sodium hydroxide (NaOH) solution [36]. If the cellulose does not dissolve in a 17.5% NaOH solution in 20 ºC, it is classified as cellulose α. Cellulose β is cellulose that dissolves to the solution, but after acidification, it precipitates. If the cellulose does not precipitate, it is classified as cellulose γ. [37]

Cellulose has four types of crystalline polyforms, but only two of them, cellulose type I and II exist in nature. Both of these types occur in parallel and antiparallel axes that is resulted from the intermolecular and intramolecular forces mentioned earlier. Cellulose type I is produced by plants, bacteria and algae and it has two suballomorphs, triclinic Iα and monoclinic Iβ. If cellulose type I is dissolved in NaOH solution, cellulose type II is obtained. When cellulose type I or II is treated with liquid ammonia, cellulose type III is produced. Unlike cellulose type II and III, type IV cellulose can be obtained through thermal processing, not via chemical treatments. [36]

Gram-negative bacteria genera, including *Gluconacetobacter*, *Acetobacter*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Azobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella,* and *Alcaligenes* can produce BC [3]. In addition to them, some Gram-positive bacteria like *Sarcina ventriculi* can synthesize BC [4]. Bacteria produce BC extracellularly, and

in static conditions, the formed BC film is translucent and gelatinous. This film is constructing of interwoven cellulose microfibrils. The length and the distribution directions of these microfibrils vary. [3] The choice of micro-organism is affecting the BC's morphology, structure, properties, and applications where it can be used [4]. By changing other factors, like culture conditions and fermentation medium, the properties can also be altered [38].

Komagateibacter xylinus (*K. xylinus*) is a common model organism, which has been used for both basic and applied studies of BC [5]. However, in literature, the naming of this bacterial species varies. This is a result of several changes in taxonomic classification made throughout history. The names used the most in literature are *Acetobacter xylinum*, *Glucoacetobacter xylinus* and *Komagateibacter xylinus*. In this day, according to the List of Prokaryotic names with Standing in Nomenclature, the name *K. xylinus* is the official one. [39] For this reason, it will be used in this work even when the writers of original articles have used the other names in their research. This decision was made to ensure the constancy of this bachelor's thesis and to honour the latest research on *K. xylinus'* taxonomy.

3.1 Culture methods of bacterial cellulose

BC can be produced using different types of culture methods, like static, agitated/shaking and bioreactor culture types. The most commonly used BC production method on a laboratory scale is a static culture. In this culture type, the BC is produced in static containers that are filled with nutrient solution and the chosen bacterial strain. The BC film is developed to the gas-liquid interface of the nutrient solution and air and the production of BC is directly affected by the surface area of this interface. The cultivation time and the thickness of the BC film are directly related to each other. [4] The productivity of static culture is not very high because of the long culture period and the required workload from laboratory personnel [5]. Because the BC film is formed to the top of the culture solution, the metabolism of the bacteria is causing the carbon dioxide entrapment inside of the BC film. When *K. xylinus* is cultivated in static culture, the produced BC consists of overlapping and intertwined cellulose chains. These chains form unorganised parallel planes. The BC produced by this method is the light-yellow colour when it is harvested from the culture medium. After the purification process including washes with hot water and sodium hydroxide, the BC colour changes from yellow to white. [4] Static BC culture and the obtained BC film are presented in Figure 1 below.

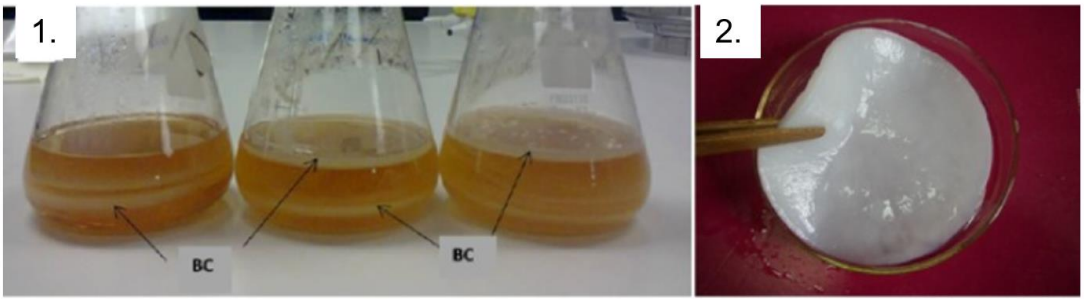

Figure 1. *Static BC culture (1.) and washed and purified BC film (2.). Pictures adapted from [4], used with the permission of Elsevier.*

In static cultures, the delivery of oxygen can be decreased due to the formation of BC film which can cause a decrease in BC production. The usage of the agitated/shaking culture method could solve this problem because in this culture type the delivery of oxygen can be increased or optimized. The agitated/shaking culture method can produce BC that forms sphere-like shapes. This shape is caused by the continuous shear force resulted from the agitated culture conditions, but also the cultivation time is influencing the process. In addition to the sphere-like shape, agitated/shaking culture can produce also ellipsoidal, stellate, fibrous, pellet-like or irregularly shaped BC. The bacteria cultivated under an agitated/shaking culture can suffer from genetic instability and the appearance of a non-cellulose mutant of the bacteria strain. This might be one of the reasons why the agitated/shaking culture method produces the approximately same amount of BC as the static culture method despite the improved delivery of the oxygen in the former one. Other drawbacks of this technique are the BC's non-Newtonian behaviour and the high shear force that are both caused by the mixing of the culture. [4]

In addition, the usage of different bioreactor cultures has been studied. With different types of bioreactors, the cultivation time and the production costs could be decreased, which could help to obtain high productivity of BC as well as the cost-efficient industrial-scale production. [4] For example, when compared to the stirred-tank reactors, the airlift bioreactor has a simpler mechanical structure, and its energy consumption is lower [40]. In addition, this reactor type can also secure the delivery of oxygen and decrease the BC's shear stress [4]. The morphology of BC while using this method is large, elliptical-shaped. The pellet size can be minimised by adding water-soluble polysaccharides to the medium. [40]

The stirred-tank and airlift reactors have one drawback: the produced BC can stick to the walls of the reactors which can cause problems. However, this situation does not occur while using rotating dick bioreactors. [40] Rotating disc bioreactors are a good choice in manufacturing the BC-based composited because the solids and fibres used in the composite can be added to the culture medium. The structure of BC produced using rotating

disc bioreactors is homogenous. Rotating disc bioreactors will not result in any notable increase of yield compared to the static culture method, unlike the modified static bioreactors do. Modified static bioreactors resemble normal static cultures, but the culture's oxygen delivery is secured. Trickling bed reactor is one example of this culture method. Using this method, one can combine the sufficient concentration of oxygen and the low shear force, which results in a higher yield of BC. The BC produced by this method is shaped to the films, and the BC has great material properties. [4]

3.2 Culture conditions during the production of bacterial cellulose

The cultivation medium contains sources of carbon and nitrogen, but usually, also some other macro- and micronutrients are added to ensure microbial growth and cellulose production. The changes made into the medium components, for example in their type or concentration in the medium, affect cellulose production. However, the optimisation of cellulose production is not that simple, because the changes do not always affect directly the yield. [5]

For carbon source, glucose and sucrose are the most usual choices. In addition to them, also the use of other carbohydrates, like fructose, maltose and xylose has been evaluated. However, the usage of glucose has its drawbacks. This is caused by the gluconic acid by-product that is produced when glucose is used as a carbon source. This by-product lowers the pH of the medium which results in the reduced production of cellulose. [5] The negative effect of the pH decrease can be prevented by adding lignosulphonate, which compounds can inhibit the formation of gluconic acid, to the medium [41].

The nitrogen source is added to the cultivation medium because nitrogen has an important role in bacteria's metabolisms acting as a primary component of proteins. For example, compounds like casein hydrolysate and peptone can be used as nitrogen source. [5] In addition to them, also the usage of yeast extract [5], different types of teas [42], soybean meal, glycine and glutamic acid [43] have been evaluated. Usually, when *K. xylinus* is used in BC production, the best results are obtained when the culture medium's carbon source is generous, but the nitrogen source is kept at a minimum [43].

In addition to the carbon and nitrogen sources, also other compounds can be added to the medium [5]. To prevent the formation of big, heterogenous lumps of BC which can result in the decrease of BC production, some water-soluble polysaccharides, like agar or acetan can be added to the medium [44]. The medium can also have compounds that act as precursor molecules. These molecules can enhance cellulose synthesis because they have metabolic driving properties. For example, some amino acids like methionine

and various vitamins increase cellulose production. [5] The addition of ethanol as a carbon source to the medium can prevent the generation of non-cellulose producing cells. With this addition, the yield of cellulose can be increased. [45] In one sturdy, the ethanol addition was reported to prevent the formation of spontaneous mutation of cell that is unable to produce cellulose [46].

However, the synthetic cultivation medium that contains the nutrients described above is expensive to use [47]. The high cost of the culture media is the primary factor to the high production costs of BC, which harms the wider use of it in different applications [48]. Because of this, the cheaper nutrient sources have been under examination [47] and by using industrial waste as a source of fermentation media, the costs of BC production can be reduced. For example, the konjac powder [49], maple syrup [50], wheat straw [51], sunflower meal hydrolysates and flour-rich hydrolysates from the confectionery industry can be utilized in BC production [38].

In addition to the culture medium composition, also the incubation temperature and the pH value of the medium are important factors. According to Chawla et al. [5], in most of the experiments, the temperature between 28 and 30 ºC give the highest BC yield. The research done by Nordin et al. gave the same kind of results: For *K. Xylinus* 0416, the optimal temperature to obtain the maximal BC production was 28 ºC [52]. Chawla et al. stated that the optimal pH value of the cultivation medium is between 4.0 and 6.0 [5]. This statement is parallel to the observations made by Zahan et al., who reported the optimal pH level of 5.0 when *K. Xylinus* 0416 is used to produce BC in a rotary disc reactor [53]. A pH lower than 4.0 can reduce the yield of BC. The decrease of pH can result from the acid fermentation by-product as discussed earlier. [5]

After the fermentation, the BC pellicle needs to be purified due to the cells and medium composition present in it. The pellicle is first mixture-washed with purified water and then treated with organic acids, potassium hydroxide and sodium hydroxide in order to get rid of the cells. BC is then washed repeatedly until neutral pH is obtained so that all the residues from the chemicals used in the purification process can be removed. [36]

3.3 Biosynthesis of bacterial cellulose

The BC biosynthesis is a well-regulated process that involves several different enzymes and proteins with catalytic and regulatory properties [5]. The biochemical pathway of BC biosynthesis is shown in Figure 2. In a very simplified way, the biosynthesis of BC goes in following steps:

The glucose molecules are first transformed to glucose-6-phosphate by the glucokinase (GK) enzyme [5]. The synthesis of cellulose begins from the glucose-6-phosphate, glycolytic intermediate of glucose. This molecule is isomerized to glucose-1-phosphate and this reaction is catalyzed by the phosphoglucomutase (PGM). [5] The obtained intermediate reacts with UTP, and this reaction forms UDP-glucose (UDPGlc). The reaction between glucose-1-phosphate and UTP is catalysed by the UTP-glucose-1-phosphate uridylyltransferase. [54] After this, the cellulose synthase (CS) is forming β-D-1→4 glucan chains from the UDPGlc's glucosyl residues. [54] UDPGlc is therefore acting as a direct cellulose precursor. In *K. xylinus*, the CS is a membrane-anchored protein that is attached to the cytoplasmic membrane. [5]

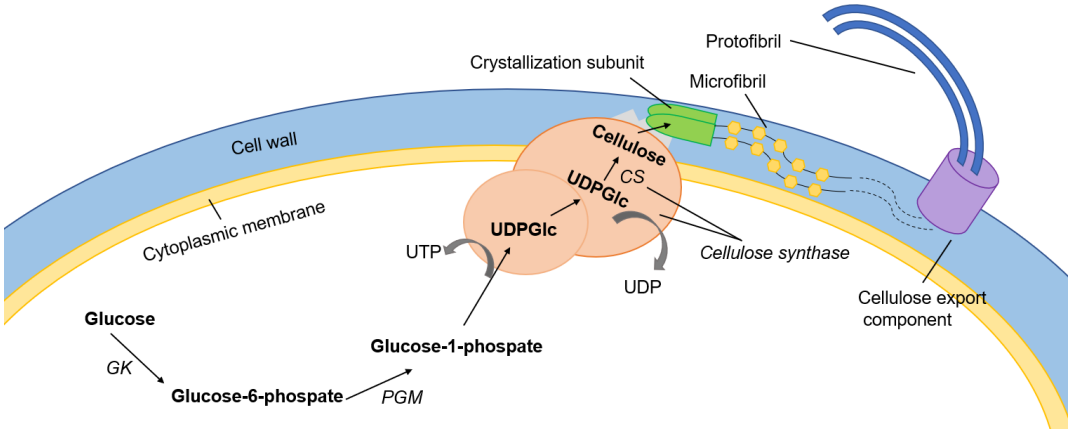

Figure 2. *Cellulose biosynthesis from glucose to the β-D-1→4 glucan chains, catalysed by glucokinase (GK), phosphoglucomutase (PGM) and cellulose synthase (CS). After these chains have been formed in the cellulose synthase, the crystallisation of the chains occurs inside of the bacteria [2]. These microfibrils are then spun out of the cells via cellulose export components and the cellulose protofibril is produced [5]. The biochemical pathway of BC synthesis is shown in the picture by bolding, based on [5,54]. The catalysing components and enzymes of this pathway are written in italics.*

An operon called *bcsABCD* is involved in BC synthesis. It contains four different genes: *bcsA*, *bcsB*, *bcsC* and *bcsD* [54]. The maximal BC production *in vivo* is obtained when all of the proteins these four genes code are present [54], but *in vitro,* just BcsA and BcsB were necessary to maintain the CS activity [55]. This suggests that the proteins coded from *bcsC* and *bcsD* are involving the process where the glucan molecules exported and packed to the cell surface. The bacteria whose *bcsC* gene was mutated were not able to produce cellulose fibrils. [54] However, the bacteria, whose *bcsD* gene was mutated, were able to produce it, but the yield of cellulose was approximately 40 % less than in unmutated bacteria [56]. Three more genes can be found from the *K. xylinus* bcs locus: *bcsZ*, *ccpA* and *bglX*. The *bcsZ* produces an endoglucanase and *bglX* a β-glucosidase. It is suspected that these enzymes are involving the hydrolysis of β-D-glucans. The *ccpA* produces protein called "cellulose-complementing protein A" which has many functions:

it is required in BC synthesis, and it is suggested to have a role in producing the crystalline ribbons by arranging the glucan chains. In addition to those, *ccpA* also influences the expression levels of *bscB* and *bscC* and interacts with the *bcsD*. [54] The schematic picture of *K. xylinus* operon is presented in Figure 3.

Figure 3. *K. xylinys E25 operon. Based on [54]*

After the precellulosic polymer has been synthesised inside of the bacterium, the chains must be extruded from the cell through cellulose export components [5]. When the molecular chains have been spun out of the cells, the chains form a protofibril that diameter is approximately 2-4 nm. These protofibrils then form ribbon-shaped microfibrils when they bundle together. The measurements of these microfibrils are approximately 4 x 80 nm. [57]

The extruded cellulose forms BC's indistinguishable gel layer. At the beginning of the cellulose gel layer formation, the entire cultivation liquid has a certain amount of dissolved oxygen that the bacteria can use for its metabolism. At this stage, the bacteria can increase their population and produce cellulose all over the liquid phase. When bacteria have used all the dissolved oxygen in the liquid, only the bacteria population near the liquid-gas-interphase can continue the cellulose production. The population size of bacteria obtains a certain equilibrium where the cells can undergo cell division, but their amount does not increase exponentially anymore. The bacterial cells below go into a dormant state. In the static condition when the cultivation is just started the thickness of the gel layer is slight at first, but after the induction period, it increases strongly. The increase is slowed down normally when the cultivation time has reached a week or ten days. [57]

3.4 Material properties and applications of bacterial cellulose

BC is a very pure material which means that it does not contain residues, like hemicellulose or lignin. It is also a hydrophobic material that has a high molecular weight. [58] The high molecular weight results from cellulose's great number of repetitive units from several hundred up to over ten thousand [59]. BC has high tensile strength (10-350 MPa [60]) and it is mechanically stable [61]. BC's Young's modulus value is approximately 15-35 GPa [62], which results in its good flexibility. The membrane of BC can have high moisture content (even 200 times of its dry mass [63]) and its permeability to gases and

liquids is good [61]. Without chemical modifications, BC is not degradable in a physiological environment. The degradation of BC would require enzymes, like cellulase, that could break down the beta acetal linkages of BC, but those enzymes are not present in the human body environment. [4]

BC is also a biocompatible and non-toxic material [61]. Biocompatibility describes material ability to perform in a specific application with an appropriate host response [64]. For example, in the context of this thesis, the biocompatibility of BC can mean that BC sutures do not cause any unwanted tissue reactions like intense inflammation or rejection when they are implanted in the body. BC's is also said to strikingly resemble collagen, which occurs naturally for example in the human body in the native extracellular matrix (ECM). The similarities with collagen molecules could be the reason why BC is so biocompatible and why the material does not show any inflammation-inducing factors. [65] It can be assumed that the similarities of collagen and BC are related to their material structure, not their chemical composition, due to the fact that the BC is a carbohydrate and collagen is a protein.

Most of the properties of BC, like its high tensile strength as well as the high polymerisation (2000-16000 repetitive units per molecular chain [66,67]) and crystallinity index (84-89 % [68]) are resulting from the BC's microfibrillar structure [5]. The properties of BC and how do they compare to the PC can be viewed from Table 4 in chapter 3.5.

Due to its versatile properties, BC has a great number of different applications. Some of these applications are presented in Figure 4 below.

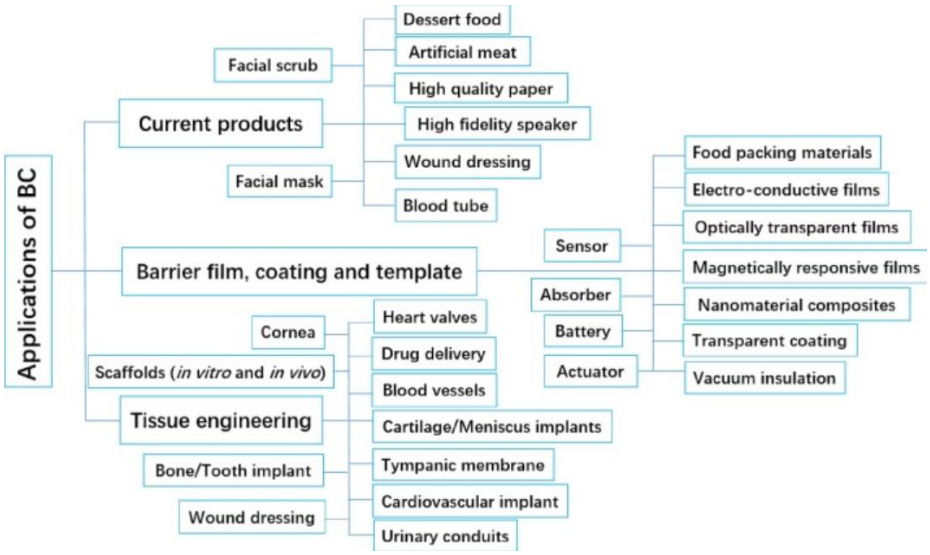

Figure 4. *Application areas of BC, a figure from [4], used with the permission of Elsevier.*

In the medical and pharmaceutical field, it can be used in wound healing and burn treatment scaffolds and, contact lens materials, artificial blood vessels, skeletal and soft tissue grafts, artificial skin, drug-delivery systems and electronics platforms [3]. BC can also be used for example in dietary fibres, nata de coco desert, food packaging, electronics, and cosmetics [69].

3.5 Comparison between plant cellulose and bacterial cellulose

Even though BC and PC share the same molecular formula of $(C_6H_{10}O_5)_n$, their physical and chemical properties are not similar. The selected and most relevant differences between the BC and PC are collected in Table 4 below.

Unlike the PC, BC does not contain lignin or hemicellulose, which makes BC's purity rate higher. BC also has higher water hydrophilicity, degree of polymerization, crystallinity, and mechanical strength. [38]The differences in their properties result from the BC's thinner nanofibrils (width approximately 1.5 nm), which causes the increase of area/surface/volume ratio. This makes the BC more elastic, formable, and strong, but also improves its water retention capacity. [6] The obtaining of PC-derived micro- or nanofibril cellulose is more difficult because the process has steps in which the plant biomass containing cellulose needs to be homogenized or fibrillated. However, the nanofibrils can be easily obtained from BC pellicles if the pellicles are still wet. [70]

Table 4: Comparison of BC and PC properties

Characteristics	Bacterial cellulose	Plant cellulose
Size of fibres	30–50 nm [71]	about 100 times bigger than BC's [63]
Tensile strength	10–350 MPa [60]	750–1080 MPa when the density was 1450–1590 kg/m^3 [72]
Young's modulus	15–35 GPa [62]	120-140 GPa when the density was 1450–1590 kg/m^3 [72]
Purity	naturally pure [2]	Contains hemicellulose, lignin, and small amounts of pectins [73]
Crystallinity	84–89% [68]	40–60% [62]
Degree of polymerization (number of repetitive units per molecular chain)	2000–16000 [66,67]	300–10000 [67]

Both the PC and BC are considered to be biocompatible [36]. For example, in the study where a PC scaffold obtained from hypanthium tissue of apples was implanted in mice, the PC was able to support blood vessel formation through the scaffolds. The study also showed that the foreign body response resulted in the scaffold disappear by 8-weeks after the implantation. After this, no immune response was observed. [74] The biocompatibility of BC in a novel peripheral nerve scaffold was studied. The cellular viability of scaffolds was studied by using Schwann cells and the blood compatibility was examined with red blood cells and a good cellular and blood compatibility of scaffolds was proved. [75]

The biodegradability rates of BC and PC are very slow in the body environment [36], due to the lack of cellulase enzyme [76]. However, PC's degradation is more difficult [36] and this can partly be explained by the presence of impurities, like hemicellulose and lignin in the composition [77]. BC's biodegradation rate is depending on four factors: molecular weight, crystallinity, hydrophilicity, and modification strategy. Higher molecular weight means more glucose units and more bonds between them that needs to be broken, thus decreasing the rate of degradation. High crystallinity index on the other hand makes the polymer chain stronger and stiffer, and so harder to break. BC is a hydrophilic material, and it can absorb water and body fluid to its structure. This causes changes in the physical structure of BC chains and the decrease of molecular weight that makes the BC degrade slightly easily. Even though BC is not normally degradable in the body environment, this property can be changed via chemical modifications, like oxidation. That enables the degradation of BC even when the cellulase enzymes is not present. [76] For example, the BC scaffold mentioned in the section above was made to be biodegradable by oxidating the BC [75].

In the next chapter, we are going to take a closer look at the usage of BC as a surgical suture material. In addition to BC, also some of the PC-derived sutures are presented. To be able to compare the BC and PC sutures and to consider if the PC sutures results can be applied also to BC, the differences and similarities of these two materials are important to keep in mind.

4. BACTERIAL CELLULOSE USAGE IN SURGICAL SUTURES

Several studies [65] in addition to existing and pending patents prove that BC can be used as a surgical suture material. In chapter 4.1, the usage of BC in surgical sutures is studied through existing research articles. Chapter 4.2 is focusing on the patents of BC-based surgical sutures. The usage of PC as a suture material is studied in the last chapter, and the possibilities to adapt these studies also with BC is discussed.

4.1 Bacterial cellulose-based surgical sutures in research

Wu et al. produced regenerated chitin (RC) sutures that were reinforced with bacterial cellulose nanocrystals (BCNCs) so that they obtained BCNC/RC filaments [78]. Chitin is a biopolymer that appears in nature as organised macrofibrils and in the crabs' and shrimps' exoskeletons. Its chemical compound is β-(1-4-)poly-N-acetyl-d-glucosamine. [79] To meet the mechanical requirements of surgical sutures, they used cellulose nanocrystals from bacterium to reinforce the material. Short (length approximately 100-300 nm) and stiff nanocrystals were composed of single crystals of cellulose. The RC and BCNC/RC fibres were produced by wet-spinning. The fibres were then twisted in bunches of 30 fibres to produce yarn, which was then chitin-coated with the coating solution. Wu et al. found that the BCNC/RC filaments surfaces were smooth, and their diameter was 20.8 ± 1.3 μm. The yarn diameter was approximately 200 μm. They were able to produce a chitin-coated BCNC/RC yarn with the highest ultimate stress value of 186.2 ± 12.4 MPa and the knot-pulling strength of 9.8 ± 0.6 N. According to the author, this value meets the USP required strength (see Table 4).

The yarn was found to have high stability in phosphate buffered saline (PBS) solution and when the degradation was studied with model enzyme lysozyme significant degradation occurred. The study also discovered that the addition of BCNC accelerated the degradation. The sutures were also found to have good biocompatibility. The authors concluded that the BCNC/RC yarn could gain its place as a good novel candidate for medical suture applications. [78]

The type of fabrication mimicking the yarn-making described in the previous example is not the only way to make surgical sutures. Guan et al. developed a hydrogel bacterial cellulose fibre whose spiral structure mimics the structure of lotus fibres in nature [80]. They named the biomimetic hydrogel fibre BHF. The BC hydrogel sheet produced by the

static fermentation acted as a preliminary material to the suture fibres. The sheet was cut into long strips that were then exposed to the opposite tangential forces that caused the strip to twist to a spiral form. The spiral form of the fibre is presented in Figure 5.

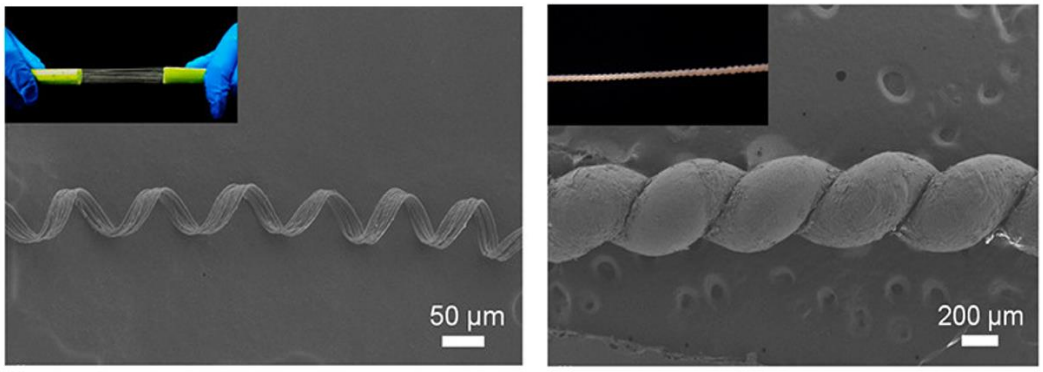

Figure 5. *Natural unstretched lotus foot fibre (left) and BHF fibre (right). The similarities of spiral structures are visible. Figure adapted from [80], used with the permission of American Chemical Society.*

During the twisting, the BC hydrogel underwent a plastic deformation, meaning that some of the hydrogen bonds in the three-dimensional hydrogel structure were broken. The hydrogel bond reconstructed when the twisting force was removed, thus fixing the spiral structure of the BC hydrogel fibre. The authors reported BHF to have a strength value of approximately 90 MPa and a toughness of approximately 116.3 MJ/m^3. For comparison, the BC hydrogel fibre that underwent only simple stretching and squeezing had a strength of approximately 83 MPa and its toughness was more than 9 times lower. The authors also found out that the chemical and mechanical stability of the BHF was good when it was soaked into PBS solution. [80]

The authors also performed *In vitro* biomechanical experiments where they compared the effects of high tensile strength applied to the two pieces of rat skin that were approximated together with BHF and commercial suture. The BHF outperformed the commercial suture, which caused grave cuts on the skin. The skin approximated with BHF did not suffer from significant cuts. The different performances between the commercial suture and the BHF are presented in Figure 6. [80]

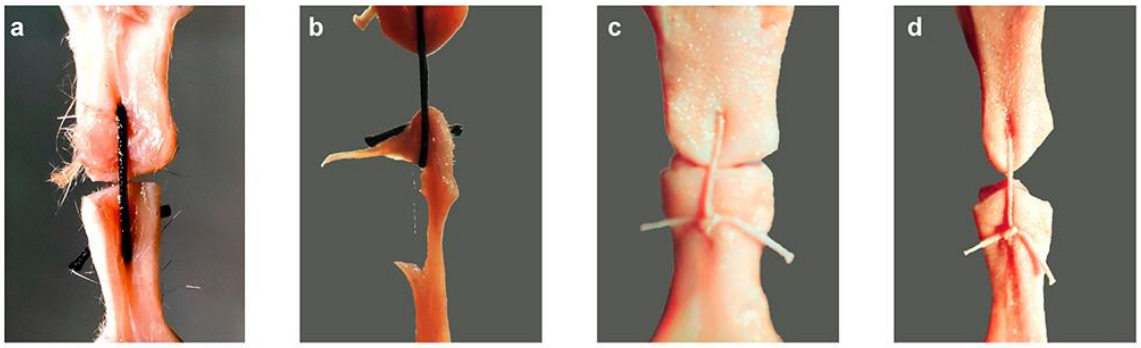

Figure 6. *The effects of the tensile strength on the approximated tissues. The white BHF suture on the right outperformed the black commercial suture on the left. In picture b, the commercial suture has caused a tissue tear when in picture d the BHF suture did not. Adapted from [80], used with the permission of American Chemical Society*

This phenomenon can be explained by examining the modulus versus strength map (Figure 7.) where the values of mammalian soft tissue, different suture materials and BHF are presented. [80]

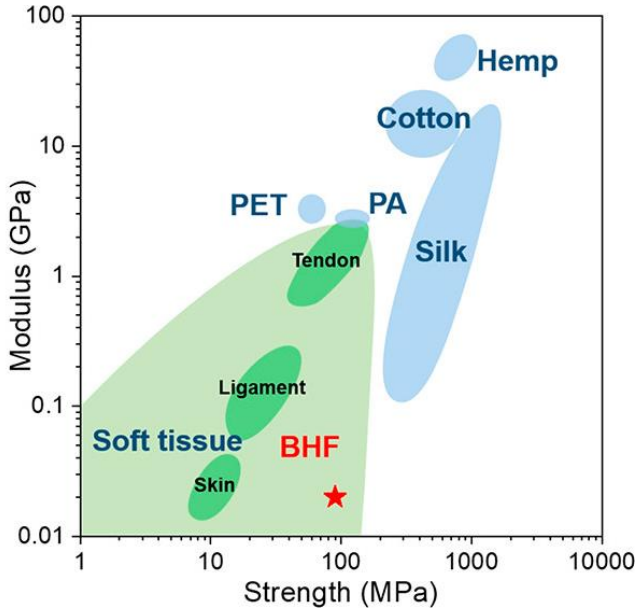

Figure 7. *The modulus-strength map of human tissues and BHF. BHF (marked with a star) resembles the soft tissues modulus-strength values (green area) than other materials (blue areas). Adapted from [80], used with the permission of American Chemical Society*

Because the BHF's modulus and strength resemble the mammalian soft tissue ones, it can tolerate greater stress without wound tearing. Because of this, the authors stated that the BHF have excellent energy dissipation properties. [80]

The authors concluded that the BHF with its favourable properties like high strength, stretchability, hydrophilicity, and biocompatibility, is a promising surgical suture material that could be used also in other biomedical applications. Because of the porous structure

of the BHF, it might perform well as a small molecule carrying material, which could enable the delivery of antibiotics or anti-inflammatory compounds to the wound site. [80]

4.2 Bacterial cellulose-based surgical suture patents

The inventions related to the BC derived materials for surgical suture applications have culminated in a few patents or their applications. In one of the patent applications, named “Biosynthetic Functional Cellulose (BC) Fibers as Surgical Sutures and Reinforcement of Implants and Growing Tissue “, invented by Gatenholm, the bacteria are fermented in permeable tubes [81]. The cultivation takes 2-5 days and during that the bacteria form a compact hydrogel. Because the cultivation happens in the tube, the obtained hydrogel is already in the form of fibre. By adjusting the length of the cultivation tube to 50 cm, a fibre with the length of the single suture package can be obtained. The diameter of the fibre can be 1-3 mm. When the cultivation is finished, the hydrogel fibre is washed to remove the bacterial cell residues. That follows by the stretching and dewatering of the fibre and finally, a well-orientated fibre structure is obtained. The fibre can also be loaded with different active agents that can for example improve the healing process or cell differentiation. [82] In this patent application, the question of how to produce a yarn-like thread from BC fibres is evaded cleverly. The method is a great demonstration of BC’s wide possibilities in both the production and the application phase.

Also, one of the other patents is based on BC hydrogels. The surgical suture is produced from BC or BC composite hydrogel. Because of the BC, the suture is reported to have a 3D network structure that makes the sutures stretchability good and the resilience rate low (varies between 5 to 30%). In the patent abstract, the low resilience rate is said to ensure that the cutting can be avoided effectively. [83] It can be assumed that the cuts mentioned in this abstract are related to similar phenomena presented in Guan et al.’s study, see Figure 6 above.

The topic of the last patent examined in this thesis was composite drug-loading fibre for surgical sutures. This fibre was a composite of cellulose, chitin, cellulose microcrystalline and an antibacterial drug named ciprofloxacin, and it was said to have good mechanical properties, high antibacterial activities, and uniform fibre structure. The fibre diameter was approximately 30 μm and its drug-releasing time can be even more than 10 days. The suture was prepared by using the wet-spinning technique. [84]

4.3 Adaption of plant cellulose-based surgical suture studies with bacterial cellulose

In addition to BC, also PC has been studied as a surgical suture material candidate [65]. In one study, performed by Guambo et al., the suitability of two natural cellulose fibres for surgical suture applications was examined. The fibres were harvested from coconut (*Cocos nucifera*) and sisal (*Agave sisalana*) and their performance was compared to the commercial silk suture. The study found out that the sisal fibre's ultimate tensile strength (138.84 ± 72.41 MPa) was superior compared to coconut fibre (only 18.72 ± 8.10 MPa). However, the sisal did not exceed the ultimate tensile strength value of silk (217.55 ± 39.58 MPa), but its crystallinity index was higher (73.65 %) than silk's (58-64 %) or coconut's (56.04 %). Vegetal cellulose fibres were concluded to be an excellent alternative to the commercially available sutures in the future [85]. As stated, the study showed that cellulose fibres can be used in surgical suture applications. This is an important conclusion although the findings of natural cellulose fibres cannot be straightforwardly applied to the BC derived suture application because the BC's 3D network does not resemble the fibre network of plants.

In the study by Mertaniemi et al., the plant-extracted native cellulose nanofibers (NFC) were used to produce cross-linked NFC threads that were then coated with human adipose mesenchymal stem cells (hASC) [86]. The hASC are used to reduce the probability of inflammation and to promote the wound healing process. By using the suture as an hASC carrier, the stem cells can be introduced to the injured area where the effects of hASCs' are most needed. The threads were produced with a 3D printer that extruded the NFC hydrogel into ethanol solution where the thread was kept for 15 minutes. After this, the NFC gel filament dried in room condition on top of the filter paper. The threads obtained with this technique had a diameter of approximately 0.1. mm. The following cross-linking of NFC was obtained by using glutaraldehyde. The stem cells adhered, migrated, and proliferated well on the top of the cross-linked NFC thread surface and their bioactivity was maintained. [86] The production process of cross-linked NFC threads is presented in Figure 8 below.

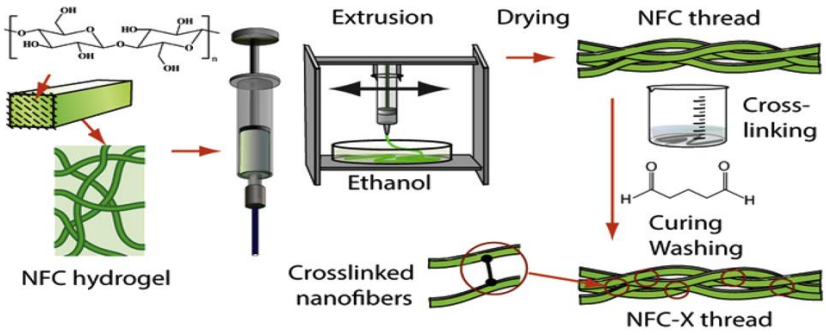

Figure 8: *Production process of the cross-linked NFC sutures. Picture adapted from [86], used with the permission of Elsevier.*

The cross-linking of NFC strongly increased the mechanical properties of the threads under wet conditions. The dry NFC without cross-linking had a tensile strength of 275 ± 25 MPa and under the wet conditions, the respective value was only 2.0 ± 0.6 MPa. By cross-linking, the dry and wet values of 227 ± 29 MPa and 111 ± 20 MPa were obtained, respectively. The hands-on performance of the cross-linked NFC suture was also studied by testing the sutures capability to pass through different muscle, fat, and skin tissues. Even the suture kept in wet cell culture conditions for over the week was able to pass the test. [86] This study is a great example of how celluloses mechanical properties can be improved with chemical treatment. Although the NFC in the study was originally obtained from plant-origin, it could be possible that the results would be similar to BC. I base this claim on the production process of NFC where the NFC hydrogel was 3D printed into a thread-like filament. Hydrogel-type cellulose can also be obtained from BC and the cross-linked NFC sutures properties were not related to for example the fibre structure of plants like in the formerly presented study by Guambo et al., is possible that the BC hydrogel would perform in similar ways when printed. In addition, as stated in chapter 3.5, the BC and PC have the same chemical formula, which leads to the expectation that the cross-linking process with glutaraldehyde would have similar results also with the BC-derived material.

As seen earlier, chemical modifications can change cellulose's properties and functionalities. Li et al. produced braided TORC sutures from regenerated cellulose that was oxidized through TEMPO-mediation [14]. The regenerated cellulose sutures in this study were obtained from the different fineness of viscose filaments that were braided into suture strands by using a circular braiding machine. Although cellulose is naturally not biodegradable, this property can be achieved through oxidation. TEMPO oxidation is a process where water-soluble and stable nitrogen radical, like 2,2,6,6-tetramethylpiperidine-1-oxyl causes polysaccharides', like cellulose's, primary hydroxyl group to oxidize

though catalytic and selective processes. [14] The production process of TORC sutures is described in Figure 9 below.

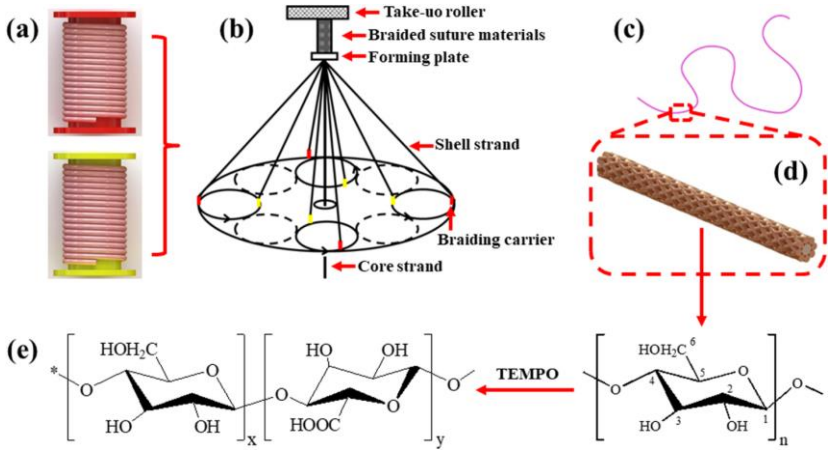

Figure 9. *The production process of TORC sutures. a: viscose filaments, b: schematic picture of the braiding machine, c and d: obtained suture, e: chemical compound after TEMPO oxidation. Picture adapted from [14], used with the permission of Elsevier.*

In this study, the oxidation times of RC sutures were varied from 15 to 90 minutes. While the oxidation time and so also carboxyl content increased, the physical properties, like strength, weight and diameter of the sutures were decreased slightly. The study was able to produce sutures with promising results of knot security and good knot-tying properties. Even after 28 days of incubation in PBS solution, the knot-pulling strength of 2 ± 0.3 N was achieved which was reported to reach the requirements of the USP. [14] This study is an excellent example of how even the most vital properties of suture material can be altered via chemical modifications. It is not clear how well this study could be recreated using BC derived materials, because the TORC suture was produced from viscose filaments. Viscose is made of regenerated cellulose fibres [87] and the possibilities to produce similar viscose filaments from BC should be studied deeper. However, the TEMPO-mediated oxidation of BC-based materials should be possible due to the same molecular compound of cellulose. By oxidating the BC sutures, the originally unachieved biodegradability could be obtained which could increase the number of possible applications and thus widen the spectrum of BC usage in the medical field.

5. CONCLUSIONS

In a conclusion, BC has many favourable characteristics that make it an attractive option for surgical suture material. BC does not contain lignin or hemicellulose, so it is pure by nature [2]. BC is also biocompatible and non-toxic [61], which makes it a good material for biomedical applications due to the lack of unwanted tissue reactions. Through chemical modification, BC's properties can be altered to better fit the wanted applications. For example, by oxidating BC, the material that is naturally non-degradable in the human body will become biodegradable in the physiological environment [76]. Because of this, BC sutures could be both absorbable and nonabsorbable which widens their possible application areas. BC's mechanical properties are also suitable for suture applications. The sutures presented by Wu et al. fulfilled the USP requirements for the suture strength [78].

Also, the BC's mouldability and the large variety of different ways to produce surgical sutures makes BC an attractive choice of material. In chapter 4, three ways to manufacture BC sutures were presented. In the study made by Wu et al. BCNC/RC sutures were obtained by using the yarn-like approach, where the fibres were first produced by wet-spinning and then twisted in bunches of 30 fibres to produce yarn [78]. Guan et al. approach the suture production from a completely different perspective: They produced BC hydrogel sheet by static fermentation and the obtained sheet was then cut into long strips that were twisted to spiral form that mimicked a structure of lotus fibre in nature. [80] The third way was introduced in the patent application by Gatenholm: the bacteria were fermented in permeable tubes, and because of this, the obtained firm BC hydrogel already in the form of fibre. By adjusting the length and the diameter of the tube, different types of sutures could be obtained. [81]

All these three ways to produce BC sutures have their advantages. The first production method with its yarn-like approach uses previously existing manufacturing methods, like wet-spinning and yarn-twisting. Because of this, it can be assumed that these sutures could be produced with the same machines and similar protocols as the other yarn-like made sutures. This might lower the discomfort of the manufacturer during the beginning of the production of this new suture type. The second example on the other hand utilises the most familiar and used way to produce BC hydrogel films. However, the cleverness of this invention arises from the problem-based approach where the wanted suture properties are obtained by biomimicking the lotus fibre, and to achieve that, the strips of BC

sheets are exposed to the mechanical modifications. In my opinion, this is a great example of what kind of novel properties the suture might obtain when the intention of making a suture that fits for all purposes is being disposed of and the precis solutions for precis problems are being pursued. Unlike these previous examples, the last example guides the form of BC hydrogel with the form of the cultivation place. Because the BC hydrogel is already in the form of suture, the time is saved because the suture requires fewer treatments and production steps. However, manufacturing sutures using this approach would require a lot of space, because only one strand of the suture is obtained from the one cultivation tube. It can be assumed that this approach requires a lot of handworks because the BC hydrogel needs to be removed from the tube, so the large batch of sutures could be hard to produce.

In this thesis, also the applicability of the results from the studies of PC usage as a suture material was discussed. The studies where the sutures were produced by obtaining the naturally occurring plant fibres of cellulose are more difficult to utilise with BC because the BC's fibre structure is not similar to that of plants. However, in the studies where the suture production is relying on for example PC hydrogels, the probability of obtaining similar results with BC are increased. This assumption is based on the same chemical composition of BC and PC hydrogels. For the same reason, the studies of the effects of different chemical modifications with PC could be applicable with BC.

The findings of this thesis strongly indicate that BC could be used as a surgical suture material. However, to the best of my knowledge, no cellulose-based suture is available in the market. Also, the number of articles about BC sutures seemed relatively small which was a surprising observation done while writing this thesis. Because of these reasons, further research is needed so that BC could establish its place in the field of surgical suture materials.

Because the selection of suture materials is wide, it could be advantageous that the sutures made by using BC would have some new clever features or manufacturing tactics, like seen in the examples above. The usage of BC in surgical sutures would fit well for the current trend in biomedical engineering where natural polymers start to replace the formerly used synthetic polymers in different medical applications [65]. In the light of the findings of this thesis and the evolving field of biomedical engineering, the sutures made of BC will probably be used in the operation rooms in the future.

6. REFERENCES

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