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BACTERIAL BIOFILMS AS WOUND HEALING DRESSING – A REVIEW

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Abstract

Tissue engineering and regenerative medicine promote skin regeneration through biomaterials that are easy to provide. Lately, many studies showed that bacterial biofilms can ensure the necessary conditions for proper healing. Several bacteria (Acetobacter spp., Lactobacillus spp., Azotobacter spp.) produce extracellular polysaccharides (cellulose, kefiran, alginate) organized in biofilms with different chemical structures. All have properties that grant medical application: cartilage and bone repair, nerve surgery and arterial stent coating. Bacterial cellulose, alginate and kefiran biofilms seem to have the qualities needed as wound healing dressings, but their characteristics and availability vary widely. The aim of this study was to summarize the current state of art on bacterial biofilms to discriminate among their specific properties and application in wound healing management. The comparison was focused on obtaining techniques, physiochemical characteristics, advantages and disadvantages of use. Cellulose, alginate and kefiran showed good results in wound healing processes, but it seems that cellulose and kefiran are the most used. Biocellulose can be obtained in multiple ways (such as stationary or agitated culture) thus the protocol varies depending on available laboratory equipment. Both cellulose and kefiran have high biocompatibility, kefiran presents antimicrobial activity as well, while cellulose can incorporate drugs. Alginate has all the properties of a wound dressing material, but it is difficult to obtain. In conclusion, bacterial cellulose seems to be the most suitable for local covering of wounds. It is studied extensively on laboratory animals and it is currently used in human medicine. However, there seems to be a lack of case studies on wound management of small animals, mainly cats and dogs.

Key words*: alginate, bacterial biofilms, cellulose, kefiran, wound healing, cellulose.*

INTRODUCTION

The main function of the skin is to protect the body against the environment and major disorders (chronic infection or necrosis).Wound healing is linked to growth and regeneration. Tissue engineering and regenerative medicine employ materials that support and accelerate healing (Nasrabadi and Ebrahimi, 2011). Thus, wound therapy remains a clinical problem and a proper, efficient management is required. The proper treatment needs to promote rapid healing and generate functional tissues (Sulaeva et al., 2015). New approaches are being developed for acute and chronic wound that avoid complications. Wound dressings and medication form an important segment of the global pharmaceutical market (Patel et al., 2012).The global market attempts to offer a variety of wound dressings for proper wound management based on different types of materials – natural or synthetic. Applicable in different forms – films, hydrocolloids and gels, they can contain drugs and bioactive substances that can accelerate wound healing process (Sulaeva et al., 2015).

Thus, the dressing of choice must ensure the necessary conditions: a moist and clean environment, blood and excess exudates absorption, infection prevention, optimal temperature, non-adhesive and rare changes (Boateng et al., 2008). Materials must be safe, biocompatible, biodegradable and non-toxic. A variety of materials meet these qualities, such as chitosan, collagen, gellan gum and bacterial biofilms (Mokhtarzadeh et al., 2016).

Biofilms are bacterial-synthesised exopolysaccharide organised into long polysaccharidic chains of sugars (glucose or galactose) or sugar derivates arranged in branches (Chawla, 2009). Their formation is an essential stage in the survival of bacteria (Sabra et al., 2001). Biocellulose is a non-toxic, hypoallergenic, non-biodegradable material, with a unique nanofiber and porous structure. These properties make it a perfect wound dressing

(Rajwade et al., 2015), being one of the best scaffold for repairing and remodelling large areas of injured skin (Mogosanu and Grumezescu, 2014).

Alginate is used for a variety of application including pharmaceutical, and biotechnology industries (Schmid et al., 2015). Usually dressing removal produces pain and destroys regenerative tissue, but alginate gels have an advantage over other scaffolds, like cotton or viscose gauze: they adsorb exudates, which prevent the fibres from sticking to the wound. Alginate gels also provide moisture and prevent drying, which benefits wound healing. They also have haemostatic properties and good permeability for oxygen that supports rapid healing (Hoefer et al., 2015).

Kefiran is an exopolysaccharide extracted from kefir grains and has superior dressing qualities. It has antibacterial, antitumoral and antifungal properties (John and Deeseenthum, 2015) and together with satisfactory mechanical resistance and good appearance makes it suitable as wound scaffold (Zolfi et al., 2014). Its applications are, however, limited by high water permeability which can be improved by incorporating hydrophobic compounds (Ghasemlou et al., 2011b).

There are many studies that describe biofilms and their main characteristics (Mogosanu and Grumezescu, 2014), but there is a lack of pertinent comparison among them. They are usually used in human medicine as wound healing materials (Mogosanu and Grumezescu, 2014) and they were largely tested on laboratory animals (Lee and Mooney, 2012; Hoefer et al., 2015; Kwak et al., 2015; Majid et al., 2016).

Studies on bacterial biofilms properties and biocompatibility reveal that they could be successfully used in veterinary medicine. However, they are not yet introduced in current veterinary practice. The three biofilms were studied extensively by many authors in respect to their mechanical properties, healing and wound dressing properties but they overlooked comparing them in regard to their use on clinical cases in wounds management of small animals. Thus, the aim of this review is to critically analyse the current knowledge on biofilms as wound dressings for veterinary use. The main bacteria species involved in biofilm production, their growth conditions and discrimination among the properties of bacterial biocellulose, alginate and kefiran are presented.

BIOFILM-PRODUCING BACTERIA AND OBTAINING TECHNIQUES

Biocellulose (BC) is an exopolysaccharide synthesised by a variety of bacteria: Gram-negatives such as *Rhizobium*, *Aerobacter*, *Agrobacterium*, *Salmonella*, *Escherichia*, *Rhodobacter*, *Acetobacter, Pseudomonas*, *Gluconacetobacter, Alcaligenes*, *Azobacter*and Gram-pozitive *Sarcina ventriculi* (Huang et al., 2013; Sulaeva et al., 2015). Compared to plant cellulose, it has superior mechanical properties and a unique structure that makes it suitable for wound dressing (Rajwade et al., 2015). *Acetobacterxylinum*, *A.hansenii* and *A. Pasteurianus* produce high yields of BC (Chawla, 2009), but only species of *Gluconacetobacter* are economically efficient (Ul-Islam et al., 2015). Relatively high levels of exopolysaccharides are produced from various sources of carbon and nitrogen (Chawla, 2009). The main stains producing BC and their cultivation conditions are systematically presented in Table 1.

The morphology is conditioned by the activity and fermentation ability of bacteria (Huang et al., 2013). Static culture was initially used, but the thickness varied a lot (Ul-Islam et al., 2015). Agitation techniques were designed to increase the yield and quality of biocellulose to commercial requirements (Czaja, 2004).

Agitated cultures of BC forma thick layer of small irregular or spherical pellets (Ul-Islam et al., 2015). The nanofibers get attached as they are synthesized through the medium, forming a deformed mass of cellulose (Huang et al., 2013). The use of high-speed agitators is a third technique used to increase the yield of BC. Static and agitated cultures cannot ensure the optimal oxygen distribution and mixture of the media. High-speed agitated cultures are produced in reactors, where oxygen is at ideal values and nutrients can be added at any time. The rotation speeds prevent the formation of BC conglomerates (Ul-Islam et al., 2015). Different strains of *Acetobacter xylinum* are commonly used to produce a reasonable amount of biocellulose from a variety of carbon sources.

Table 1. Main bacterial stains producing bacterial cellulose

Bacteria species	Medium	Carbon source	Supplement	Type of culture	Temper-ature	Culture time	pH	Reference
Gluconacetobacter swingsii spp.	Hestrin and Schramm	glucose	-	static				
	sugar cane juice	glucose, fructose, sucrose	-	static	28°C	13 days	3.5	Castro et al. (2011)
	pineapple peel juice	glucose, fructose, sucrose	-	static				
Gluconacetobacter xylinum BRC-5	Hestrin and Schramm	glucose	-	static	30°C	14 days		Cai and Kim (2009); Kim et al. (2010)
Acetobacter xylinum FF-88	coconut milk	sucrose	-	static	-	10 days	3	Nakagaito et al. (2005)
Acetobacter xylinum TISTR 975	yeast extract powder	glucose	-	agitated	30°C	24 h	6	Maneerung et al. (2008)
Acetobacter spp. A10	Hestrin and Schramm	glucose	-	static	32°C	9 days	6.7	Kwak et al. (2015)
Acetobacter xylinum NQ5	Hestrin and Schramm	glucose	0.1% cellulose enzyme (*Trichoderma resei*)	static	28°C	3 days	-	Czaja (2004)
				agitated	28°C	7 days	-	
Acetobacter xylinum X2	green tea powder	sucrose	-	static	-	7 days	4.5	Wan et al. (2007)
Acetobacter xilinum NBRC 13693	Hestrin and Schramm	fruit juice	disodium hydrogen phosphate	-	30°C	14 days	6	Kurosumi et al. (2009)
	Hestrin and Schramm	sugar reagent (glucose, fructose, sucrose)	nitrogen				-	
	-	fruit juice	-				6	
Acetobacter xylinum E25	Hestrin and Schramm	glucose	-	static and horizontal fermenters	30°C	48-64 h	3.22	Krystynowicz et al. (2002)
	Hestrin and Schramm	glucose	ethanol 1%		30°C	48-64 h	3.12	
	Yamanaka	sucrose	-		30°C	48-64 h	3.61	
	Yamanaka	sucrose	ethanol 1%		30°C	48-64 h	3.28	
	Yamanaka (optimized)	sucrose, fructose	fructose, yeast extract, ammonium sulfate		30°C	48-64 h	4.98	
	Yamanaka (optimized)	sucrose, fructose	ethanol 1%, fructose, yeast extract, ammonium		30°C	48-64 h	4.78	

It can be produced by various culture methods to produce reasonable economic quantities and to achieve desirable mechanical properties.

Alginate. Bacterial alginate was discovered by Linker and Jones back in 1964, by extracting exopolysaccharides from a *Pseudomonas aeruginosa* mucoid stain (Hoefer et al., 2015). Alginate is an anionic linear polymer formed by β-1,4-linked mannuronic acids and α-L-guluronic acid. The molecular mechanisms involved in biosynthesis is extensively studied (Hay et al., 2014). Microbial alginate is restricted to the *Pseudomonas* and *Azotobacter* species. More efficient large scale production is specific to algae. However, bacterial alginate has constant composition and yield, thus optimised larger scale production would make it a more desirable product (Sabra et al., 2001; Schmid et al., 2015).

Pseudomonas and *Azotobacter* have virtually identical genes involved in alginate biosynthesis, but the process differs. Alginate production is influenced by 12 genes (algD-algA) under strict control of alginate promoter (algD) which encode enzymes involved in precursor synthesis and encoding proteins that modify the alginate structure as travelling the periplasm (algI, algJ, algF, algL, algV and algG) (Remminghorst and Rehm, 2006). The production of bacterial alginate could be expanded by expressing biosynthesis genes and inactivate negative regulators (Schmid et al., 2015). Genetic engineering of *A.vinelandii* can control the molecular weight, degree of acetylation, monomer composition and sequence structure of alginate (Remminghorst and Rehm, 2006). Thus, new techniques must be developed to obtain alginate with optimal properties and yields.

Azotobacter vinelandii is cultivated on Burk`s medium (Hoefer et al., 2015). The pH is adjusted to 7 ± 2 with $NaOH_2$ (Gómez-Pazarín et al., 2016) or HCl and autoclaved for 15 min at 121°C (Hoefer et al., 2015). Cultures are grown at 29°C for 72h (Gómez-Pazarín et al., 2016) in an orbital incubator with a 25mm shaking diameter. Carbon sources (sucrose and glycerol) are then added. The cultures are grown at 30°C for 48h. Favourable development conditions are supplemented by growing under strict oxygen control (Hoefer et al., 2015; Gómez-Pazarín et al., 2016). After 48h the bacteria is incubated in the shaker at 30°C to dissolve the cell-associated alginate and then the suspension is diluted with NaCl. The bacteria are separated by centrifugation at 4°C for 40 min. Then, by adding ice-cold ethanol, the alginate in the supernatant is precipitated and collected by repeated centrifugation. Alginate is washed 2 times with ethanol before drying overnight (Hoefer et al., 2015).

Future biotechnological research should aim at improving bacterial production stains by genetically engineering to obtain alginate suitable for high value wound dressings (Schmid et al., 2015; Mokhtarzadeh et al., 2016).

Kefiran is a heteropolysaccharide soluble in water, isolated from kefir grains and produced by several *Lactobacillus* species: *L. kefiranofaciens*, *L. parakefir*, *L. kefirgranum*, *L. parakefir*, *L. kefir* and *L. delbrueckii* subsp. *bulgaricus* (Vinderola et al., 2006; Patel et al., 2012). It contains glucose and galactose in approximately equal amounts and it encapsulates acetic acid bacteria and yeasts, involved in the fermentation process. Visco-elastic properties of acid milk films are improved by glycerol (Patel et al., 2012).

First the kefir grains - the starter cultures - are kept until they are cultured, in skimmed milk, at room temperature (Ghasemlou et al., 2011a). Kefir grains are obtained by growing the *Lactobacillus* spp. in lactic acid whey broth (LAW). The pH is adjusted to 5.5 with liquid DL-lactic acid syrup. Distilled water is added and the solution is boiled for 30 min. The precipitate is centrifuged for 25 min at 4°C. Fermentation occurred at 25°C, under anaerobic conditions and the pH is adjusted daily at 5.5 with KOH (Vinderola et al., 2006). The kefir grains are usually collected when they reach a 2 cm diameter (Shahabi-Ghahfarrokhi et al., 2015).

The polysaccharides are extracted by dissolving kefir grains in boiling water 1:100 for 1h (Ghasemlou et al., 2011a; Zolfi et al., 2014) or 1:10 for 30 min (Shahabi-Ghahfarrokhi et al., 2015; Blandon et al., 2016) and agitated. Then the mixture is centrifuged for 15 min at 20°C (Ghasemlou et al., 2011a; Zolfi et al., 2014). The polysaccharides are precipitated by adding equal volume of 96% cold ethanol and kept

overnight at -20°C (Zolfi et al., 2014). Then the mixture is centrifuged again for 20 min at 4°C to separate the precipitated carbohydrate. The precipitates are washed with water for removing impurities. The process is repeated three or four times. The resulting solution is concentrated precipitated polysaccharides and is hereafter called kefiran (Vinderola et al., 2006; Ghasemlou et al., 2011a; Zolfi et al., 2014; Shahabi-Ghahfarrokhi et al., 2015; Blandon et al., 2016).

Film preparation begins with weighing the amount of film-forming kefiran aqueous solution, with different concentrations. Then glycerol is added to the mixture as a plasticizer at various levels (15-35%) (Ghasemlou et al., 2011b) or equal amount of glycerol to that of kefiran (Zolfi et al., 2014). The mixture is then agitated using a magnetic stirrer for 15 minutes. The filmogenic solutions obtained are cast in Petri dishes and dried at 30°C for 30 min (Zolfi et al., 2014) or at 40°C for 6h (Piermaria et al., 2011) in a ventilated oven to remove the air bubbles (Zolfi et al., 2014; Sulaeva et al., 2015). The resulted films are removed from the plates and stored at 20°C and humidity at 75% (Piermaria et al., 2011).

Plasticizer must be added into the film to achieve flexibility otherwise they are fragile and cracked during drying (Ghasemlou et al., 2011b; Zolfi et al., 2014). Ghasemlou et al. (2011) used different concentrations of Kefiran (1%, 2%, 3%) and showed that biofilms containing 2% were taken easily from plates, but, on the contrary, films with 1% were thick and difficult to handle (Ghasemlou et al., 2011b).

BACTERIAL BIOFILMS PROPERTIES

Biocellulose is produced as a gel at the interface of air-liquid of the proper medium. Culture time and carbon source in the medium influence the thickness of BC (Ul-Islam et al., 2015). Scanning electronic microscope (SEM) studies reveal a 3D network structure with 30 to 100 nm fibre diameter (Yang et al., 2012) and 120-160 nm pore size (Shahmohammadi Jebel and Almasi, 2016). Bacterial cellulose has a porous structure, which gives it water absorption properties (Ul-Islam et al., 2015) up to 350% its own weight in 24h with a water vapour transmission rate of 112.14 g x m^2/h (Kwak et al., 2015). The tensile straight ranges widely between 12.13 MPa (Kim et al., 2010) and 450 MPa, the strain reaches up to 12.53% and the crystallinity about 17.63% (Kwak et al., 2015).

Drying method influences the structure of biofilm: an uniform pores distribution and greater number of pores are present when freeze-drying biocellulose compared to air-drying (Rajwade et al., 2015).

The biodegradability of biocellulose was studied *in vitro* by immersing the membrane for 8-12 weeks in phosphate buffered saline solution at 37°C temperature and 7.25 pH. The studies showed a modest fragmentation of the film and formation of woolly aggregates (Rajwade et al., 2015).

Biocompatibility of cellulose was studied as a substitute for dura mater membrane in dogs (Rajwade et al., 2015). Research showed no pathological inflammation when implanting cellulose in the nasal dorsum of rabbits, and the results showed little fragmentation of the biofilm after 6 months (Rajwade et al., 2015). Other studies were made on rats, by implanting subcutaneous BC (Kalia et al., 2011). There were no signs of biodegradability after 12 weeks of implantation (Rajwade et al., 2015).

Biocellulose has unique mechanical properties such as ultrafine 3D network structure, with various pore geometry, is highly purified, has high water absorption ability (over 100 times its own weight) and high crystallinity.

Alginate has a smooth and uniform surface, with an ordered fibre structure, resulting in transparent biofilms that can easily be removed from plates (Zhang et al., 2015). SEM studies showed a porous microfiber structure (Mogosanu and Grumezescu, 2014). The tensile straight of bacterial alginate is 6.51 MPa (Zhang et al., 2015) in contrast to 2.6 MPa of algae alginate (Hoefer et al., 2015). The ability to absorb exudates is an important feature of alginate. A comparison between bacterial and algae alginate was studied by immersing both biofilm types in a 0.9% saline solution, containing calcium. After 30 min, both biofilms turned into hydrogels, but bacterial alginate absorbed a larger amount of solution. This changed their microscopic structure, the fibres almost disappeared. Marine alginate kept its

fibre structure and absorbed less saline solution (Hoefer et al., 2015). Alginate is highly soluble in water (~99.5%), but the solubility can be reduced by adding lipids (Zhang et al., 2015). In contrast, Mogosanu and Grumezescu (2014) observed a porous structure, no adhesive properties and water absorption up to 20 times its weight.

The pH can influence viscosity: it increases with the decrease of pH, reaching a peak at pH 3-3.5 (Hay et al., 2013). Alginate did not show any bacteria-inhibition properties (Zhang et al., 2015), but it can retain and inactivate bacteria inside its structural matrix (Spasojevic et al., 2016). By adding antibacterial agents this disadvantage can be removed (Zhang et al., 2015). The antimicrobial activity of alginate-lignin compound was tested on bacteria active in chronic wounds: *Enterobacter cloacae*, *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Micrococcus flavus*, *Listeria monocitogenes* and *Staphylococcus aureus.* It was concluded that lignin has little antimicrobial activity, but in association with alginate, the effect is synergetic (Spasojevic et al., 2016).

Alginate forms strong thermostable gels by interacting with various cations, especially Ca^{2+}. This aspect grants encapsulation properties. It is suitable for medical delivery systems because it is permeable to liquids and small molecules (i.e. drugs) (Mokhtarzadeh et al., 2016).

The biocompatibility of alginate was largely investigated *in vivo* and *in vitro* studies (Lee and Mooney, 2012; Spasojevic et al., 2016), but there are disagreements about the effect of its composition on tissue response. Some studies show that alginate can be immunogenic and can induce cytokine production (Lee and Mooney, 2012), in contrast, others observed no such effect (Spasojevic et al., 2016). The immunogenic response could be assigned to remaining impurities because highly purified alginate induced no body reaction in animal tissues (Lee and Mooney, 2012). Alginate-lignin compound revealed no cytotoxic effect when tested on cervix carcinoma and human conjunctival epithelial cells. Furthermore, no damage on wounds or nearby skin was observed when tested *in vivo* on sterile wounds induced by incision on rat skin (Spasojevic et al., 2016). Similarly, no important inflammatory reaction was noticed when alginate gel was subcutaneously injected to mice (Lee and Mooney, 2012).

Alginate yield can be increased by genetic modification of bacteria strains but even so it cannot reach a reasonable economic scale. Alginate forms transparent gel like films with a fibre porous structure, good mechanical properties and the ability to absorb exudates. It has a good biocompatibility and although it has no antimicrobial activity it has the ability to incorporate drugs, fact that substitutes this lack.

Kefiran. Studies reveal the use of polysaccharides to prepare films with different properties increase significantly. Kefiran finds increasing use because of its texture and promising mechanical properties. Biofilms have good appearance although are highly permeable to water vapour and the control of moisture in wound healing is a desirable propriety (Ghasemlou et al., 2011a).

SEM reveals that kefiran biofilms have smooth uniform surface, with compact structure, after being plasticized with glycerol (Ghasemlou et al., 2011b). The structure of kefiran can be changed by varying the concentration of glycerol (Piermaria et al., 2011) which makes the biofilm more compact (Piermaria et al., 2009). An increased amount of plasticizer increases the moisture content from 17.95% to 37.04%. The plasticizer acts as a water scavenging agent: the plasticity increases with the increase of water content (Ghasemlou et al., 2011b).

An increasing polysaccharide concentration increases the film thickness from 1.9±1.2 μm to 2.1±1.3 μm (Piermaria et al., 2009). Sugar and polyols, used as plasticizer, lead to thicknesses varying from 22 to25μm, while sucrose generated a 31μm film (Piermaria et al., 2011).

Transparency is an important propriety, pure kefiran biofilms transparency varies between 2.714±00.15 A600/mm (Piermaria et al., 2009) but also depends on the plasticizer used, ranging between 1.88 A600/mm to3.30 A600/mm (Piermaria et al., 2011).

Glycerol influenced the mechanical properties of kefiran film as well. A considerable tensile straight was shown in films with no glycerol and lower elongation at break (Piermaria et al., 2009). Thus, plasticizers affect the tensile

straight and elongation at break: tensile straight decreases with an increase of glycerol (Ghasemlou et al., 2011b). The tensile straight of pure kefiran ranged from11.18±2.2 MPa (Ghasemlou et al., 2011b) to 40.92±7.83 MPa (Piermaria et al., 2009). The plasticized biofilm had a variable tensile straight depending on glycerol concentration 8.85±1.64 at 15% and 5.04±2.1 at 35% (Ghasemlou et al., 2011b).

Elongation at break was 116.69±14.48 % in glycerol enriched kefiran compared to 2.70±0.47% in pure film (Piermaria et al., 2009). Another study observed 39.56± 11.13% in pure kefiran biofilms and as high as 162.45 ± 6.09% in films containing 35% glycerol (Ghasemlou et al., 2011b). Thus, plasticized biofilms have elongation values higher than cellophane (20%) or polystyrene (1%), but much lower than low-density polyethylene (500%) (Ghasemlou et al., 2011b).

The water solubility of kefiran depends on temperature. It is relatively soluble at 25 to 37°C and totally dissolved at 100°C (Ghasemlou et al., 2011b). Adding glycerol increased solubility (Piermaria et al., 2009; Ghasemlou et al., 2011b).

X-ray diffraction patterns revealed that the degree of crystallinity was less than 3.1% and no significant differences were observed among biofilms with different plasticizers (Piermaria et al., 2011).

Kefiran biofilms are extremely permeable to water vapour, which limits its applications (Ghasemlou et al., 2011b). To remedy this disadvantage hydrophobic compounds are often incorporated in biofilms to enhance water barrier properties.

Lactic and acetic acids in kefiran could induce antibacterial and wound healing activity (John and Deeseenthum, 2015). Natural antibiotics and inhibitory substances (lactic acid, acetic acid, bacteriocins, reuterin, hydrogen peroxide) from kefiran have good action over pathogens (Rahimzadeh et al., 2015).

Kefiran biocompatibility was tested in several studies (Huseini et al., 2012; Majid et al., 2016) it decreased blood pressure and cholesterol, also slowed tumour growth. It was used as an oral antigen and conferred systemic immunity by releasing cytokines into the blood (Patel et al., 2012).

Kefiran films find increasing use in wound healing management with satisfactory mechanical properties and good appearance. It is permeable to water vapour fact that limits its application since the control of moisture is a desirable propriety. Hydrophobic compounds are added to remedy this lack. Kefiran has antimicrobial activity because of lactic and acetic acids in its composition.

BIOFILMS AS WOUND DRESSING MATERIALS

Bacterial cellulose was first described as a wound dressing material back in the early 1980s (Sulaeva et al., 2015). The perfect wound dressing material has a unique 3D nanofiber network, with a porous structure and different pore size. The structure can be modified by varying the carbon source, pH, temperature, culture time or production method. The best choice seems to be wound scaffold (Rajwade et al., 2015) because it is a never-dried membrane, with exceptional mechanical strength and physiochemical properties (Mogosanu and Grumezescu, 2014).

Biocellulose is a suitable scaffold material for chronic wounds, being a non-degrading material. It deteriorates very slowly in the body because of its crystallinity and lack of enzymes able to digest the glycosidic bonds (Rajwade et al., 2015).

Bacterial cellulose is usually used as healing dressing for chronic wounds because it reduces pain and accelerates healing. It stimulates granulation and epithelisation processes (Mogosanu and Grumezescu, 2014).

Sprague Dawley (SD) rats with inflicted burn skin injuries were treated for 15 days with biocellulose films and gauze dressing (Kwak et al., 2015). The severity score of skin injury was lower in the BC group throughout the study, the thickness of dermis and epidermis was significantly higher, as well, angiogenesis was pronounced, many new blood vessels were observed and a remarkable level of collagen was expressed in the group treated with BC (Kwak et al., 2015).

Biocellulose can incorporate different active molecules like vitamins, enzymes, antioxidants, drugs, fact that expand its qualities (Mogosanu and Grumezescu, 2014).

Alginate is used as wound dressing because of its haemostatic properties in bleeding and burn wounds, being a very absorbent natural fibre (Mogosanu and Grumezescu, 2014). Alginate can absorb body fluids or water up to 20 times its own weight. Hydrophilic alginate biofilms area moist environment, which is perfect for proper wound healing. Films have a porous structure and no adhesive properties, so a second dressing is needed to secure and protect the biofilm (Mogosanu and Grumezescu, 2014). *In vivo* and *in vitro* studies showed that calcium mediates wound healing, by supporting the fibroblast production, and alginate dressings contains calcium ions. Further *in vitro* studies (Lee and Mooney, 2012) revealed that the mobility of fibroblast did not increase. This suggested that calcium ions released from alginate dressings can increase only some cells involved in the process of wound healing (Lee and Mooney, 2012). Other studies concluded that alginate activates human macrophages to generate tumour necrosis factor (TNFa), this induced inflammatory responses - an important step in injury healing (Lee and Mooney, 2012; Mogosanu and Grumezescu, 2014).

Different composite alginate materials were obtained by adding compounds that increased the antimicrobial properties and wound healing properties: zinc, silver, chitosan (Mogosanu and Grumezescu, 2014).The alginate biofilms became firmer by adding guluronic acid and alginate-mannuronate gel become softer and more flexible as they absorbed wound exudates (Boateng et al., 2008).

Alginate can be used as a proper wound dressing because it forms gels and it is highly absorbent in contact with wound exudations. Alginate gel is very hydrophilic (Mogosanu and Grumezescu, 2014), this restrains wound secretions, but also protects the tissue from microbial contamination. Alginate forms a protective gel-like biofilm in contact with the exudates and blood in wounds, it also maintains optimum healing temperature and a favourable moisture, ensuring proper healing (Boateng et al., 2008; Lee and Mooney, 2012). Alginate has gelling properties because of the calcium ions in its composition. Calcium also forms crosslinks with alginic acid polymers that lead to a slow degradation of the biofilm. These properties make alginate an ideal scaffold in wound healing management (Boateng et al., 2008; Hoefer et al., 2015).

Kefiran can produce films that have satisfactory mechanical characteristics, but are very permeable to water vapour, fact that limits its application (Ghasemlou et al., 2011a).

Kefiran is reported to have wound healing, antimicrobial, antifungal and antitumoral properties (Ghasemlou et al., 2011a).

Kefir films have great potential in wound healing management increasing epithelisation, scar formation and decreasing inflammation (Nasrabadi and Ebrahimi, 2011). Kefir extracts also hastens wound healing by stimulating the immune system against pathogens (Rahimzadeh et al., 2014).

A remarkable shorter healing time and decreased wound size was noticed in biofilm containing kefir extracts fermented for 96 h, compared to lower fermentation time (Huseini et al., 2012; Rahimzadeh et al., 2015).

Wound healing experiments were made on Wistar rats with induced diabetic cutaneous injuries. The results showed that the group treated with kefiran presented an increased inflammation and an improved accelerated healing process, compared to control groups (Majid et al., 2016). Similar studies were made, on induced thermal wounds. The results showed that the inflammation decreased, scar formation and epithelisation increased significantly (Nasrabadi and Ebrahimi, 2011; Huseini et al., 2012; Rahimzadeh et al., 2014). Other studies on rats with burn injuries revealed that kefiran had better wound healing properties than sulfadiazine treatment or clostebol-neomycin emulsion (John and Deeseenthum, 2015).

Kefiran could be the best choice as a wound dressing material due to its antibacterial properties, ability to accelerate wound healing and reduce inflammation.

CONCLUSIONS

Biocellulose is synthesized by a variety of bacteria species, in contrast to alginate or kefiran that are produced by *Pseudomonas* or *Azotobacter* and *Lactobacillus* species. BC can be produced at economic scale, depending on the culture method. Alginate yield can be increased by genetic modifications of bacteria

strains, but even so, it does not reach commercial level. Kefiran biofilms are easy to obtain, from common bacteria species, but glycerol must be added to obtain desired properties.

Cellulose biofilm has a 3D nanostructure with porous structure, good tension straight and low elongation at break. Also, it can absorb exudates, it is a never-drying material and it is not biodegradable. Neither cellulose, nor alginate have antibacterial properties, but active agents can be encapsulated and delivered. Alginate absorbs water, it has encapsulation properties, but it can generate immunogenic responses, if it is not highly purified. Kefiran films can be manipulated only if plasticizer is added; this also gives good tensile straight and low elongation at break. Kefiran has good antibacterial properties due to lactic and acetic acids. Both alginate and kefiran are soluble in water and lipids should be added in their structure.

Based on the ease of obtaining, the main properties, biocompatibility and its unique structure, biocellulose should be the best choice as a wound dressing material.

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