

Microemulsion systems for the incorporation of poorly-water soluble compounds into bacterial cellulose

Field of the invention

The present invention relates to a fiber-layer patch comprising or consisting of

- a) a bacterial cellulose layer, and
- b) an oil-in-water microemulsion or water-in-oil microemulsion, each comprising or consisting of
at least one surfactant,
at least one co-surfactant,
at least one oil,
water,
optionally at least one additive, and
at least one active ingredient.

Furthermore, the present invention pertains to a method for manufacturing the patch according to the invention, wherein said is obtained by absorption loading comprising the following steps:

- I) providing and optionally squeezing a never-dried patch of bacterial cellulose,
- II) inserting the never-dried and optionally squeezed patch of bacterial cellulose into a container containing the microemulsion so that the bacterial cellulose is fully covered with the microemulsion,
- III) stirring the microemulsion which contains the patch of bacterial cellulose for at least 48h.

Finally, the present invention refers to the use of the patch according to the present invention for wound healing, drug delivery, skin care, aesthetic treatments and tissue regeneration.

Description of the related art

Treatment and management of chronic, non-healing wounds continues to be a major challenge for healthcare providers and plays a critical role in maintaining patients' quality of life. The effective pharmacological treatment of wounds remains challenging, as the systemic application of suitable drugs is compromised by severe side effects and the inherent difficulties of wounds as drug targets.

Therefore, the topical administration can be an option. Unfortunately, many challenges remain in the context of successfully delivering active ingredients (AIs) to wounds. The use of conventional, non-sustained dosage forms that need daily application is not reconcilable with a desired reduction of dressing change frequency to increase patients compliance. Moreover, systemic drug delivery to wounds is particularly challenging due to obstacles such as ineffective vascularization of the wound bed and thus impaired transport of active agents through the blood flow. Bacterial cellulose (BC) as a patch is known in the art but the incorporation of AIs into the hydrophilic material still possess a problem. These problems propose the need for a suitable patch.

The inventor of the present invention surprisingly found, that microemulsions comprising AIs are a suitable solution to provide an AI to BC-patches.

Summary

Therefore, in a first aspect the present invention refers to a fiber-layer patch comprising or consisting of

- a) a bacterial cellulose layer, and
- b) an oil in water microemulsion or water in oil microemulsion, each comprising or consisting of
at least one surfactant,
at least one co-surfactant,
at least one oil,
water,
optionally at least one additive, and
at least one active ingredient.

In a second aspect the present invention pertains to a method for manufacturing the patch according to the invention, wherein said is obtained by absorption loading comprising the following steps:

- I) providing and optionally squeezing a never-dried patch of bacterial cellulose,
- II) inserting the never-dried and optionally squeezed patch of bacterial cellulose into a container containing the microemulsion so that the bacterial cellulose is fully covered with the microemulsion,
- III) stirring the microemulsion which contains the patch of bacterial cellulose for at least 48h.

In a third aspect the present invention refers to the use of the patch according to the present invention for wound healing, drug delivery, skin care, aesthetic treatments and tissue regeneration.

These and other aspects, embodiments, features, and advantages of the invention will become apparent to a person skilled in the art through the study of the following detailed description and claims. Any feature from one aspect of the invention can be used in any other aspect of the invention. Furthermore, it will readily be understood that the examples contained herein are intended to describe and illustrate the invention but not to limit the invention and that, in particular, the invention is not limited to these examples.

Brief description of the drawings

Fig. 1 shows the transparency of native bacterial cellulose and bacterial cellulose loaded with microemulsion (BC-ME); transmission at 650 nm.

Fig. 2 shows the downregulation of TNF α in stimulated monocytes by BC-ME- hydrocortisone and BC-ME-dexamethasone and permeation through Strat-M® membranes in comparison to control without API and vehicle control with and without stimulation (none) determined by ELISA. Data is shown as mean $\pm$ SD from four different donors following three technical replicates in vertical diffusion cell testing.

Detailed description

Numerical ranges that are indicated in the format "from x to y" also include the stated values. If several preferred numerical ranges are indicated in this format, it is self-evident that all ranges that result from the combination of the various endpoints are also included.

"At least one" means one or more, i.e. 1, 2, 3, 4, 5, 6, 7, 8, 9 or more. "At least one", as used herein in relation to any component, refers to the number of chemically different molecules, i.e. to the number of different types of the referenced species, but not to the total number of molecules. For example, "at least one surfactant" means that at least one type of molecule falling within the definition for a surfactant is used but that also two or more different types of surfactants falling within this definition can be present but does not mean that only one or more molecules of one type of surfactant are present.

All percentages given herein in relation to the compositions or formulations relate to wt.-% relative to the total weight of the respective composition, if not explicitly stated otherwise.

In the following, "bacterial cellulose" is referred to as "BC".

In the following, "active ingredient" is referred to as "AI".

In the following, "active pharmaceutical ingredient" is referred to as "API".

In the following, "microemulsion" is referred to as "ME".

Microemulsions are thermodynamically stable, optically isotropic, and translucent mixtures of oil and water stabilized by at least one surfactant. They form spontaneously at specific compositions of the two phases, an amphiphilic surfactant and often one cosurfactant. The microstructure of microemulsions can be classified either as bicontinuous or dispersed, depending on concentration and character of its constituents. While bicontinuous microemulsions are characterized by continuous domains of water and oil, dispersed microemulsions more closely resemble droplet structures.

The fiber layer patch comprises or consists of

- a) a bacterial cellulose layer, and
- b) an oil in water microemulsion or water in oil microemulsion, each comprising or consisting of
 - at least one surfactant,
 - at least one co-surfactant,
 - at least one oil,
 - water,
 - optionally at least one additive, and
 - at least one active ingredient.

In an embodiment the bacterial cellulose is expressed in bacteria of the genus *Komagataeibacter*, preferably *Komagataeibacter xylinus*, *Komagataeibacter rhaeticus*, *Komagataeibacter intermedius* or *Komagataeibacter hansenii*, more preferably *Komagataeibacter xylinus*.

In an embodiment the bacterial cellulose is expressed in a mixture of bacterial strains, preferably *Komagataeibacter* and *Lactobacillus*, more preferably *Komagataeibacter xylinus* and *Lactobacillus mali*.

In an embodiment the BC is produced from a bacterial culture in a cultivation medium, which is not actively stirred, shaken, or otherwise moved during BC-synthesis, in which, however, the BC is continuously produced by the bacterial culture. The fact that the cultivation medium is not actively stirred, shaken, or otherwise moved during BC-synthesis does neither exclude harvesting or removal of BC from the bacterial culture and/or reaction vessel during BC-synthesis nor adding cultivation medium and/or constituents of the cultivation medium during BC-synthesis. Particularly, in a "continuous semi-static" process as used herein, preferably the BC is harvested or removed from the bacterial culture and/or reaction vessel more than once during BC-synthesis, particularly regularly, step-wise and/or continuously. Particularly, in a "continuous semi-static" process as used herein, preferably the cultivation medium and/or constituents of the cultivation medium are added more than once, preferably regularly, step-wise and/or continuously during the BC-synthesis. A "continuous semi-static" process as used herein, preferably is not a batch production method.

Providing BC in a continuous semi-static process may comprise at least one of the following steps:

- i) providing a reaction vessel comprising cultivation medium,
- ii) inoculating the cultivation medium with a BC-producing bacterial strain or a mixture of bacterial strains,
- iii) bacterial synthesis of BC in the reaction vessel.

Production of the BC can preferably be done at a cell count of from 10^4 to 10^7 cells/ml of culturing medium during the culturing. Preferably, the step ii) of inoculating the cultivation medium with a BC-producing bacterial strain comprises providing a stock culture having about 10^6 cells/ml and inoculating the cultivation medium with the stock culture resulting in a cell concentration of about 5×10^3 cells/ml. The properties of the obtained BC content of the article may also be influenced by the volume ratio of the cultivation medium to the bacterial strain stock culture used for inoculation. In an embodiment the volume ratio is at least 2:1, preferably at least 5:1, more preferably at least 10:1, most preferably at least 15:1. Preferably, the volume ratio is at most 50:1, more preferably at most 30:1, and most preferably at most 20:1.

Step iii) of synthesis of BC comprises adding cultivation medium, or preferably selectively adding ingredients of the cultivation medium, into the reaction vessel more than once, preferably regularly, step-wise and/or continuously during BC-synthesis. Particularly, step iii) of synthesis of BC in the reaction vessel preferably comprises incubating a bacterial culture in a cultivation medium, which is not actively stirred, shaken, or otherwise moved during BC-synthesis. The fact that the cultivation medium is not actively stirred, shaken, or otherwise moved during BC-synthesis does neither exclude harvesting or removal of BC from the bacterial culture and/or reaction vessel during BC-synthesis nor adding cultivation medium and/or constituents of the cultivation medium during BC-synthesis. Additionally or alternatively, step iii) of synthesis of BC in the reaction vessel preferably comprises a continuous production of BC. Additionally or alternatively, step iii) of synthesis of BC in the reaction vessel preferably comprises harvesting and/or removing BC from the bacterial culture and/or reaction vessel during BC-synthesis more than once, preferably regularly, step-wise and/or continuously during BC-synthesis. Additionally or alternatively, step iii) of synthesis of BC in the reaction vessel preferably comprises adding cultivation

medium and/or constituents of the cultivation medium to the bacterial culture and/or reaction vessel more than once, preferably regularly, step-wise and/or continuously during the BC-synthesis.

In an embodiment the cultivation medium is liquid.

In an embodiment the cultivation medium comprises a carbon source, a nitrogen source and a vitamin source and optionally a buffer system.

In an embodiment the carbon source is selected from one or more sugars and their derivatives, preferably glucose, the nitrogen source is peptone, the vitamin source is yeast extract and the buffer system is disodium hydrogen phosphate and citric acid.

In an embodiment the cultivation medium comprises the carbon source in an amount of at least 10 g/l, preferably at least 15 g/l and at most 30 g/l, preferably at most 25 g/l based on the volume of the cultivation medium. Most preferably, the cultivation medium comprises the carbon source in an amount of 20 g/l.

In an embodiment the cultivation medium comprises the nitrogen source in an amount of at least 2 g/l, preferably at least 4 g/l and at most 10 g/l, preferably at most 7 g/l based on the volume of the cultivation medium. Most preferably, the cultivation medium comprises the nitrogen source in an amount of 5 g/l.

In an embodiment the cultivation medium comprises the vitamin source in an amount of at least 2 g/l, preferably at least 4 g/l and at most 10 g/l, preferably at most 7 g/l based on the volume of the cultivation medium. Most preferably, the cultivation medium comprises the vitamin source in an amount of 5 g/l.

In an embodiment the cultivation medium comprises the buffer system in an amount of at least 2 g/l, preferably at least 4 g/l and at most 10 g/l, preferably at most 5 g/l based on the volume of the cultivation medium. Most preferably, the cultivation medium comprises the buffer system in an amount of 4.5 g/l.

In an embodiment the cultivation medium comprises 20 g/l glucose, 5 g/l peptone, 5 g/l yeast extract, 3.4 g/l disodium hydrogen phosphate and 1.15 g/l citric acid.

In an embodiment the cultivation medium is free of ginseng extracts.

In an embodiment the cultivation temperature is at least 20°C, preferably at least 25°C, more preferably at least 28°C. and at most 36°C, preferably at most 33°C, more preferably at most 30°C.

In an embodiment the cultivation time is at least 1 day, preferably at least 3 days, more preferably at least 7 days, and most preferably at least 10 days and at most 1 year, preferably at most 6 month, more preferably at most 30 days, still more preferably at most 25 days, and most preferably at most 20 days.

In an embodiment the culture volume is at least 20 ml, preferably at least 500 ml, more preferably at least 2000 ml, still more preferably at least 4000 ml, and most preferably at least 10,000 ml and at most 200 l, preferably at most 180 l, more preferably at most 100 l, and most preferably at most 50 l.

In an embodiment the cultivation vessel has a synthesis area of at least 1 cm², preferably at least 10 cm², more preferably at least 100 cm², and at most 50,000 cm², preferably at most 20,000 cm², more preferably at most 1,000 cm², still more preferably at most 500 m², still more preferably at most 100 m².

In an embodiment the patch of bacterial cellulose has a width of 20 to 300 mm, preferably 75 to 240 mm.

In an embodiment the patch of the bacterial cellulose has a height of 20 to 300 mm, preferably 35 to 175 mm.

In an embodiment the patch of the bacterial cellulose has a thickness of 0.5 to 10 mm, preferably 0.5 to 7 mm, more preferably 1 to 5 mm.

In an embodiment the BC-layer comprises or consists of a three-dimensional cellulose network and water.

In an embodiment the microemulsion comprises of 5 to 60 wt%, preferably 10 to 55 wt%, more preferably 15 to 52.5 wt%, most preferably 33.3 to 50 wt% of at least one surfactant and 0.5 to 20 wt%, preferably 1 to 15 wt%, more preferably 5 to 10 wt%, most preferably of 6.7 to 10 wt% of at least one co-surfactant and 0.5 to 40 wt%, preferably 1 to 35 wt%, more preferably 2 to 35 wt%, most preferably of 5 to 35 wt% of at least one oil and 0 to 60 wt%, preferably 0.1 to 55 wt% of at least one additional additive and 0.005 to 10 wt%, preferably 0.01 to 5 wt%, more preferably 0.025 to 1 wt%, most preferably of 0.05 to 0.5 wt% of at least one active ingredient and the remainder is water, wherein the total weight of components is 100 wt%.

In one embodiment, microemulsions with more than 30 wt% water, less than 41.7 % surfactant, less than 8.3 wt% co-surfactant and less than 20 wt% oil are particularly suitable for fast and easy loading of the cellulose and faster release of the active ingredient due to their lower viscosity.

In one embodiment, microemulsions with less than 30 wt% water, more than 41.7 % surfactant, more than 8.3 wt% co-surfactant and more than 20 wt% oil are particularly suitable for improved stability of the active ingredient due to their lower water content and sustained release of the active ingredient due to their higher viscosity.

In an embodiment the at least one surfactant is selected from polyoxyglycerides, polysorbates, polyoxyethylene castor oil derivatives or mixtures thereof, preferably selected from caprylocaproyl polyoxyglycerides, lauroyl polyoxyglycerides, linoleoyl polyoxyglycerides, oleoyl polyoxyglycerides, stearyl polyoxyglycerides, polyoxyethylene 20 sorbitan monolaurate, polyoxyethylene (4) sorbitan monolaurate, polyoxyethylene 20 sorbitan monopalmitate, polyoxyethylene 20 sorbitan monostearate, polyoxyethylene (4) sorbitan monostearate, polyoxyethylene 20 sorbitan tristearate, polyoxyethylene 20 sorbitan monoleate, polyoxyethylene 20 sorbitan trioleate, polyoxyethylene 20 sorbitan monoisostearate, macrogolglycerol ricinoleate, macrogolglycerol hydroxystearate, polyethoxylated castor oil or mixtures thereof, most preferably caprylocaproyl polyoxyl-8-glycerides.

In an embodiment the at least one co-surfactant is selected from 1,2-propanediol, glycerol, ethanol, polyethylene glycol, propan-2-ol, surfactant or mixtures thereof, preferably diethylene glycol monoethyl ether.

In an embodiment the at least one oil is selected from polyglyceryl-3 oleate, 1-methylethyl tetradecanoate, triheptanoin, glyceryl tricaprylate or mixtures thereof, preferably oleoyl macrogol-6 glycerides, polyglyceryl-3-dioleate or mixtures thereof.

In an embodiment the at least one active ingredient is a lipophilic pharmaceutical active compound. The lipophilic pharmaceutical active compound may show a solubility of less than 0.1 mg of pharmaceutically active compound, preferably of the pure pharmaceutically active compound, in 1 ml water at 37 °C (as defined for poorly insoluble drugs in the USP). The determination of the solubility of the pharmaceutically active compound is well known to a person skilled in the art. For instance, an excess amount of the pharmaceutically active compound is placed in a certain amount of water and mixed. The dissolved amount of the pharmaceutically active compound is then determined by a suitable analytical method, for instance by spectrometry.

In an embodiment the at least one active ingredient is an active pharmaceutical ingredient and/or an cosmetic ingredient, preferably corticosteroids, antibiotics, antifungals, antipsoriatics, proteolytic enzymes, emollients, photoprotectives, cicatrizants, antihistamines, chemotherapeutics, cytostatics, antiseptics, disinfectants, anti-acne-preparations, antihidrotics, calcineurin inhibitors, anti-dermatitis agents, anti-rosacea agents, vitamins, humectants, virutatics, beta-blockers, immunosuppressants, antiglaucoma preparations, miotics, antioxidants, growth factors, steroid hormones or mixtures thereof, more preferably a glucocorticoid and/or a local anesthetic, still more preferably cortisone, prednisone, prednisolone, methylprednisolone, betamethasone, betamethasone valerate, betamethasone dipropionate, clobetasole propionate, triamcinolone, deflazacort, fludrocortisone acetate, deoxycorticosterone acetate, methylprednisolone aceponate, mometasone furoate, aldosterone, lidocaine, benzocaine, prilocaine, cinchocaine, polidocanol, beclometasone or mixtures thereof, most preferably hydrocortisone or dexamethasone or mixtures thereof.

In an embodiment the at least one active ingredient is a mixture of glucocorticoids and local anesthetics.

In an embodiment the microemulsion is located between the fibers of the bacterial cellulose, preferably as a dispersed or bicontinuous microstructure.

In an embodiment the ratio of fiber-layer patch to the microemulsion is 1:1 to 1:1000, preferably 1:2 to 1:100, more preferably 1:5 to 1:50, wherein the ratio is quantified by weight of the fiber layer patch and weight of the microemulsion.

In an embodiment the fiber-layer patch has a storage stability at 40°C and 70% relative humidity of at least 300 days, preferably at least 200 days, more preferably at least 100 days, most preferably at least 90 days, wherein the storage stability is assessed by measuring the transmission at 650 nm before and after the period of storage whereat the transmission is not allowed to change more than 10% of the total transmission after the storage period.

In an embodiment the active ingredient incorporated in the fiber-layer patch has a storage stability at 40°C and 70% relative humidity of at least 300 days, preferably at least 200 days, more preferably at least 100 days, most preferably at least 90 days, wherein the storage stability is assessed by measuring the content of the active ingredient before and after the period of storage using a UHPLC-system coupled with ESI-MS and UV-detection at maximum excitation wavelength of the active ingredient and a gradient

of eluent A (0.1% formic acid in water) and eluent B (acetonitrile), whereat the content has to be >90% of the content before the period of storage.

In an embodiment the fiber-layer patch is stable after sterilization by autoclaving and by e-beam irradiation wherein the stability is assessed by measuring the transmission at 650 nm before and after the process of sterilization whereat the transmission is not allowed to change more than 10% of the total transmission after the process of sterilization.

In an embodiment the patch according to the invention has a transmission of 15 to 90%, preferably 20 to 80%, more preferably 30 to 80%, most preferably 46.85 to 77.37%.

For measurement, the patches were transferred to a 24-well plate (Greiner, Nuertingen, Germany) and the mean transmission at 650 nm was measured at five points located on the surface of the patches (Tecan Infinite® M Nano, Tecan, Männedorf, Switzerland). Aliquots of bidestillated water in the same layer thickness as the patches were used as positive control.

For the dermal application, the transmission is of considerable benefit, as it allows inspection of wounds and treated skin areas during the application, without removal of the wound dressing.

In an embodiment the patch according to the invention has an anti-inflammatory activity.

In an embodiment the patch according to the invention decreases the concentration of TNF α . To confirm the anti-inflammatory activity of the patches according to the invention a two-part test design was used. Release and drug permeation of the API was conducted in vitro in a vertical diffusion cell setup using Strat-M® membranes as synthetic skin models, to obtain receptor media samples containing the API. Next, the obtained receptor media samples were investigated regarding their effect on the release of TNF α as a pro-inflammatory indicator species by the determination of TNF α downregulation in LPS-stimulated monocytes.

In an embodiment the fiber-layer patch according to the invention is a transdermal drug delivery device, preferably a dermal drug delivery device, more preferably an active wound dressing.

Furthermore the present invention provides a method for manufacturing the fiber-layer patch according to the present invention comprising the following steps:

- I) providing and optionally squeezing a never-dried patch of bacterial cellulose,
- II) inserting the never-dried and optionally squeezed patch of bacterial cellulose into a container containing the microemulsion so that the bacterial cellulose is fully covered with the microemulsion,
- III) stirring the microemulsion which contains the patch of bacterial cellulose for at least 48h.

In an embodiment the patch of bacterial cellulose is not dry.

In an embodiment the microemulsion of step II) is manufactured as follows: the surfactant, co-surfactant and oil are mixed until a homogenous mixture is obtained. Under stirring, deionized water is added dropwise. The end point of adding water is represented at the transition point of transparency to turbidity. In a next step the active ingredient is dissolved under stirring.

In an embodiment the microemulsion loaded bacterial cellulose is manufactured as follows: The optionally squeezed bacterial cellulose patch is brought into contact with the microemulsion, preferably submersed in the microemulsion and stirred.

In an embodiment the volume of the microemulsion is 0.5 to 1000 mL, preferably 2 to 100 mL, more preferably 5 to 50 mL.

In an embodiment the microemulsion which contains the patch of bacterial cellulose is stirred for 0.5 to 240 hours, preferably 0.5 to 120 hours, more preferably 1 to 72 hours, most preferably 48 hours.

In an embodiment the microemulsion which contains the patch of bacterial cellulose is stirred at 0°C to 80°C, preferably 10°C to 60°C, more preferably 20°C to 50°C, most preferably 20°C.

In an embodiment the microemulsion which contains the patch of bacterial cellulose is stirred at 50 to 2000 rpm, preferably 200 to 1500 rpm, more preferably 500 to 1200 rpm, most preferably 1000 rpm.

In addition the present invention provides the use of the fiber-layer patch according to the invention for wound healing, drug delivery and tissue regeneration.

Examples

1. Preparation of the API-loaded BC-patch

The surfactant:cosurfactant mixture (Smix) consisted of Labrasol® as main surfactant and Transcutol® P as cosurfactant in the ratio of 5:1 (w/w), while Oil phase (Omix) consisted of Plurol Oleique® CC 497 and Labrafil® M 1944 CS in 1.4:1 ratio (w/w). Smix and Omix were mixed until homogenous mixtures of 10 mL were obtained. Deionized water was added drop-wise under stirring. The total quantity of added deionized water was recorded at the transition point of transparency to turbidity, which represented the end point of adding water. Five mixtures (ME-A, ME-B, ME-C, ME-D, ME-E) with varying ratios of deionized water, Smix and Omix were prepared as listed in Table 1. The components were weighed in glass vials. They are all spontaneously formed MEs after short periods of stirring. All formulations were stirred for 60 min at minimum to ensure complete homogenization.

Table 1: Composition of the prepared microemulsions

Microemulsion	Smix [wt%]	Water [wt%]	Omix [wt%]
ME-A	40.0	55.0	5.0
ME-B	45.0	42.5	12.5
ME-C	50.0	30.0	20.0
ME-D	55.0	17.5	27.5
ME-E	60.0	5.0	35.0

For the preparation of API loaded microemulsions, micronized hydrocortisone (HC) powder (Euro OTC Pharma GmbH, Germany) was added to the microemulsions at 0.5 wt% and dissolved under stirring. Also micronized dexamethasone (Dex) powder (Euro OTC) was added to another microemulsion at 0.05 wt% and dissolved under stirring. The obtained samples were labeled and are referred to as:

ME-A; ME-B; ME-C; ME-D; ME-E (blank MEs without API),

ME-A-HC; ME-B-HC; ME-C-HC; ME-D-HC; ME-E-HC (HC loaded MEs),

ME-A-DEX; ME-B-DEX; ME-C-DEX; ME-D-DEX; ME-E-DEX (DEX loaded MEs).

The BC was synthesized under static cultivation of bacteria strain *Komagataeibacter xylinus* DSM 14666 as described in the description. The microemulsion loaded BC was obtained by absorption loading technique. Aliquots of 10 g of all five MEs were transferred separately to glass vials. For each individual sample, a circular BC patch of 1.0 ± 0.2 g was dehydrated to a weight of 0.20 ± 0.01 g with manual pressure and submersed in the ME aliquots. The absorption loading was performed for 72 h on a magnetic stirrer at 1000 rpm. BC-microemulsion samples containing hydrocortisone and dexamethasone were prepared in the same way using hydrocortisone and dexamethasone loaded microemulsions. The obtained BC patches are referred to as:

BC-ME-A; BC-ME-B; BC-ME-C; BC-ME-D; BC-ME-E (ME loaded BC without API),

BC-ME-A-HC; BC-ME-B-HC; BC-ME-C-HC; BC-ME-D-HC; BC-ME-E-HC (ME loaded BC containing HC),

BC-ME-A-DEX; BC-ME-B-DEX; BC-ME-C-DEX; BC-ME-D-DEX; BC-ME-E-DEX (ME loaded BC containing DEX).

2. Measurement of the transmission

It could be observed that loading of BC with the microemulsions led to an increase in transmission, which was subsequently quantified spectrophotometrically. While for native BC a relatively low transmission of $12.70 \pm 2.17\%$ was found, there was a remarkable increase in transmission for BC-ME, ranging from $50.12 \pm 3.27\%$ for BC-ME-A, up to $74.94 \pm 2.43\%$ for BC-ME-E.

3. Anti-inflammatory activity

Several patches were tested regarding their effect on the release of $\text{TNF}\alpha$ as a pro-inflammatory indicator species by the determination of $\text{TNF}\alpha$ downregulation in LPS-stimulated monocytes. As shown in Figure 2, the API loaded BC patches led to the significant downregulation of $\text{TNF}\alpha$ in comparison to the control without APIs. The biological, anti-inflammatory activity of glucocorticoid loaded BC-ME was thus successfully demonstrated.

Claims

1. A fiber-layer patch comprising or consisting of
 - i) a bacterial cellulose layer, and
 - ii) an oil-in-water microemulsion or water-in-oil microemulsion, each comprising or consisting of at least one surfactant, at least one co-surfactant, at least one oil, water, optionally at least one additive, and at least one active ingredient.
2. The fiber-layer patch according to claim 1, wherein the bacterial cellulose is expressed in *Komagataeibacter*.
3. The fiber-layer patch according to any of the preceding claims, wherein the microemulsion consists of:
 - a) 5 to 60 wt% of at least one surfactant;
 - b) 0.5 to 20 wt% of at least one co-surfactant;
 - c) 0.5 to 40 wt% of at least one oil;
 - d) 0 to 60 wt% of at least one additional additive;
 - e) 0.005 to 10 wt% of at least one active ingredient, and
 - f) the remainder is water; wherein the total weight of components a) to f) is 100 wt.-%.
4. The fiber-layer patch according to any of the preceding claims, wherein the at least one surfactant is selected from polyoxylglycerides, polysorbates, polyoxyethylene castor oil derivatives or mixtures thereof.
5. The fiber-layer patch according to any of the preceding claims, wherein the at least one co-surfactant is selected from diethylene glycol monoethyl ether, 1,2-propanediol, glycerol, ethanol, polyethylene glycol, propan-2-ol, polyoxylglycerides, polysorbates, polyoxyethylene castor oil derivatives or mixtures thereof.
6. The fiber-layer patch according to any of the preceding claims, wherein the at least one oil is selected from oleoyl macrogol-6 glycerides, polyglyceryl-3 oleate, 1-methylethyl tetradecanoate, triheptanoin, glyceryl tricaprylate or mixtures thereof.
7. The fiber-layer patch according to any of the preceding claims, wherein the at least one active ingredient is selected from corticosteroids, antibiotics, antifungals, antipsoriatics, proteolytic enzymes, emollients, photoprotectives, cicatrizants, antihistamines, chemotherapeutics, cytostatics, antiseptics, disinfectants, anti-acne-preparations, antihidrotics, calcineurin inhibitors, anti-dermatitis agents, anti-rosacea agents, vitamins, humectants, virutatics, beta-blockers,

immunosuppressants, antiglaucoma preparations, miotics, antioxidants, growth factors, steroid hormones, local anesthetics or mixtures thereof.

8. The fiber-layer patch according to any of the preceding claims, wherein the microemulsion is located between the fibers of the bacterial cellulose as a dispersed or bicontinuous microstructure.
9. The fiber-layer patch according to any of the preceding claims, wherein said patch has a storage stability of at least 90 days.
10. The fiber-layer patch according to any of the preceding claims, wherein said patch is stable under sterilization.
11. The fiber-layer patch according to any of the preceding claims, wherein the ratio of fiber-layer patch to microemulsion is 1:1 to 1:1000.
12. The fiber-layer patch according to any of the preceding claims, wherein said patch is a transdermal drug delivery device, dermal drug delivery device or active wound dressing.
13. A Method for manufacturing the fiber-layer patch according to any of claims 1 to 12, comprising the following steps:
 - I) providing and optionally squeezing a never-dried patch of bacterial cellulose,
 - II) inserting the never-dried and optionally squeezed patch of bacterial cellulose into a container containing the microemulsion so that the bacterial cellulose is fully covered with the microemulsion,
 - III) stirring the microemulsion which contains the patch of bacterial cellulose for at least 48h.
14. Use of the patch according to any of claims 1 to 12 for wound healing, drug delivery, skin care, aesthetic treatments and tissue regeneration.