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(54) **Title:** MEMBRANE CONSTRUCTION WITH FIBER LAYERS FOR BLOOD FILTRATION

(57) **Abstract:** The invention relates to a membrane construction for the separation of blood cells from blood plasma, comprising multiple layers wherein at least of the layers is a support layer; at least one of the layers is a nanoweb made of polymeric nanofibers wherein the nanoweb comprises a mean flow pore size in the range from 0.3 to 3 μm measured according to standard ASTM E 1294-89, a basis weight in the range 1 to 20 g/m^2 measured according to standard ASTM D 3776 and a porosity in the range from 60 to 95 % measured according to standard ASTM D 2873-94; and wherein the nanofibers have a number average diameter in the range from 100 to 600 nm; characterised in that the support layer is a porous layer with a mean flow pore size of between 5 and 15 μm and $\text{h.P.A}/100$ is more than 5 μL , wherein h is the thickness of the support layer, P the porosity (%) of the support layer and A the surface of the support layer in the membrane construction.



MEMBRANE CONSTRUCTION WITH FIBER LAYERS FOR
BLOOD FILTRATION

The invention relates to a membrane construction for the separation of blood cells from blood plasma, a membrane cassette comprising said membrane construction, a device comprising said membrane construction or said membrane cassette and the uses thereof such as for example blood filtration, diagnostic devices, bio-separation of cell cultures and bio-fermentation. The invention relates in particular to a membrane for a point of care (POC) device. POC devices will enable patients to monitor themselves with a few drops of blood and communicate results with clinicians, ultimately giving patients a greater control of their own health and access to their personal health data. In addition, POC devices may also support the emergence of personalized medicine in which treatments are tailored to the genetic profile of patients and/or to their specific metabolism of drugs. A POC device is attractive because it requires only a few microliter of blood from a patient. To analyses proteins, related certain diseases in a patient's blood, red blood cells (RBC's) have to be separated from the plasma, which is generally done by filtration for small quantities of blood. However, human blood with a volume percentage of RBC's (the hematocrit or Hct) of between 35 and 55 vol% may quickly block a filter, which implies an undesirably long time to separate RBC's from the plasma. Separation of blood and plasma has to be done within about 300 seconds in order to avoid hemolysis of RBC's. With hemolysis, the plasma loses its quality for a subsequent analysis, as protein recovery is poor.

Membrane constructions for blood filtration are known from WO2011151314. WO2011151314 describes a membrane construction comprising multiple layers wherein at least one of the layers is a nanoweb made of polymeric nanofibers, the mean flow pore size of the nanoweb is in the range from 50 nm to 5 μ m, the number average diameter of the nanofibers is in the range from 100 to 600 nm, the basis weight of the nanoweb is in the range from 1 to 20 g/m², the porosity of the nanoweb is in the range from 60 to 95 %, at least one of the layers is a support layer and preferably but not necessarily the nanoweb is hydrophilic.

A disadvantage of the membrane construction described in WO2011151314 is, that the time to separate RBC's from plasma is too long, reason for which the blood has to be diluted first.

An object of the present invention is to provide a membrane construction whereby time to separate plasma from RBC's in blood for small quantities, typically below 100 μ L, with an Hct of 55 vol% is below 300 seconds with good protein

recovery.

According to the present invention, this object (amongst other objects), is achieved by the membrane construction for the separation of blood cells from blood plasma, comprising multiple layers wherein:

- 5 - at least of the layers is a support layer;
- at least one of the layers is a nanoweb made of polymeric nanofibers wherein the nanoweb comprises a mean flow pore size in the range from 0.3 to 3 μm measured according to standard ASTM E 1294-89, a basis weight in the range 1 to 20 g/m^2 measured according to standard ASTM D 3776 and a porosity in
10 the range from 60 to 95 % measured according to standard ASTM D 2873-94 and
 wherein the nanofibers have a number average diameter in the range from 100 to 600 nm;

characterised in that the support layer is a porous layer with a mean flow pore size of
15 between 5 and 15 μm and $h.P.A/100$ is more than $5\mu\text{L}$, wherein h is the thickness of the support layer, P the porosity (%) of the support layer and A the surface of the support layer in the membrane construction.

Advantageously, when $h.P.A/100$ is more than $5\mu\text{L}$ and defined by a thickness h of about 250 μm and a porosity P of at least 80%, the membrane construction according
20 to the present invention provides good results when filtrating blood. The best results have been obtained when the thickness h in the range 200 to 400 μm , the porosity P in the range 80% to 97% and the surface A is in the range 0.2 to 1.8 cm^2 ,

As a result of a support layer being a porous layer with a mean flow pore size of between 5 and 15 μm and a $h.P.A/100$ being more than $5\mu\text{L}$, with the
25 membrane construction of the invention RBC's can be separated from plasma in less than 100 sec with good protein recovery. Preferably, $h.P.A/100$ is more than $15\mu\text{L}$ in order to ensure an optimum separation.

With membrane construction is meant a collection of layers together forming the membrane construction. With 'multiple layers' is meant at least two layers.
30 Each of the layers differ in mean flow pore size and/or type of material.

It is known to the skilled person how to prepare a membrane construction comprising multiple layers, for example multiple layers can be made using phase inversion (e.g. as described in US6045899) or for example by spinning the nanoweb on the same place while moving the support layer or vice versa, moving the
35 spinning head above a fixed support layer, or by laminating the support layer with the

nanoweb. In order to attach the nanoweb to the other layers, hot laminating may be used and/or glue may for example be applied onto the support material and/or the support layer may be in a hot-melt state when the nanoweb is applied thereon.

In the context of the invention, with nanoweb made of polymeric
5 nanofibers is meant a nonwoven web comprising primarily, or even exclusively polymeric nanofibers.

The mean flow pore size of the nanoweb is in the range from 0.3 to 3 μm .

In the context of the present invention, the mean flow pore size is
10 determined according to standard ASTM E 1294-89, "standard test method for pore size characteristics of membrane filters using automated liquid porosimeter" by using automated bubble point method from ASTM designation F 316 using a capillary flow porosimeter (model number CFP-34RTF8A-3-6-L4, Porous Materials, Inc. (PMI), Ithaca, N.Y.).

15 The mean flow pore size can also be advantageously measured indirectly with airflow techniques via the air permeability, such as Gurley or Airflux. The method that is applied for the air permeability, and from which the values have been derived for mean flow pore size values reported herein, is the Gurley test method according to ISO 5636-5. As a standard measuring set up a measuring area of 6.45
20 cm^2 and a load of 567 grams is used, and the time needed for 50 ml of air to be permeated is measured. The air permeability thus measured is expressed in s/50ml, (wherein s = seconds and ml = millilitre). For micro-porous membranes with relative large pore sizes, for example with a mean flow pore size of about 1 μm or more, the measuring area can be reduced, for example to 1 cm^2 , and the volume of air to be
25 permeated can be increased, for example 100 ml or 200 ml, to thus allowing the permeation time to be measured more accurately. The thus obtained measuring values can be recalculated to the corresponding value for the standard measuring set up, and also these modifications can be applied in accordance with ISO 5636-5. The relation between the Gurley (50 cc) number and air permeability is described in ISO 5636-5.
30 The air permeability measured with Gurley, and expressed in s/50ml, can be translated via an empirical relation into pore size in μm , by dividing the number 1,77 by the Gurley number.

Advantageously, the mean flow pore size of the nanoweb is at least 0.3, for example at least 0.4, for example at least 0.5 μm . The at least 0.3 μm mean
35 flow pore size provides a good flux into the membrane and reduces residence time,

thereby limiting protein adsorption at the surface of the membrane construction. A maximum of 1.5 μm mean flow pore size allows an optimal filtration by removing the suitable components of the blood (such red blood cells). For example, the mean flow pore size is at most 0.9, for example at most 0.8, for example at most 0.7 μm . The preferred range of the nanoweb is between 0.4 and 1.5 μm , providing the best results in blood filtration.

The mean flow pore size of the nanoweb may be reduced by calendering the nanoweb and/or the nanoweb in combination with the support layer. This may increase the strength of the nanoweb and/or the nanoweb in combination with the support layer. Calendering is the process of passing sheet material (in this case the nanoweb) through a nip between rolls or plates. The calendering process also affects the porosity P and the thickness h of the support, and the resulting values need to match the previous requirements.

The mean flow pore size (of the nanoweb) is influenced by a combination of the thickness of the nanoweb and the number average diameter of the nanofibers. For example, by increasing the thickness, the mean flow pore size may be reduced. By reducing the number average diameter of the nanofibers, the mean flow pore size can also be reduced.

In the context of the present invention, the mean flow pore size is defined by a thickness h in the range 200 to 400 μm , a porosity P in the range 80% to 97% and surface A in the range 0.2 to 1.8 cm^2 .

With 'basis weight of the nanoweb' is meant the weight per square meter. The basis weight of the nanoweb is in the range from 1 to 20 g/m^2 . The basis weight can be measured according to standard ASTM D-3776, which is hereby incorporated by reference. Preferably, the basis weight of the nanoweb is in the range 2 to 6 g/m^2 .

A nanofiber web may be prepared from nanofibers using methods known to the person skilled in the art, for example via multi-nozzle electrospinning, for example as described in WO2005/073441, hereby incorporated by reference; via nozzle-free electrospinning, for example using a NanopiderTM apparatus, bubble-spinning or the like; or via electroblowing, for example as described in WO03/080905, hereby incorporated by reference; via melt-blowing or via centrifuge-spinning.

The desired basis weight can be achieved by adjusting the flow rate of an electrospinning process using to spin the nanofiber and/or by adjusting the speed of the support layer onto which the nanoweb is spun.

The porosity of the nanoweb is in the range from 60 to 95%. The porosity of the nanoweb is the difference between 100% and the solidity of the nanoweb. The solidity can be calculated by dividing the basis weight of the nanoweb sample in g/m^2 , determined as described herein, by the polymer density of the polymer from which the nanofiber is made in g/cm^3 and by the sample thickness in μm and multiplying by 100, i.e. $\text{solidity} = (\text{basis weight}/(\text{density} \times \text{thickness})) \times 100$. Porosity = $100\% - \text{\% solidity}$. Porosity is determined according to standard ASTM D 2873-94, or by standard ASTM F 2450-10 which methods are hereby incorporated by reference.

The porosity of the nanoweb is in the range from 60 to 95%, for example the porosity of the nanoweb is for example at least 65%, for example at least 70%, for example at least 80%.

Sample thickness is determined by ASTM D-645 (or ISO 534), which method is hereby incorporated by reference, under an applied load of 50kPa and an anvil surface area of 200mm^2 . The density (ρ) of the polymer composition is measured as described in ISO1183-1:2004.

The surface A of the filter in the membrane construction, for example any filtration device, such as a cartridge.

Polymer density is measured as described in ISO1183-1:2004.

The term 'nanofibers', as used herein, refers to fibers having a number average diameter of at most 1000nm ($1\mu\text{m}$).

To determine the number average diameter of the fibers, ten (10) scanning electron microscopy (SEM) images at 5,000x magnification were taken of each nanofiber sample or web layer thereof. The diameter of ten (10) clearly distinguishable nanofibers was measured from each photograph and recorded, resulting in a total of one hundred (100) individual measurements. Defects were not included (i.e. lumps of nanofibers, polymer drops, intersections of nanofibers). The number average diameter d of the fibers was calculated from the one hundred (100) individual measurements.

Preferably, the number average diameter of the nanofibers present in the membrane construction according to the present invention is in the range 100 to 600 nm, preferably the number average diameter of the nanofibers is at most 500 nm, more preferably at most 400 nm. Advantageously, the number average diameter of the nanofibers is at least 150, more advantageously, at least 200nm. When the number average diameter of the nanofibers is in the range 200 nm to 400 nm, preferably 200-300 nm, the membrane construction presents a minimized clogging and/or an optimal

flux and/or an efficient filtration (which can also be designated as optimal retention of the particulate to be filtrated).

The nanofiber diameter can be reduced e.g. reducing the solution concentration, reducing the polymer molecular weight, or modifying the process conditions (applied voltage, solution flow rate, spinning distance).

The desired number average diameter of the nanofiber can be achieved by routine experimentation. Factors that may influence the number average diameter of the nanofiber are the viscosity of the polymer solution used to make the nanofibers (usually between 200 and 1000mPa.s), the electrical voltage, the flow rate of the polymer solution and the choice of polymer.

The polymeric nanofiber may be prepared from any polymer material. Examples of polymer materials include but are not limited to polyacetals, polyamides, polyesters, polyolefins, cellulose ethers and esters, polyalkylene sulfides, polyarylene oxides, polysulfones, modified polysulfone polymers and mixtures thereof. Examples of materials that fall within these generic classes include poly(vinylchlorides), polymethylmethacrylates and other acrylic resins, polystyrenes and copolymers thereof, for example ABA type block copolymers, poly(vinylidene fluorides), poly(vinylidene chlorides), polyvinylalcohols.

When the polymeric nanofiber is made of polyamide, the membrane shows less clogging of the material to be filtrated than with other polymers. Good results in blood filtration have been obtained when the polymeric nanofiber is prepared from a polyamide chosen from the group of aromatic polyamides, semi-aromatic polyamides, aliphatic polyamides, mixtures and copolyamides thereof. Preferably the polyamide is from the group of aliphatic polyamides, mixtures and copolyamides thereof. Aliphatic polyamides are preferred over aromatic and semi-aromatic polyamides when used for electrospinning of the nanofibers since aromatic and semi-aromatic polyamides usually require more hazardous solvents or from a polyvinylalcohol. The polyamides may be crystalline, semi-crystalline or amorphous. Preferably, the polymeric nanofiber is prepared from a semi-crystalline polyamide.

Examples of semi-aromatic polyamides, include terephthalic acid based polyamides, for example polyamide 4,T polyamide 6,T/6,6, polyamide 4,10, polyamide 9,T, polyamide 6T/6I or PAMXD,6, PAMXDT or copolyamides thereof; aromatic polyamides, also known as polyaramides, for example polyparaphyleneterephthalamide (PPTA, commercially available as for example KevlarTM, TwaronTM or TechnoraTM) or polyparaphyleneisophthalamide (PPIA,

commercially available as NomexTM); aliphatic polyamides, for example polyamide 2 (polyglycine), polyamide-3, polyamide-4, polyamide-5, polyamide-6, polyamide-2,6, polyamide-2,8, polyamide-6,6, polyamide-4,6 or polyamide 6,10 or copolyamides thereof, for example polyamide 6/6,6, polyamide 4,6/6; or copolyamides of semi-
5 aromatic and/or aromatic and/or aliphatic polyamides.

Preferred thermoplastic polyamides include but are not limited to polyamide 6; polyamide 6,6; polyamide 4,6; polyamide 4,10; polyamide 6,10; polyamide 4,6, copolyamides and/or mixtures thereof, more preferably polyamide-6, polyamide 4,6, copolyamides and/or mixtures thereof. If the nanoweb is made from
10 nanofibers made from these preferred thermoplastic polyamides, the nanoweb may have a high wettability and a high (tensile) strength.

Wettability (with a liquid of choice for example blood or water) of the base material of the nanoweb can be determined by measuring the advanced contact angle according to ASTM D7334-08 (using said liquid of choice). The lower the
15 advanced contact angle of the nanoweb with said liquid of choice, the higher the wettability of the nanoweb. The wettability should be chosen such that the nanoweb can be impregnated with water and blood.

Tensile strength can be measured on a tensile tester (Zwick) at a constant rate of elongation of 2 inches per minute. Samples are cut to a size of
20 1inchx8inches, being longer in the direction of loading. The gage length of samples was 6 inches and the starting width of samples was 1 inch. The tensile strength is defined as the maximum load supported by a sample piece of the nanoweb divided by its cross-sectional area. Samples are tested in both the X (length) and the Y (width) direction.

25 Preferably, the polyamide has a carbon/nitrogen (C/N) ratio of at most 9, more preferably, the polyamide has a C/N ratio in the range of from 4-8.

Preferably, the nanofibers are prepared from polyamide-6, polyamide-6,6, polyamide-4,6 or copolymers thereof, more preferably from polyamide-4,6.

Polyamide-4,6 is a class of polyamides commercially available under the trademark
30 StanylTM from DSM, the Netherlands. An advantage of the use of polyamide-6, polyamide-6,6, polyamide-4,6 or copolymers thereof and in particular of polyamide-4,6 to prepare the nanofibers may be an improved hydrophilicity of the nanoweb, which makes the nanoweb even more suitable for the uses described herein. Furthermore, advantages may be improved tensile strength and/or an increased thermal stability .

35 As used herein, the term polyamide encompasses for example

polyamides comprising proteins such as for example silk or keratin as well as modified polyamides, such as for example hindered phenol end capped polyamides.

In the polymer solution comprising the polymeric material of choice used to prepare the nanofibers, additives may be present. Suitable additives include but are not limited to: surface tension agents or surfactants, for example perfluorinated acridine, crosslinking agents, viscosity modifiers, for example hyperbranched polymers such as hydroxylfunctional hyperbranched polyester amide polymers as described in WO1999/016810, carboxyfunctional hyperbranched polyester amide polymers as described in WO2000/056804, dialkylamide functional hyperbranched polyester amide polymers as described in WO2000/058388, ethoxyfunctional hyperbranched polyester amide polymers as described in WO2003/037959, heterofunctionalized hyperbranched polyester amides as described in WO2007/098889 or secondary amide hyperbranched polyester amides as described in WO2007/144189, electrolytes, antimicrobial additives, adhesion improvers, for example maleic acid anhydride grafted rubber or other additives to improve adhesion with a polypropylene or polyethylene terephthalate substrate, nanoparticles, for example nanotubes or nanoclays, and so on. Examples of electrolytes include water soluble metal salts, for example metal alkali metal salts, earth alkali metal salts and zinc salts, LiCl, HCOOK (potassium formate), CaCl₂, ZnCl₂, KI₃, NaI₃. Preferably, an electrolyte is present in an amount in the range of from 0 to 2 wt% relative to the total weight of the polymer solution. The water soluble salt may be extracted with water from the nanofibers produced, thereby obtaining microporous nanofibers.

Nanofibers may be prepared using methods known to the skilled person, for example, they may be produced using electrospinning, such as classical electrospinning or electroblowing, and sometimes also by meltblowing processes. Classical electrospinning is illustrated in US 4,127,706, hereby incorporated by reference. Electrospinning, as well as centrifuge spinning, can operate from solutions or from melts.

The preparation of nanofibers using an electrospinning process comprising the steps of:

- applying a high voltage between a spinneret comprising a series of spinning nozzles and a collector, or between a separate electrode and a collector
- feeding a stream of polymer solution comprising a polymer and a solvent, or a polymer melt, to the spinneret

- whereby the polymeric solution or melt exits from the spinneret through the spinning nozzles and transforms under the influence of the high voltage into charged jet streams,
- whereby the jet streams are being deposited on or taken up by the collector or a support layer
- whereby the polymer in the jet stream solidifies prior to or while being deposited on or taken up by the collector or the support layer whereby the nanofibers are formed.

After preparation of the nanofibers, the nanofibers may be post-stretched, post-coated, washed, dried, cured, annealed and/or post condensed. It may be advantageous to dry the nanofibers to remove residual solvents which may interfere with the analysis of the blood plasma obtained after filtration using the membrane construction of the invention.

A detailed description on how polyamide-4,6 nanofibers may be prepared is for example given by Huang, C. et al., 'Electrospun polymer nanofibres with small diameters', Nanotechnology, vol. 17 (2006), pp2558-2563.

Hydrophilicity respectively hydrophobicity of a surface can be determined via the advanced contact angle made by a liquid, for example water using ASTM D7334-08. If the surface, for example the nanoweb shows an advanced contact angle with water of at least 90°, the surface, for example the nanoweb is defined herein as hydrophobic, if the surface, for example the nanoweb shows an advanced contact angle with water of less than 90°, the surface is defined herein as hydrophilic. Preferably, the nanoweb is hydrophilic, more preferably, the nanoweb has a contact angle as measured with water using ASTM D7334-08 of less than 80°, for example less than 70°, for example less than 60°, for example less than 50°, for example less than 45°. The hydrophilicity of the nanoweb should be chosen such that the nanoweb can be impregnated with water and blood.

The membrane construction of the invention comprises at least one support layer. The support layer is a porous layer with a mean flow pore size of between 5 and 15 µm and $h.P.A/100$ is more than 5µL, wherein h is the thickness of the support layer, P the porosity (%) of the support layer and A the surface of the support layer in the membrane construction (i.e. A is the top surface of the support layer). Preferably, the support layer is a non-woven. With a non-woven, an advantageously high porosity can be obtained.

Surprisingly only for a membrane construction with a support layer

with a mean flow pore size between 5 μm and 15 μm , preferably between 5 μm and 10 μm and with h.P.A/100 being more than 5 μL , the time to separate RBC's from plasma is less than 100 sec. Preferably, h.P.A/100 is more than 15 μL . Advantageously, h.P.A/100 is at most 200 μL .

5 In a special embodiment, the membrane construction comprises more than one support layer, wherein the support layers form a gradient pore structure. With 'gradient pore structure' is meant that the mean flow pore size in the membrane construction increases with the distance to the top layer of the membrane construction, e.g. the nanoweb. In that case, the sum of h.P.A/100 for the different layers should be
10 more than 5 μL , preferably even more than 10 μL . Preferably h.P.A/100 is less than 100 μL , more preferably less than 50 μL , as no blood will pass the membrane construction by gravity or capillary forces only without an additional driving force like e.g. a vacuum or an extra pressure.

The porosity of the support layer is preferably at least 50%, for
15 example at least 60%, for example at least 70%, for example at least 80%, for example at least 90%. Preferably the porosity of the support layer is at least 92%.

The support layer is preferably also hydrophilic because outstanding results have been obtained when combining at least one hydrophilic support layer and at least one layer being a nanoweb: the membrane construction comprising such a
20 structure provide the advantage of less clogging and/or less adhesion of proteins in the membrane. The support layer may be prepared from hydrophilic materials or if the support layer is prepared from hydrophobic material, the support layer may be coated with a hydrophilic coating as described herein.

More preferably, the hydrophilic support layer has a contact angle as
25 measured with water using ASTM D 7334-08 below 80°, preferably below 70°, more preferably below 60°, most preferably below 50°, even most preferably below 45°. When the nanoweb is made of polymeric nanofibers comprising a polyamide such as defined in the context of the present invention, the improved properties of the membrane with respect to clogging and decreased protein adhesion even better.

30 The membrane construction of the invention comprises at least one support layer. The support layer may be any substrate on which the nanoweb can be added, for example a non-woven cloth, any fibrous substrate, or a filter or membrane layer, for example a microporous membrane. Examples of non-woven cloths include for example a meltblown nonwoven cloth, needle-punched or spunlaced nonwoven cloth,

woven cloth and knitted cloth. Preferably, the membrane construction according to the present invention comprises a support layer which is a non-woven layer based on micro-fibers with a number average diameter between 1 to 10 μm .

According to an embodiment of the present invention, the support
5 layer can comprise micro-glass.

Examples of any fibrous substrates include, paper, any fibrous substrate comprising selected from the group of materials comprising glass, silica, metals, ceramic, silicon carbide, carbon, boron, natural fibers such as cotton, wool
10 hemp or flax, artificial fibers, such as viscose or cellulosic fibers, synthetic fibers, for example polyester, polyamides, polyacrylics, chlorofibers, polyolefines, synthetic rubbers, polyvinylalcohol, aramides, fluorofibers, phenolic.

If a microporous membrane is used as the at least one support layer, the membrane may be prepared from any polymer, for example a nylon, polyamides, pref aliphatic polyamides, for example polyamide 6, polyamide-4,6, copolymers or
15 mixtures thereof., a polyolefin or a halogenated vinyl polymer, preferably from a polyolefin or polytetrafluoroethylene (PTFE), more preferably from a polyethylene (PE), most preferably from ultra high molecular weight polyethylene (UHMWPE), which has a weight average molecular weight (M_w) of at least $0.5 \cdot 10^6$ g/mol. Microporous membranes made from UHMWPE is for example available from Lydall, the
20 Netherlands under the name SoluporTM. Preferably, microporous membranes made from highly stretched UHMWPE are used as microporous membrane.

The amount of polyolefin or halogenated vinyl polymer present in the microporous membrane is for example at least 20wt%, for example at least 50wt% relative to the total weight of the microporous membrane.

25 Microporous membranes may be prepared using methods known to the skilled person. For example in US 3,876,738 it is described that microporous films may be produced by a process of quenching a polymer solution cast in a quench bath containing a non-solvent system for the polymer to form micropores in the resulting polymer film. For example, US5693231 describes a process for the preparation of
30 microporous nylon membranes, US5264165 describes a process for the preparation of polyamide-4,6 microporous membranes.

Preferably, the nanoweb and the support layer are in contact with one another, as this may provide mechanical support and/or a reduced amount of so-called 'dead volume', that is the amount of liquid to be separated that stays inside the
35 membrane construction rather than flowing through.

The basic weight of the support layer is in the range of from 5 to 400 g/m², preferably 20 to 100 g/m².

The membrane construction may comprise further layers besides the nanoweb and the support layer. These layers may be layers to increase the separation of the components to be separated and/or to increase the tensile strength of the membrane construction. For example, the membrane construction may further comprise, a 'functional' membrane layer, a further nanoweb layer, a textile layer.etc.This textile layer is preferably in contact with the support layer if a microporous support layer is present in the membrane construction according to the invention. The textile layer may also be the support layer if no microporous support layer is present in the membrane construction of the invention. In that case, the textile layer and the nanoweb are preferably in contact with one another.

The textile layer can for example be any non-woven support or any fibrous substrate as described above.

In case a nanoweb is spun directly onto a support surface with a mean flow pore size of between 5 -15 µm, then multiple nanoweb forming a nanofiber gradient may be used. WO2008/142023 A2 describes for example how to spin a multiple layer gradient nanoweb. For instance, a two layer nanoweb, wherein for examples one layer is prepared from nanofibers having a number average diameter in the range of from 500 to 600 nm and the top layer is prepared from nanofibers having a number average diameter in the range of from 100 to 200 nm, may be used.

As defined herein, two layers are preferably in contact with one another by being bonded, adhered or laminated together.

In a special aspect, at least one of the layers of the membrane construction is coated. With 'coated' is meant that the at least one layer is contacted with a coating solution, such that the coating solution impregnates the layer. So, for example the nanoweb layer and/or the support layers and/or any other further layer of the membrane construction may be coated, or treated with antifouling e.g. PEG, proteins or amino acids. A coating may affects the porosity P and the thickness h of the support. Once coated, the membrane should pass the previous requirements in terms of pore size, porosity, thickness and weight per unit of surface.

Examples of coating solutions include antifouling coating solutions, for example antibiofouling coating solutions such as for example described in WO2006/016800.

In a preferred embodiment at least one of the layers of the membrane

construction is coated with an antibiofouling coating. By coating at least one of the layers of the membrane construction with an antibiofouling coating, protein recovery in the blood plasma is increased. If the membrane construction is used for diagnostics, this will enhance the analysis resolution. If the membrane construction is used for dialysis, the efficiency of the dialysis will be increased. A description on how to impregnate a membrane layer is for example given in WO2009/063067.

In one aspect, the invention relates to a membrane cassette comprising the membrane construction of the invention.

With membrane cassette is meant a construction (housing) containing one or more membrane constructions of the invention.

In another aspect, the invention relates to a device comprising the membrane construction of or the membrane cassette of the invention.

Such devices may be devices used in plasma and serum separation in for example diagnostics; pre-analytical systems, such as blood collection devices, for example tubes and capillaries), micro fluidic point of care biosensor or Dry Plasma Spots.

The invention will now be elucidated with the following examples, without however being limited thereto.

Examples

The time to separate blood from plasma is measured with a card ridge test method described below.

Cart ridge test method

A filter with a diameter of 10 mm is mounted on a card-ridge with a two-sided sticky tape (outer diameter 10 mm, inner diameter 8 mm). This card ridge is an injection moulded part comprising a filter mounting surface, a flow channel and a chamber R. The card ridge is horizontally positioned during the test.

Plasma that has passed the filter drops on a round flat surface with a diameter of 8 mm, comprising 6 grooves of each 0.15 mm width and 0.02 mm deep, separating the surface in 6 equal radial parts.

The center of said surface is connected with a channel with a length of 24 mm, a width of 0.6 mm and height of 0.08 mm. Via this channel, plasma that has passed the filter is guided to a chamber of 1.6x1.6 mm² and a height of 0.08 mm.

Two drops of blood with a volume of 50 μL and a Hct% as given in Table 1 are applied with an Eppendorf pipette on the filter. The Start Time is the time wherein the first liquid has passed the first 4 mm in the channel. The Fill Time is the time wherein the chamber R is completely filled with plasma.

5 Comparative Experiments 1 - 7 were carried out with a Pall GF filter. The Pall GF filter is a filter made from polyethersulfone with a thickness of 340 μm and a variable pore size ranging from 80 μm to 3 μm .

10 In the Comparative Experiments 8 -10 according WO2011151314 a Novatex support layer (non-woven PA66/PA6) with a thickness of 200 μm and a PA nanoweb layer with a thickness of 10 μm is used. The base weight of the layer of nanofibers is 2 g/m^2 .

The Examples I to VII according to the invention are based on a Lypore 9858 PEG microglass support layer with a base weight of 49.5 g/m^2 (density 2.5 g/cm^3) and a PA 46, 70 μm thick layer of nanofibers with a base weight of 2 g/m^2 .

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	Properties Support					Properties Nanoweb			Blood	Filtration	
	Thickness	Base weight	MFP	Porosity	h.p.A/100	Thickness	MFP	Fibre Thickness		Start time	Fill time
	μm	g/m ²	μm	%	μL	μm	μm	nm	Hct		sec
Cart ridge without filter									%		
Comp. Exp. 1	340	59	80	87	23		2.9		plasma	2.5	10-20
Comp. Exp. 2	340	59	80	87	23		2.9		plasma	6	14
Comp. Exp. 3	340	59	80	87	23		2.9		10	8	21
Comp. Exp. 4	340	59	80	87	23		2.9		32	13	37
Comp. Exp. 5	340	59	80	87	23		2.9		44	50	X
Comp. Exp. 6	340	59	80	87	23		2.9		47	120	X
Comp. Exp. 7	340	59	80	87	23		2.9		50	154	X
Comp. Exp. 8	200	70	38.5	86	13	10	0.4	70	55	171	X
Comp. Exp. 9	200	70	38.5	86	13	10	0.4	70	plasma	9	20
Comp. Exp. 10	200	70	38.5	86	13	10	0.4	70	10	15	37
Example I	250	53	6.5	92	18	20	1.5	350	32	X	X
Example II	250	53	6.5	92	18	20	1.5	350	plasma	10	25
Example III	250	53	6.5	92	18	20	1.5	350	10	12	35
Example VI	250	53	6.5	92	18	20	1.5	350	32	18	43
Example V	250	53	6.5	92	18	20	1.5	350	47	24	54
Example VI	250	53	6.5	92	18	20	1.5	350	48	34	95
Example VII	250	53	6.5	92	18	20	1.5	350	50	44	101
	250	53	6.5	92	18	20	1.5	350	55	52	155

Table 1.

From this table it can be learned that blood with more than 44 Hct % does pass the Pall filter, but does not reach the chamber.

This was due to blockage of the filter and subsequent hemolysis. Blood with more than ca. 30 Hct% does not even pass the comparative Novatex filter.

CLAIMS

1. Membrane construction for the separation of blood cells from blood plasma, comprising multiple layers wherein
 - 5 - at least of the layers is a support layer;
 - at least one of the layers is a nanoweb made of polymeric nanofibers wherein the nanoweb comprises a mean flow pore size in the range from 0.3 to 3 μm measured according to standard ASTM E 1294-89, a basis weight in the range 1 to 20 g/m^2 measured according to standard ASTM D 10 3776 and a porosity in the range from 60 to 95 % measured according to standard ASTM D 2873-94 ; andwherein the nanofibers have a number average diameter in the range from 100 to 600 nm measured by scanning electron microscopy; characterised in that the support layer is a porous layer with a mean flow pore size of between 15 5 and 15 μm and $h.P.A/100$ is more than $5\mu\text{L}$, wherein h is the thickness of the support layer, P the porosity (%) of the support layer and A the surface of the support layer in the membrane construction.
2. Membrane construction according to claim 1, wherein the mean flow pore size of the nanoweb is in the range 0.4 to 1.5 μm .
- 20 3. Membrane construction according to claim 1 or 2, wherein the number average diameter of the nanofibers is in the range 200 to 300 nm.
4. Membrane construction according to claim any one of claims 1 to 3, wherein the polymeric nanofibers are polyamide nanofibers.
5. Membrane construction according to any one of claims 1 to 4, wherein 25 $h.P.A/100$ is more than 15 μL .
6. Membrane construction according to any one of claims 1 to 5, wherein $h.P.A/100$ calculated with a thickness h of about 250 μm and a porosity P of at least 80%.
7. Membrane construction according to any one of claims 1 to 6, wherein the 30 support layer is a non-woven layer based on micro-fibers with a number average diameter between 1 to 10 μm .
8. Membrane construction according to any one of claims 1 to 7, wherein the support layer comprises micro-glass.
9. Membrane construction according to any one of claims 1 to 8, wherein the 35 support layer is hydrophilic.

10. Membrane cassette for the separation of blood cells from blood plasma comprising the membrane construction of claim 1-9.11.
11. Device for the separation of blood cells from blood plasma, comprising the membrane construction of claim 1-9, or the membrane cassette of claim 10.
- 5 12. Method for the filtration of blood comprising introducing blood through the membrane construction according to any one of claims 1 to 9, or the membrane cassette according to claim 10, thereby separating blood cells from blood plasma.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/077030

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01D69/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01D A61M D04H D01F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/151314 A1 (DSM IP ASSETS BV [NL]; DULLAERT KONRAAD [BE]; DORSCHU MARKO [NL]; QIU) 8 December 2011 (2011-12-08) cited in the application page 12, line 18; claims 1,6, 11-13 page 11, lines 5-9; example 3 example B the whole document -----	1-12
X	US 6 048 464 A (TANAKA JUN [JP] ET AL) 11 April 2000 (2000-04-11) claim 1; example 1 the whole document -----	1-12
X	JP 2004 215873 A (KAWASUMI LAB INC) 5 August 2004 (2004-08-05) JPO machine translation; paragraph [0001]; table 2 the whole document -----	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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