Med J. 26: 250-8. Wisher M. (2002) Biosafety and product release testing issues relevant to replication-competent oncolytic viruses, Review. Cancer Gene Ther. 9: 1056-61).

[015] A membrane pore size rating of 0.1 µm indicates that a membrane has been validated to remove *Mycoplasma*. (See, Roche, K.L.: Levy, R.V., Methods to Validate Microporous Membranes for the Removal of *Mycoplasma*, BioPharm 1992, 5, (3), 22-33)

[016] For example, membranes having a pore size rating of 0.1 um can be used to filter media, nutrient and cell culture fluid delivered to cells living and growing inside of a bioreactor. Membranes currently exist that have a specific Log Reduction Value (LRV) for *A. Laidlawii*, a test microorganism for *Mycoplasma*. While it is customarily accepted that LRV >8 is sufficient to claim "full" retention of *Mycoplasma*, filters having a lower LRV are often used instead in liquid filtration because of greater permeability and higher throughput.

[017] WO/2009/032040, assigned to Millipore Corporation and titled, SERUM-FREE GROWTH MEDIUM FOR *ACHOLEPLASMA LAIDLAWII* AND METHODS FOR RETENTION TESTING STERILIZING GRADE FILTERS, fully incorporated by reference herein in its entirety, teaches the full retention of *Mycoplasma* by a filtration medium can be validated to produce sterile effluent after being challenged with >1 10^9 x cfu/mL *Acholeplasma laidlawii* (*A. laidlawii*; ATCC 23206).

[018] For example, two membranes having a pore size rating of 0.1 µm, Durapore® W and Express SHR, each available from Millipore Corporation, Billerica, MA, USA, have *Mycoplasma* LRVs of 4 and 6, respectively. While Durapore® MV, also available from Millipore Corporation, claims full *Mycoplasma* retention (LRV >8), it has a lower permeability and capacity in media filtration compared to Durapore® VV and Express SHR.

[019] Synthetic polymers have been formed into webs of very small diameter fibers, i.e., on the order of a few micrometers or less than 1 µm, using various processes including melt blowing, electrostatic spinning and electroblowing. Such webs have been shown to be useful as liquid barrier materials and filters. Often they are combined with stronger sheets to form composites, wherein the stronger sheets provide the strength to meet the needs of the final filter product.

adjacent to each other. Layering membrane filters to improve particle retention is commonly used in virus filtration and is practiced commercially in Millipore's product lines of Viresolve® NFP and Viresolve Pro®. Layering filtration media of the same or different composition is also used to improve filter throughput. Examples of such layered filters are Millipore's Express® SHC and SHRP product lines. Other considerations for choosing a multi-layered filtration product include economics and convenience of media and device manufacturing, ease of sterilization and validation. The fibrous filtration media of the present invention can be used in single-layer or in a multi-layer configuration.

[081] Test Methods

[082] Basis Weight was determined by ASTM D-3776, which is hereby incorporated by reference and reported in g/m^2.

[083] Porosity was calculated by dividing the basis weight of the sample in g/m^2 by the polymer density in g/cm^3, by the sample thickness in micrometers, multiplying by 100, and subtracting the resulting number from 100, i.e., porosity=100-[basis weight/(density×~~.times.~~thickness)×~~.times.~~100].

[084] Fiber Diameter was determined as follows. Ten scanning electron microscope (SEM) images at 40,000.times. Magnification was taken of each nanofiber layer sample. The diameter of ten (10) clearly distinguishable nanofibers were measured from each SEM image and recorded. Defects were not included (i.e., lumps of nanofibers, polymer drops, intersections of nanofibers). The average fiber diameter for each sample was calculated.

[085] Thickness was determined by ASTM D1777-64, which is hereby incorporated by reference, and is reported in micrometers.

[086] Mean flow bubble point was measured according to ASTM Designation 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 custom-built capillary flow porosimeter, in principle similar to a commercial apparatus from Porous Materials, Inc. (PMI), Ithaca, N.Y. Individual samples of 47 mm in diameter (9.6 cm^2 measurable area) were wetted with isopropyl alcohol. Each sample was placed in a holder, and a differential pressure of air was applied and the fluid removed from the sample. The differential

[095] The following Examples of the present invention will demonstrate that an electrospun nanofiber mat can possess both high permeability and high bacteria retention at the same time.

[096] Hereinafter the present invention will be described in more detail in the following examples. The invention will be further clarified by the following examples which are intended to be exemplary of the invention.

[097] **EXAMPLES**

[098] Example 1

[099] An electrospinning process and apparatus for forming a nanofiber web as disclosed in WO 2006/131081 was used to produce the nanofiber layers and mats of the Examples below.

[0100] Nanofiber layers were made by electrospinning a solution of Nylon 6 polymer. Nylon 6 was supplied by BASF Corp., Florham Park, NJ, USA, under the trademark Ultramid B24. A solvent mixture of acetic and formic acid, weight ratio 2:1, was used to prepare solutions of Nylon, with concentrations ranging from 8 to 16%.

[0101] A 10 wt.% solution of Nylon was electrospun at 82 kV and distance between solution and ground electrode 155 mm, for 45 minutes. By way of example only, samples were tested for *A. ~~Laidiawii~~ Laidlawii* retention using standard Millipore procedures described above. A representative sample is compared to the closest Durapore® membrane, MVPP, in Table I below.

[0119] The disclosure set forth above may encompass multiple distinct inventions with independent utility. Although each of these inventions has been disclosed in its preferred form(s), the specific embodiments thereof as disclosed and illustrated herein are not to be considered in a limiting sense, because numerous variations are possible. The subject matter of the inventions includes all novel and nonobvious combinations and subcombinations of the various elements, features, functions, and/or properties disclosed herein. The following claims particularly point out certain combinations and subcombinations regarded as novel and nonobvious. Inventions embodied in other combinations and subcombinations of features, functions, elements, and/or properties may be claimed in applications claiming priority from this or a related application. Such claims, whether directed to a different invention or to the same invention, and whether broader, narrower, equal, or different in scope to the original claims, also are regarded as included within the subject matter of the inventions of the present disclosure.

[0120] Notes

1. A method of removing microorganisms from a liquid sample comprising the steps of:

a) providing a liquid sample containing microorganisms,

b) providing a porous nanofiber containing filtration medium having a microorganism Log Reduction Value (LRV) greater than about 8, a porosity from about 80% to about 95%, and a liquid permeability greater than about 300 LMH/psi, wherein the nanofiber has a fiber diameter from about 10 nm to about 1 ,000 nm, and

c) passing the liquid sample containing microorganisms through the filtration medium resulting in the full retention of microorganisms.

2. The method of Note 1, wherein the filtration medium has a mean flow bubble point, as tested with isopropanol, in the range from 1 to 100 psi.

3. The method of Note 1, wherein the filtration medium has a thickness ranging from about 1 µm to about 500 µm.

4. The method of Note 1, wherein the nanofiber containing filtration medium is formed by a process selected from the group consisting of electrospinning and electroblowing.

5. The method of Note 1, wherein the microorganism is bacteria.

6. The method of Note 1, wherein the microorganism is Mycoplasma.

7. The method of Note 1, wherein the nanofiber comprises a polymer selected from the group consisting of polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyetherimide, polyacrylonitrile, poly(ethylene terephthalate), polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, polyvinyl chloride), polyvinyl alcohol), poly(vinylidene fluoride), polyvinyl butylene) and copolymers, derivative compounds, or blends thereof.

8. The method of Note 1, wherein the nanofiber comprises an aliphatic polyamide.

9. The method of Note 1, wherein the nanofiber comprises a blend of polymers or copolymers.

10. The method of Note 1, wherein the nanofiber is disposed on a porous support.

11. The method of Note 1, wherein the porous support comprises one or more layers selected from the group consisting of spunbonded nonwovens, meltblown nonwovens, needle punched nonwovens, spunlaced nonwovens, wet laid nonwovens, resin-bonded nonwovens, woven fabrics, knit fabrics, paper, and combinations thereof.

12. The method of Note 6, wherein the full retention of Mycoplasma by the filtration medium occurs when the filtration medium can be validated to produce sterile effluent after being challenged with $\geqslant$1 109 x cfu/mL Acholeplasma laidlawii.

13. A porous nanofiber filtration medium having a microorganism LRV greater than about 8 for use in fully retaining microorganisms removed from a liquid sample passing through the filtration medium, the filtration medium comprising:

a nanofiber containing substrate wherein the nanofiber has a fiber diameter from about 10 nm to about 1 ,000 and the substrate has a,

i) a porosity ranging from about 80% to about 95%, and

ii) a liquid permeability greater than about 300 LMH/psi.

14. The filtration medium of Note 13, having a thickness ranging from about 1 μm

to about 500 μm.

15. The filtration medium of Note 13, further comprising: iii) a mean flow bubble point, as tested with isopropanol, in the range from 1 to 100 psi.

16. The filtration medium of Note 13, wherein the nanofiber comprises a polymer selected from the group consisting of polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyethehmide, polyacrylonitrile, poly(ethylene terephthalate), polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, polyvinyl chloride), polyvinyl alcohol), poly(vinylidene fluoride), polyvinyl butylene) and copolymers, derivative compounds, and blends thereof.

17. The filtration medium of Note 13, wherein the nanofiber comprises an aliphatic polyamide.

18. The filtration medium of Note 14, wherein the nanofiber containing substrate is formed by a process selected from the group consisting of electrospinning and electroblowing.

19. The filtration medium of Note 14, wherein the nanofiber is disposed on a porous support.

20. The filtration medium of Note 19, wherein the porous support comprises one or more layers selected from the group consisting of spunbonded nonwovens, meltblown nonwovens, needle punched nonwovens, spunlaced nonwovens, wet laid nonwovens, resin-bonded nonwovens, woven fabrics, knit fabrics, paper, and combinations thereof.

21. The filtration medium of Note 14, wherein the microorganism is bacteria.

22. The filtration medium of Note 14, wherein the microorganism is Mycoplasma.

23. The filtration medium of Note 22, wherein the full retention of Mycoplasma by the filtration medium occurs when the filtration medium can be validated to produce sterile effluent after being challenged with $\geqslant$1 109 x cfu/mL Acholeplasma laidlawii.

24. A filtration device having a microorganism LRV greater than about 8 for use in fully retaining microorganisms removed from a liquid sample passing through the device, the filtration device comprising:

a nanofiber containing filtration medium having a microorganism Log Reduction Value (LRV) greater than about 8, wherein the nanofiber has a fiber diameter from about 10 nm to about 1 ,000 nm, and the filtration medium has,

i) a porosity ranging from about 80% to about 95%, and

ii) a liquid permeability greater than about 300 LMH/psi.

25. The device of Note 24, wherein the filtration medium has a thickness ranging from about 1 µm to about 500 µm.

26. The device of Note 24, wherein the filtration medium further comprises: iii) a mean flow bubble point, as tested with isopropanol, in the range from 1 to 100 psi.

27. The device of Note 24, wherein the filtration medium is formed by a process selected from the group consisting of electrospinning and electroblowing.

28. The device of Note 24, wherein the filtration medium is an electrospun polymeric fibrous mat.

29. The device of Note 24, wherein the filtration medium is disposed on a porous support.

30. The device of Note 24, wherein the microorganism is Mycoplasma.

31. The device of Note 24, wherein the full retention of Mycoplasma by the filtration medium occurs when the filtration medium can be validated to produce sterile effluent after being challenged with ⩾1 109 x cfu/mL Acholeplasma laidlawii.

32. A method of removing microorganisms from a liquid sample comprising the steps of:

a) providing a liquid sample containing microorganisms,

b) providing a porous nanofiber containing filtration medium having a microorganism Log Reduction Value (LRV) greater than about 9, a porosity from about 80% to about 95%, and a liquid permeability greater than about 1 ,000 LMH/psi, wherein the nanofiber has a fiber diameter from about 10 nm to about 1 ,000 nm, and

c) passing the liquid sample containing microorganisms through the filtration medium resulting in the full retention of microorganisms.

33. The method of Note 32, wherein the filtration medium has a mean flow bubble point, as tested with isopropanol, in the range from 1 to 100 psi.

34. The method of Note 32, wherein the filtration medium has a thickness ranging from about 1 μm to about 500 μm.

35. The method of Note 32, wherein the filtration medium is an electrospun polymeric fibrous mat.

36. The method of Note 32, wherein the microorganism is bacteria.

37. The method of Note 36, wherein the bacteria is Brevundimonas diminuta.

38. The method of Note 32, wherein the nanofiber comprises a polymer selected from the group consisting of polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyetherimide, polyacrylonitrile, poly(ethylene terephthalate), polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, polyvinyl chloride), polyvinyl alcohol), poly(vinylidene fluoride), polyvinyl butylene) and copolymers, derivative compounds, and blends thereof.

39. The method of Note 32, wherein the nanofiber comprises an aliphatic polyamide.

40. The method of Note 32, wherein the filtration medium is disposed on a porous support.

41. The method of Note 40, wherein the porous support comprises one or more layers selected from the group consisting of spunbonded nonwovens, meltblown nonwovens, needle punched nonwovens, spunlaced nonwovens, wet laid nonwovens, resin-bonded nonwovens, woven fabrics, knit fabrics, paper, and combinations thereof.

42. The method of Note 32, wherein full retention of Brevundimonas diminuta by the filtration medium occurs when the filtration medium meets the requirements of the Standard Test Method for Determining Bacterial Retention Of Membrane Filters Utilized For Liquid Filtration (ASTM F838-83 test), and can be validated to produce sterile effluent after being challenged with $\geqslant$107 CFU/cm2 Brevundimonas diminuta.

43. A liquid filtration nanofiber containing medium having a microorganism LRV greater than about 9 for use in fully retaining microorganisms removed from a liquid sample passing through the filtration medium, the filtration medium comprising:

a nanofiber containing mat wherein the nanofiber has a fiber diameter from about 10 nm to about 1 ,000 nm, and the mat having a,

i) a porosity ranging from about 80% to about 95%, and

ii) a liquid permeability greater than about 1 ,000 LMH/psi.

44. The filtration medium of Note 43, has a thickness ranging from about 1 μm to about 500 μm.

45. The filtration medium of Note 43, further comprising: iii) a mean flow bubble point, as tested with isopropanol, in the range from 1 to 100 psi.

46. The filtration medium of Note 43, wherein the nanofiber comprises a polymer selected from the group consisting of polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyetherimide, polyacrylonitrile, poly(ethylene terephthalate), polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, polyvinyl chloride), polyvinyl alcohol), poly(vinylidene fluoride), polyvinyl butylene) and copolymers, derivative compounds, and blends thereof.

47. The filtration medium of Note 43, wherein the nanofiber comprises an aliphatic polyamide.

48. The filtration medium of Note 43, wherein the mat is an electrospun polymeric porous mat.

49. The filtration medium of Note 43, wherein the nanofiber is disposed on a porous support.

50. The filtration medium of Note 43, wherein the porous support comprises one or more layers selected from the group consisting of spunbonded nonwovens, meltblown nonwovens, needle punched nonwovens, spunlaced nonwovens, wet laid nonwovens, resin-bonded nonwovens, woven fabrics, knit fabrics, paper, and combinations thereof.

51. The filtration medium of Note 43, wherein the microorganism is bacteria.

52. The filtration medium of Note 51, wherein the bacteria is Brevundimonas diminuta.

53. The filtration medium of Note 52, wherein full retention of Brevundimonas diminuta by the filtration medium occurs when the filtration medium meets the

requirements of the Standard Test Method for Determining Bacterial Retention Of Membrane Filters Utilized For Liquid Filtration (ASTM F838-83 test), and can be validated to produce sterile effluent after being challenged with ≥107 CFU/cm2 Brevundimonas diminuta.

54. A liquid filtration device having a microorganism LRV greater than about 9 for use in fully retaining microorganisms removed from a liquid sample passing through the device, the filtration device comprising:

a nanofiber containing filtration medium having a microorganism Log Reduction Value (LRV) greater than about 9, wherein the nanofiber has a fiber diameter from about 10 nm to about 1 ,000 nm, and the filtration medium has,

i) a porosity from about 80% to about 95%, and

ii) a liquid permeability greater than about 1 ,000 LMH/psi.

55. The device of Note 54, wherein the filtration medium has a thickness ranging from about 1 μm to about 500 μm.

56. The device of Note 54, wherein the filtration medium further comprises: iii) a mean flow bubble point, as tested with isopropanol, in the range from 1 to 100 psi.

57. The device of Note 54, wherein the filtration medium is an electrospun polymeric porous mat.

58. The device of Note 54, wherein the filtration medium is disposed on a porous support having one or more layers.

59. The device of Note 54, wherein the microorganism is Brevundimonas diminuta.

60. The device of Note 59, wherein full retention of Brevundimonas diminuta by the filtration medium occurs when the filtration medium meets the requirements of the Standard Test Method for Determining Bacterial Retention Of Membrane Filters Utilized For Liquid Filtration (ASTM F838-83 test), and can be validated to produce sterile effluent after being challenged with ≥107 CFU/cm2 Brevundimonas diminuta.

61. The device of Note 54, wherein the nanofiber comprises a polymer selected from the group consisting of polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyetherimide, polyacrylonitrile, poly(ethylene terephthalate),

polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, polyvinyl chloride), polyvinyl alcohol), poly(vinylidene fluoride), polyvinyl butylene), and copolymers, derivative compounds, and blends thereof.

62. The device of Note 54, wherein the nanofiber comprises an aliphatic polyamide.

63. The method of Note 32, using the filtration device of Note 54.

What is claimed is:

1. A porous nanofiber filtration medium having a microorganism LRV greater than 8 capable of fully retaining microorganisms removed from a liquid sample passing through the filtration medium by size-based separation, the filtration medium comprising:

a nanofiber containing layer wherein the nanofiber has a fiber diameter from 10 nm to 1,000 nm and the layer has:

i) a porosity ranging from 80% to 95%, and

ii) a liquid permeability greater than 300 LMH/psi, and

iii) a mean flow bubble point, as tested with isopropanol, in the range from 1 psi to 100 psi.

2. The filtration medium according to claim 1, having a thickness ranging from 1 μm to 500 μm.

3. The filtration medium according to claim 1, wherein the nanofiber comprises a polymer selected from the group consisting of polyimide, aliphatic polyamide, aromatic polyamide, polysulfone, cellulose acetate, polyether sulfone, polyurethane, poly(urea urethane), polybenzimidazole, polyethehmide, polyacrylonitrile, poly(ethylene terephthalate), polypropylene, polyaniline, poly(ethylene oxide), poly(ethylene naphthalate), poly(butylene terephthalate), styrene butadiene rubber, polystyrene, poly(vinyl chloride), poly(vinyl alcohol), poly(vinylidene fluoride), poly(vinyl butylene) and copolymers, derivative compounds, and blends thereof, and the nanofiber containing layer is formed by a process selected from the group consisting of electrospinning and

electroblowing.

4. The filtration medium according to claim 1, wherein the nanofiber containing layer is disposed on a porous support having one or more layers, and the porous support comprises one or more layers selected from the group consisting of spunbonded nonwovens, meltblown nonwovens, needle punched nonwovens, spunlaced nonwovens, wet laid nonwovens, resin-bonded nonwovens, woven fabrics, knit fabrics, paper, and combinations thereof.

5. The filtration medium according to claim~~s~~ 1, wherein the filtration medium is an electrospun polymeric fibrous mat.

6. The filtration medium according to claim 1, wherein the microorganism is bacteria.

7. The filtration medium according to claim 1, wherein the microorganism is Mycoplasma.

8. The filtration medium according to claim 7, wherein the full retention of Mycoplasma by the filtration medium occurs when the filtration medium can be validated to produce sterile effluent after being challenged with ~~>~~≥1 x 10^7 cfu/mL Acholeplasma laidlawii.

9. The filtration medium according to claim 1, ~~whereim~~ wherein the filtration medium has a microorganism LRV greater than 9.

10. A method of removing microorganisms from a liquid sample comprising the steps of:

a) providing a liquid sample containing microorganisms,

b) providing a porous nanofiber containing filtration medium according to claim 1, and

c) passing the liquid sample containing microorganisms through the filtration medium resulting in the full retention of microorganisms by size-based separation.

11. The method of claim 10 wherein the nanofiber containing filtration medium has a microorganism Log Reduction Value (LRV) greater than 9.

12. A method of removing bacteria from a liquid sample comprising the steps of:

a) providing a liquid sample containing bacteria,

b) providing a porous nanofiber containing filtration medium according to claim 11, and

c) passing the liquid sample containing bacteria through the filtration medium resulting in the full retention of microorganisms by size-based separation.

13. The method according to claim 12 wherein full retention of Brevundimonas diminuta by the filtration medium occurs when the filtration medium meets the requirements of the Standard Test Method for Determining

Bacterial Retention Of Membrane Filters Utilized For Liquid Filtration (ASTM F838-83 test), and can be validated to produce sterile effluent after being challenged with ~~>~~≧10^7 CFU/cm^2 Brevundimonas diminuta.

14. A filtration device having a microorganism LRV greater than 8 capable of fully retaining microorganisms removed from a liquid sample passing through the device, the filtration device comprising:

a nanofiber containing filtration medium according to claim 1,

i) a porosity ranging from 80% to 95%, and

ii) a liquid permeability greater than 300 LMH/psi.

15. The device according to claim 14, ~~whereim~~ wherein the nanofiber filtration medium has a microorganism LRV greater than 9.

16. The device according to claim 15, wherein the microorganism is Brevundimonas diminuta.

17. The device of claim 14, wherein the filtration medium is disposed on a porous support having one or more layers, and the porous support comprises one or more layers according selected from the group consisting of spunbonded nonwovens, meltblown nonwovens, needle punched nonwovens, spunlaced nonwovens, wet laid nonwovens, resin-bonded nonwovens, woven fabrics, knit fabrics, paper, and combinations thereof.