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(54) **SEEDING IMPLANTABLE MEDICAL DEVICES WITH CELLS**

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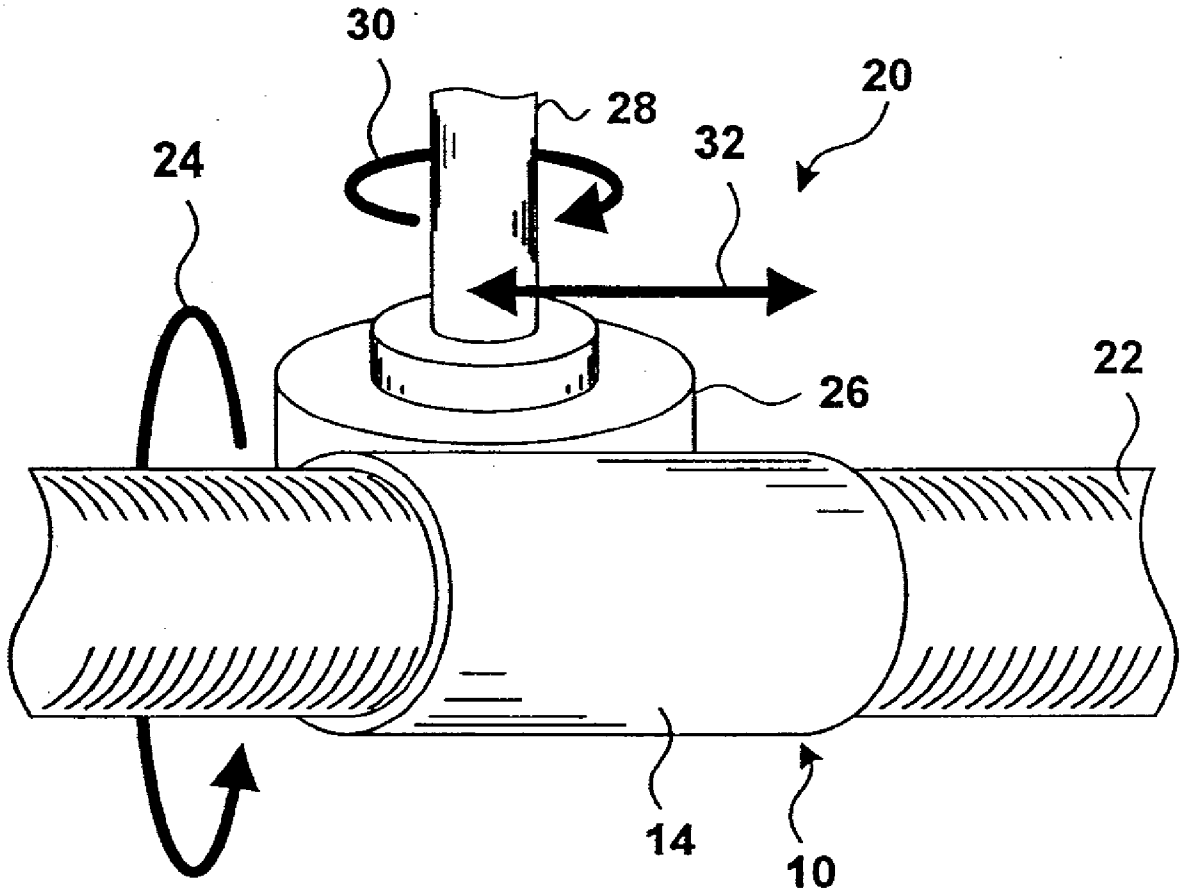
A61F 2/82	(2006.01)
A61F 2/24	(2006.01)

(52) **U.S. Cl.** **600/36**; 623/1.41; 623/2.42

(57) **ABSTRACT**

The invention is directed to apparatus and methods for seeding an implantable medical device, such as a vascular prosthesis, with cells, such as endothelial cells. The invention supports techniques for seeding a luminal surface of the device with axial centrifugation. Cells are introduced in suspension into the lumen of the device, and the device is subjected to centrifugation around a longitudinal axis defined by the lumen. Axial centrifugation causes the cells to concentrate toward the luminal surface. Shortly after axial centrifugation, the seeded device can be presented for implantation in a patient.



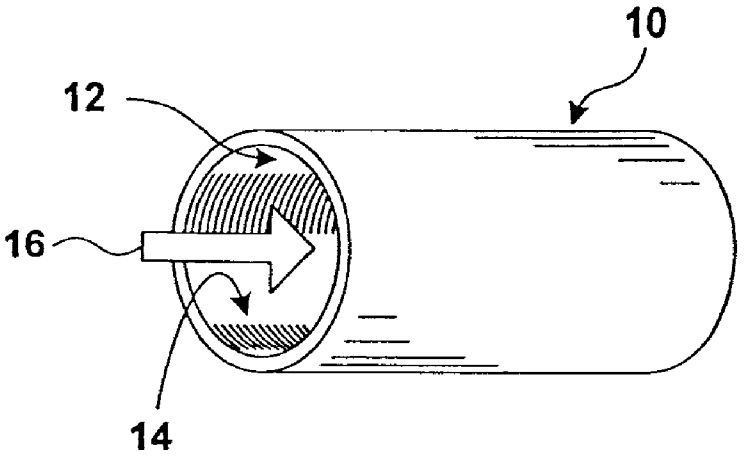


FIG. 1

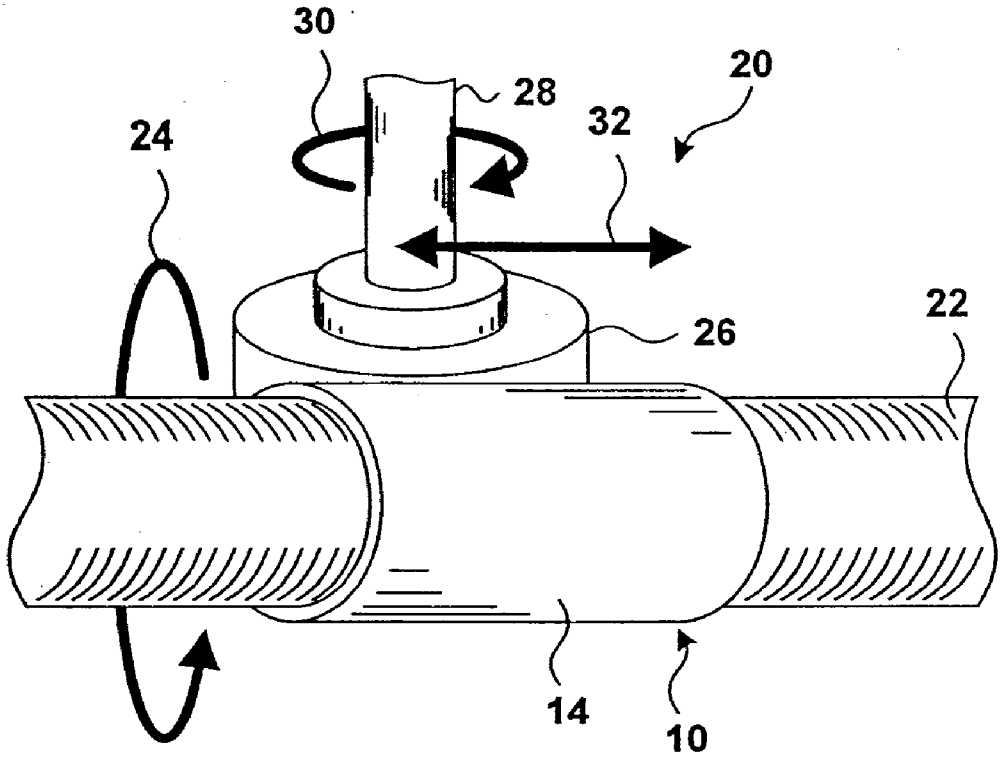


FIG. 2

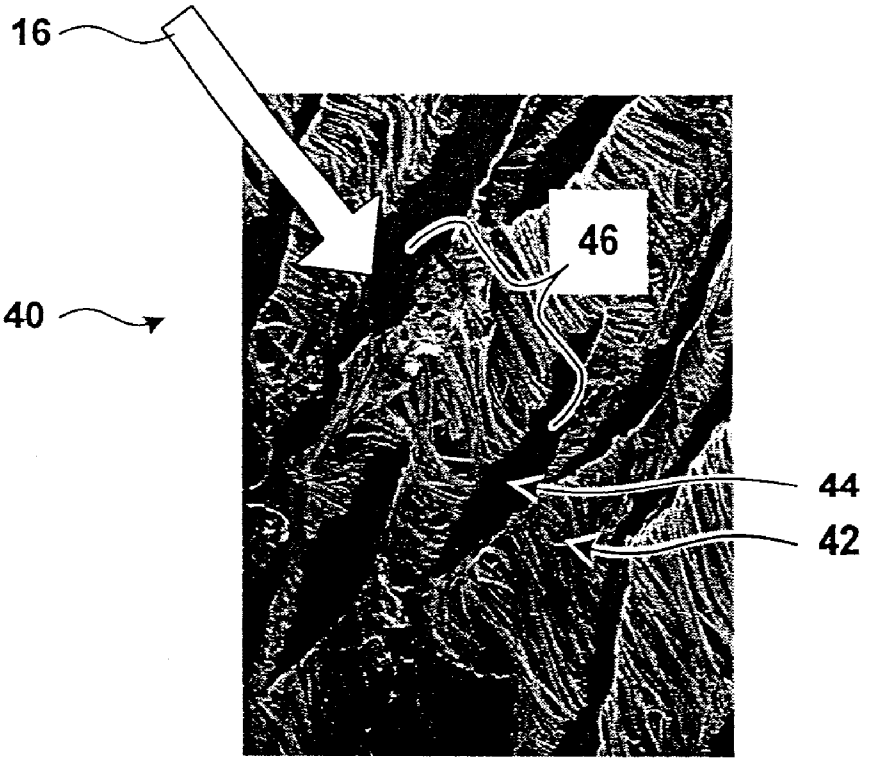


FIG. 3

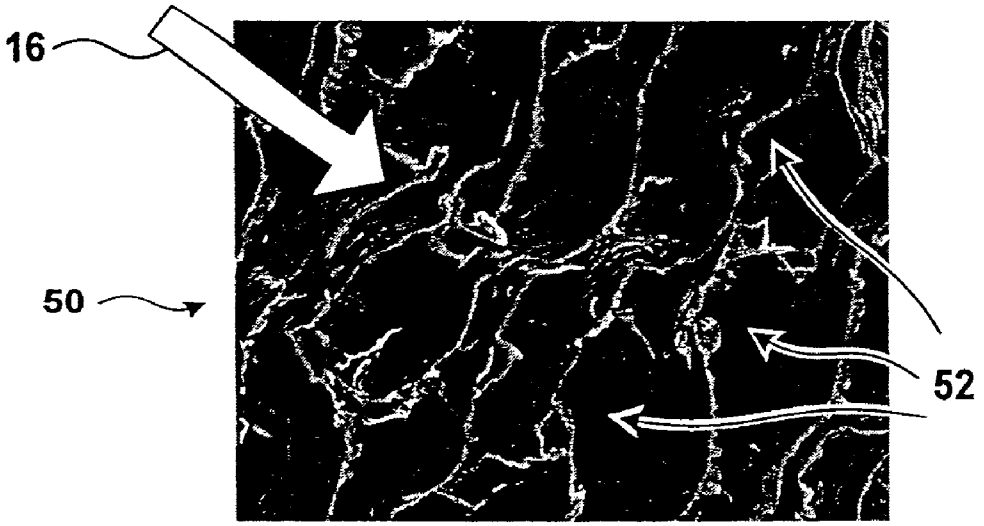


FIG. 4

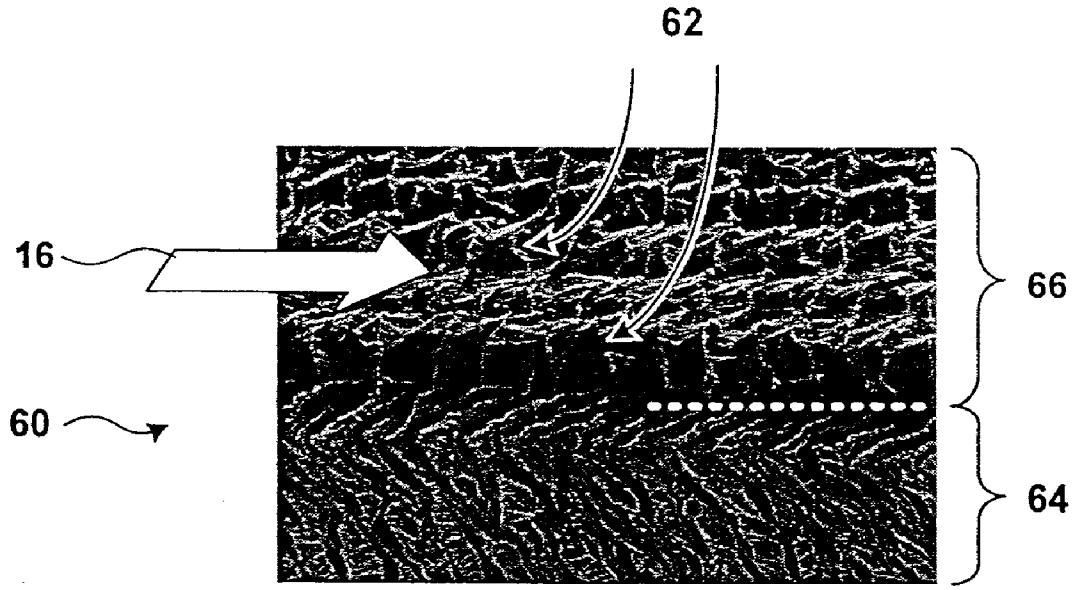


FIG. 5

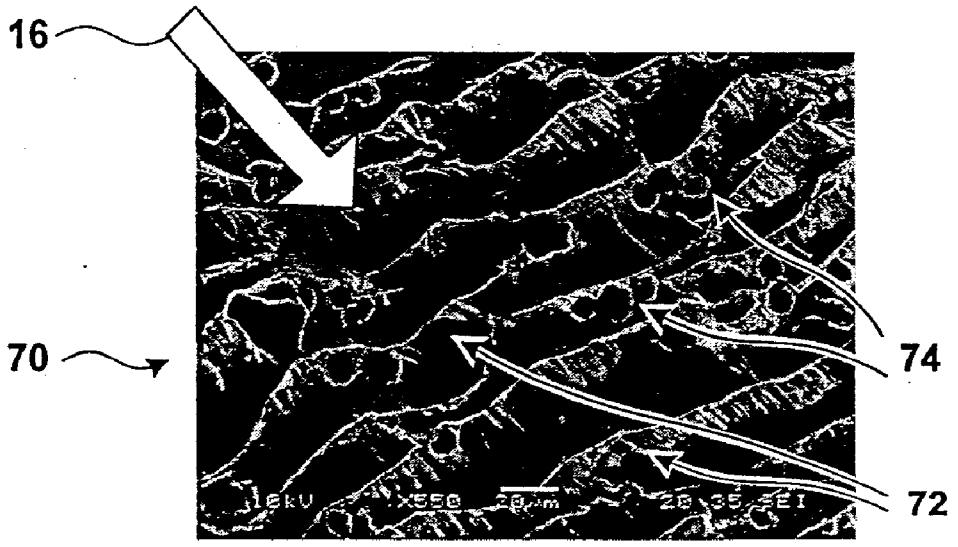


FIG. 6

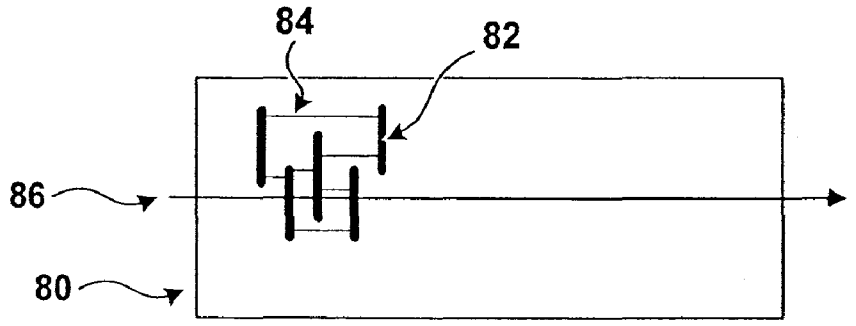


FIG. 7

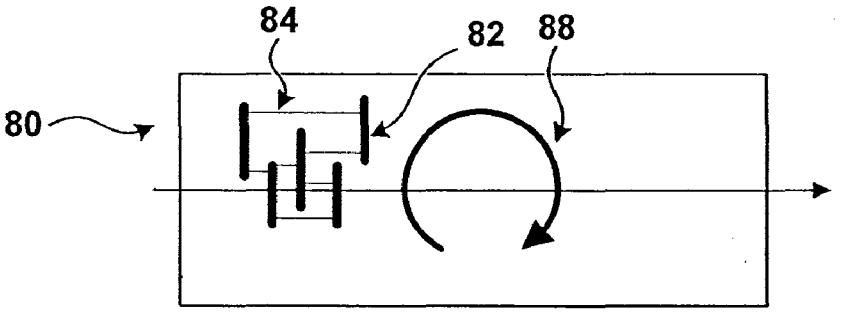


FIG. 8

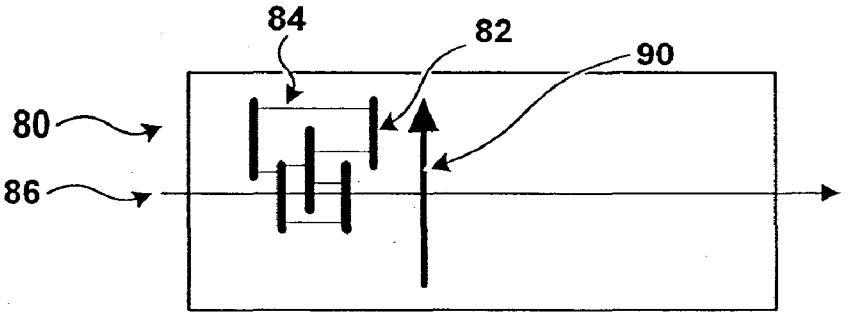


FIG. 9

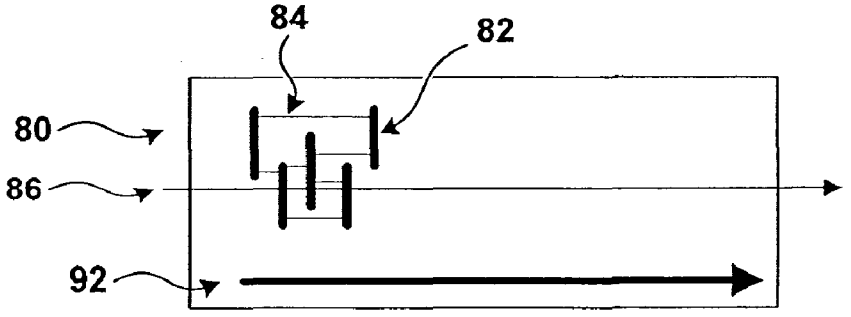


FIG. 10

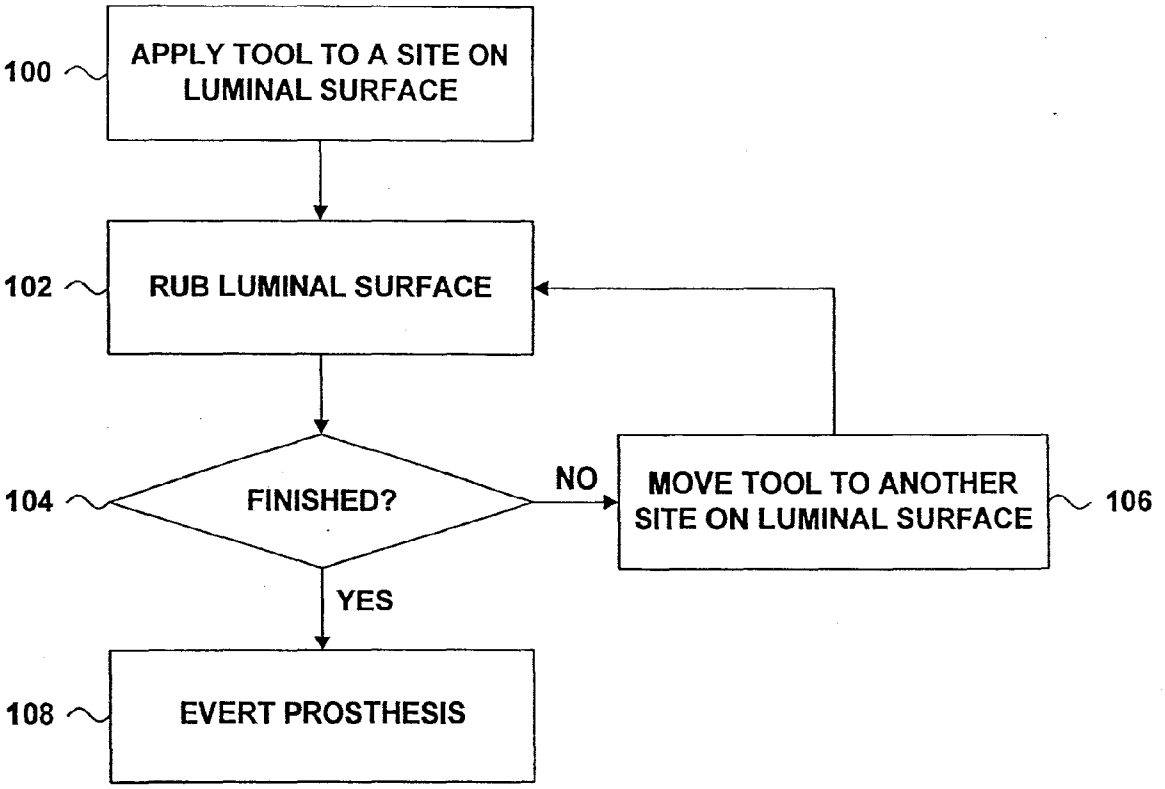

100
APPLY TOOL TO A SITE ON LUMINAL SURFACE
102
RUB LUMINAL SURFACE
104
FINISHED?
NO
MOVE TOOL TO ANOTHER SITE ON LUMINAL SURFACE
106
YES
108
EVERT PROSTHESIS

FIG. 11

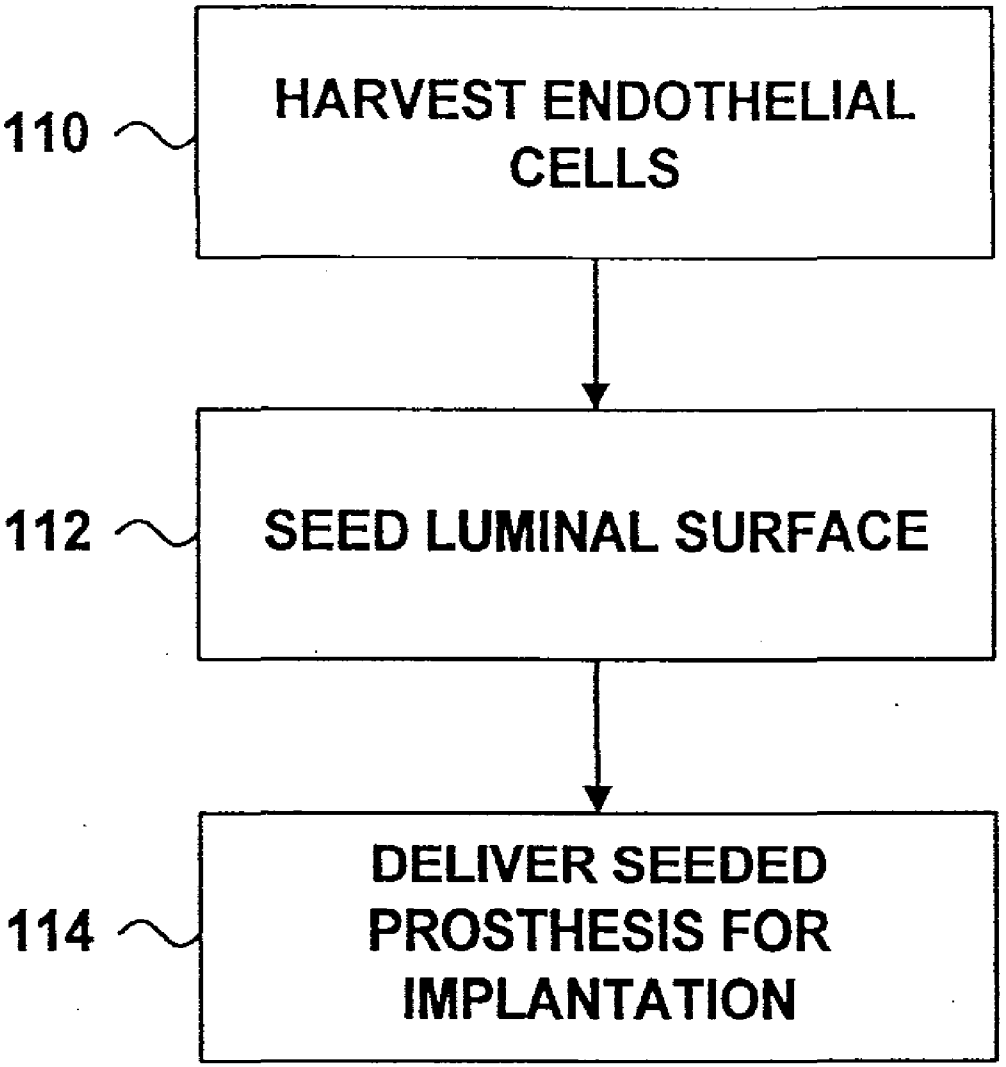

110
HARVEST ENDOTHELIAL CELLS
112
SEED LUMINAL SURFACE
114
DELIVER SEEDED PROSTHESIS FOR IMPLANTATION

FIG. 12

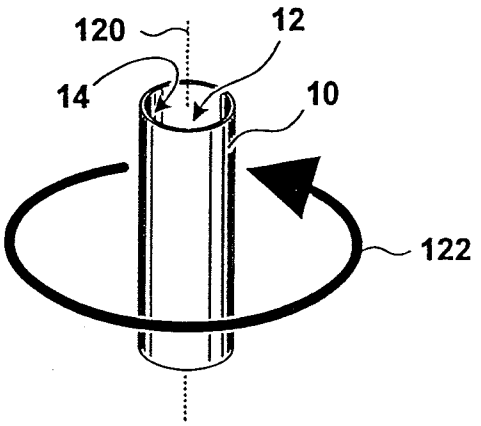


FIG. 13

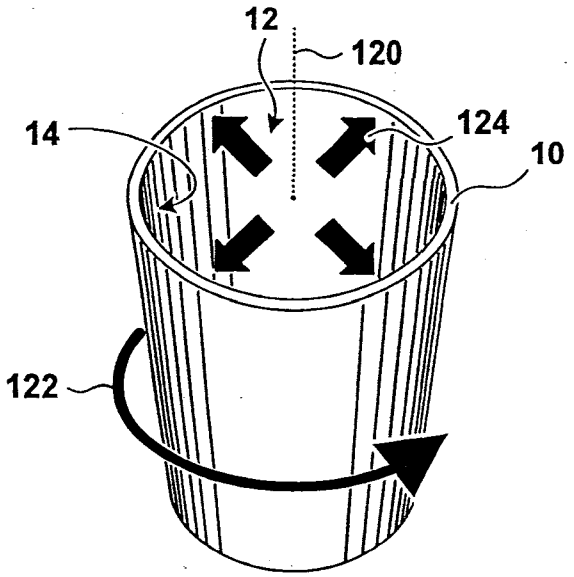


FIG. 14

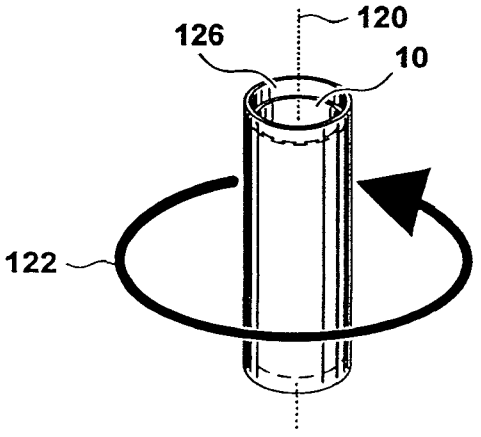


FIG. 15

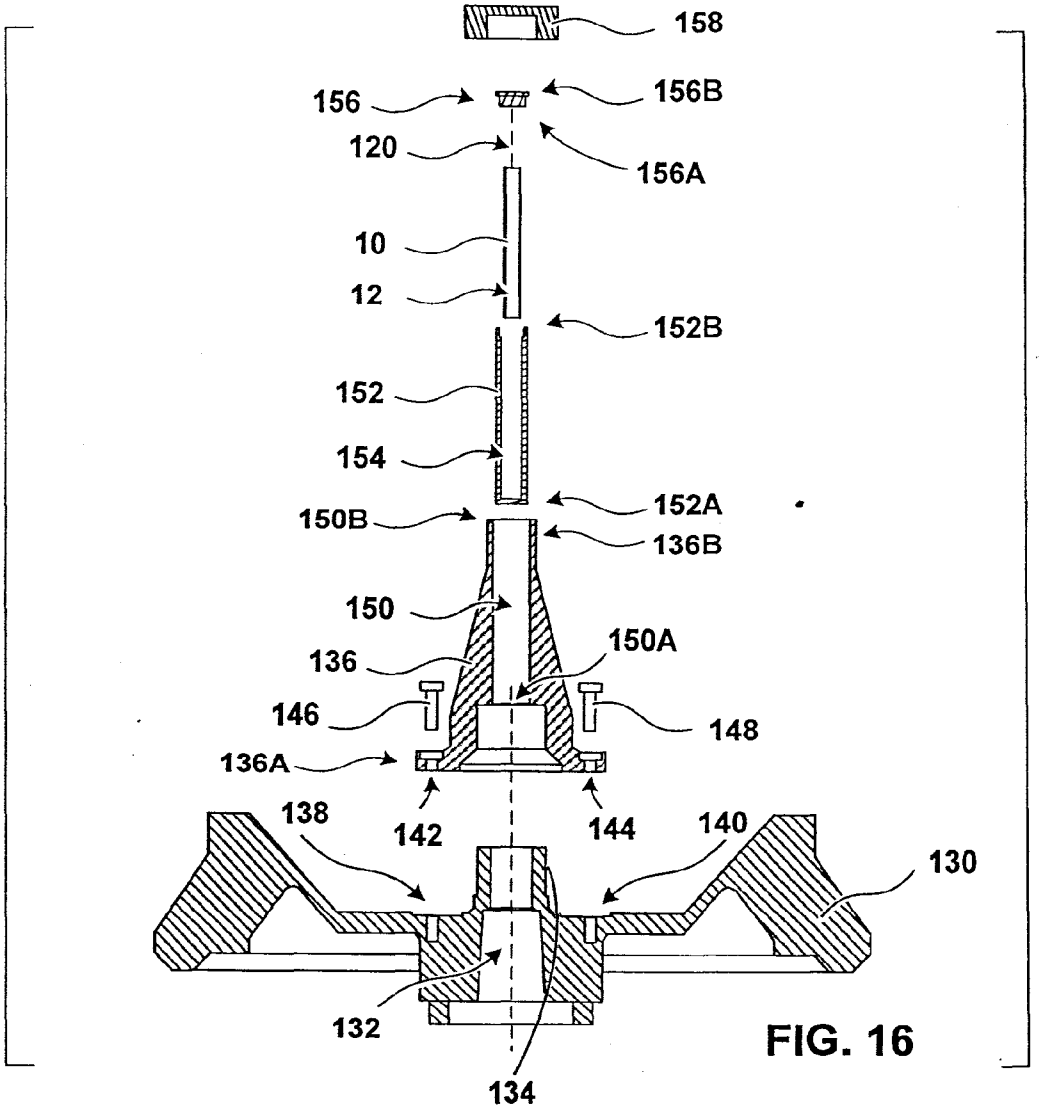

158
156
156B
120
156A
10
12
152B
152
154
152A
150B
136B
150
150A
136
146
148
136A
138
142
144
140
130
132
134

FIG. 16

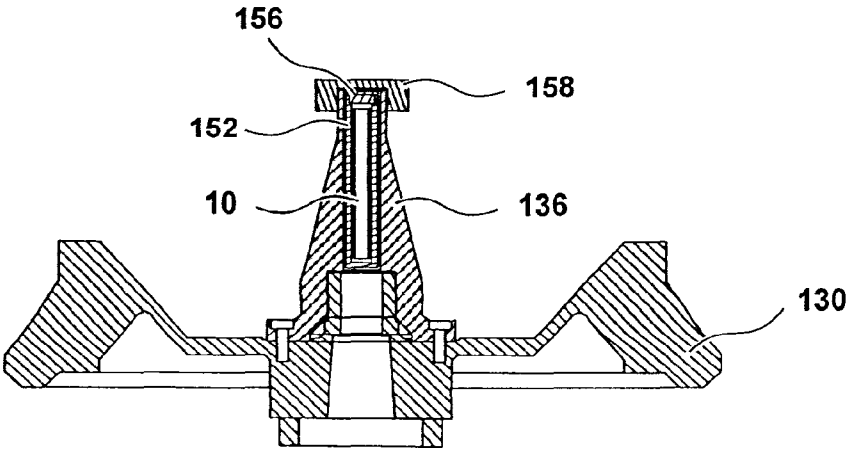

156
158
152
10
136
130

FIG. 17

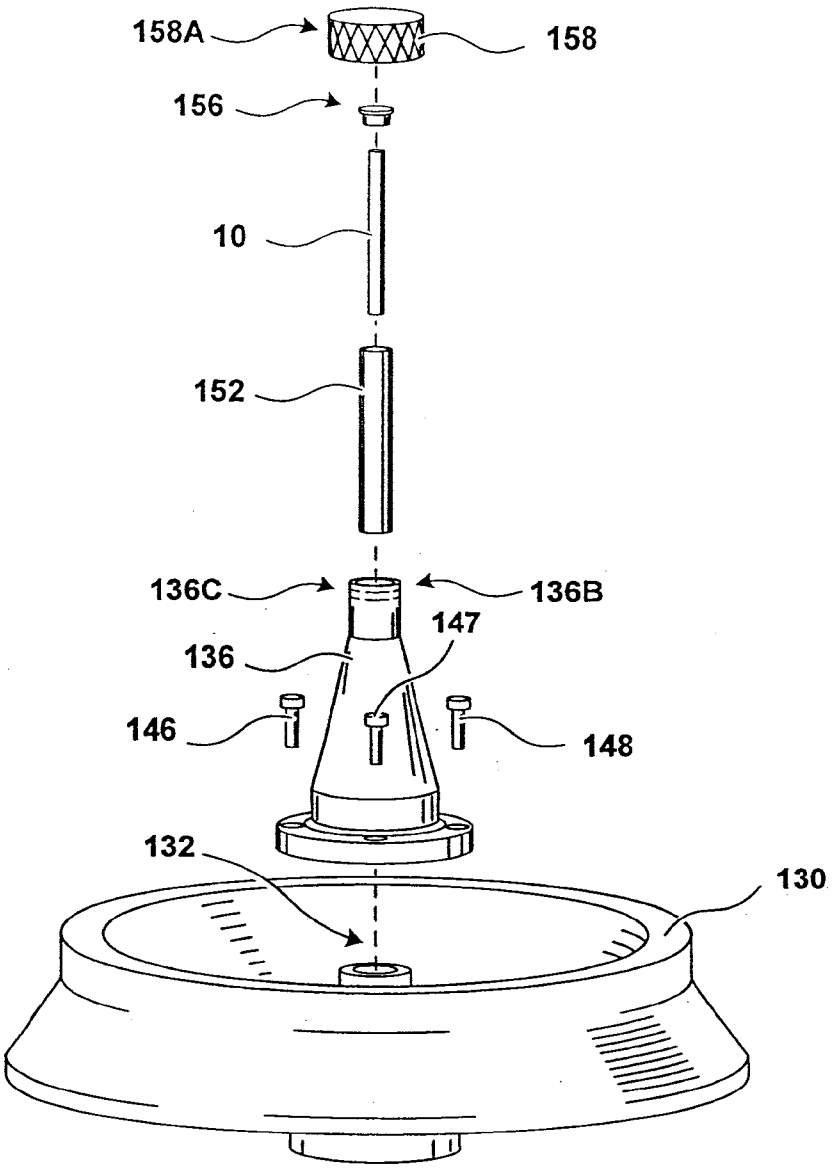

158A
158
156
10
152
136C
136B
147
136
146
148
132
130

FIG. 18

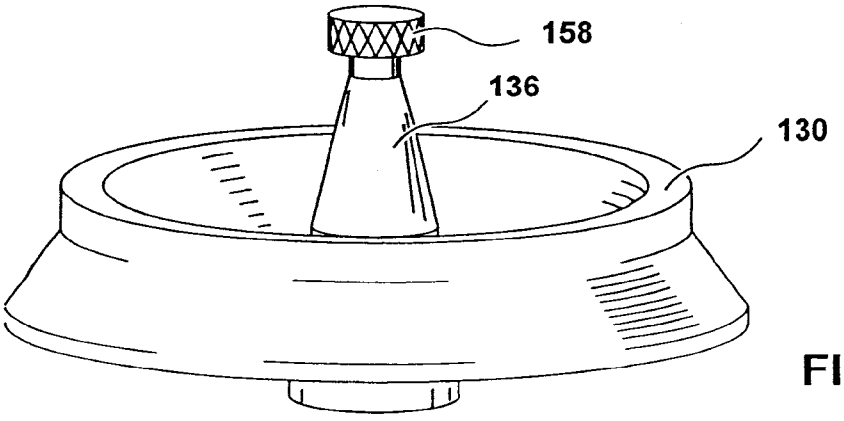

158
136
130

FIG. 19

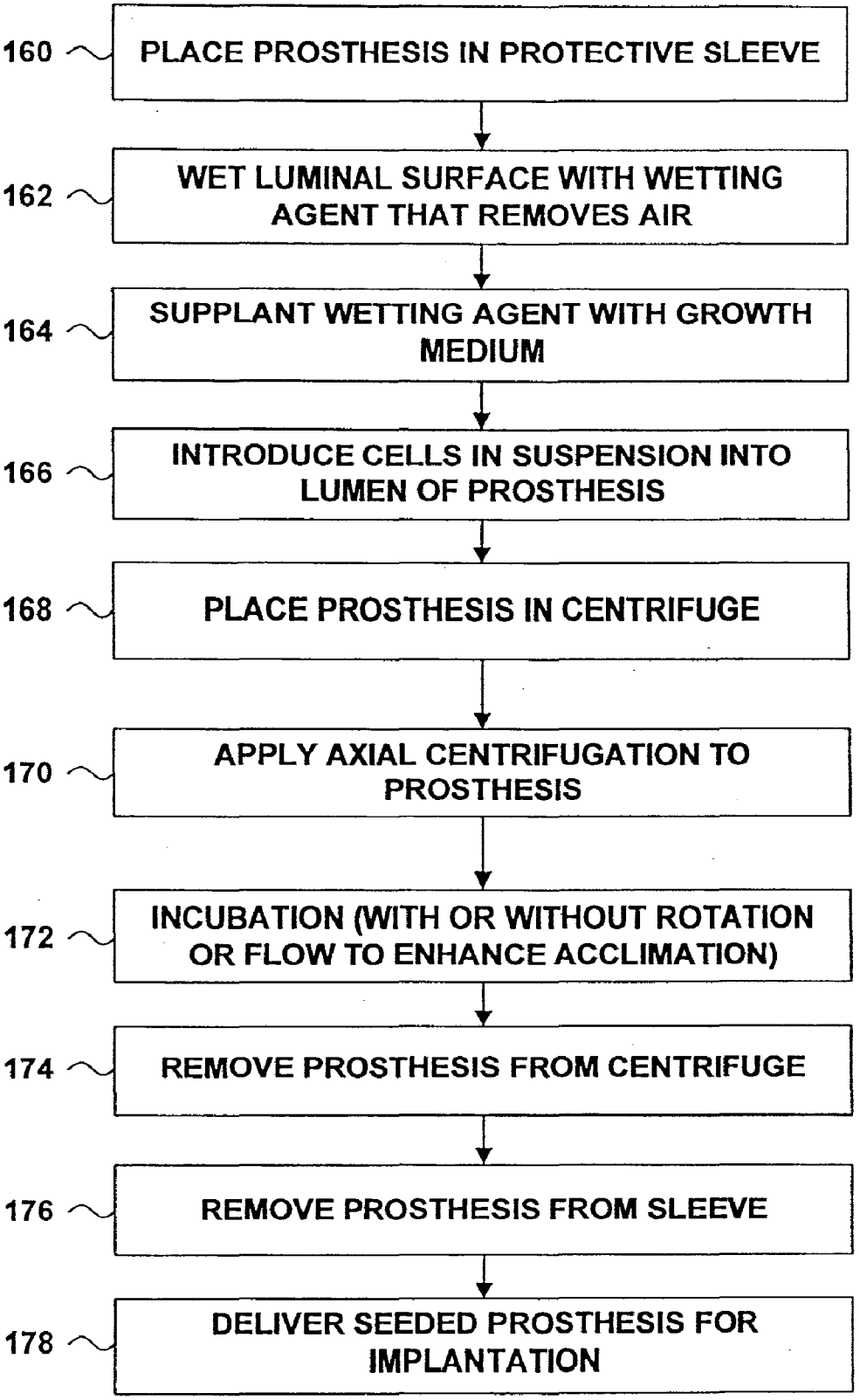

160
PLACE PROSTHESIS IN PROTECTIVE SLEEVE
162
WET LUMINAL SURFACE WITH WETTING AGENT THAT REMOVES AIR
164
SUPPLANT WETTING AGENT WITH GROWTH MEDIUM
166
INTRODUCE CELLS IN SUSPENSION INTO LUMEN OF PROSTHESIS
168
PLACE PROSTHESIS IN CENTRIFUGE
170
APPLY AXIAL CENTRIFUGATION TO PROSTHESIS
172
INCUBATION (WITH OR WITHOUT ROTATION OR FLOW TO ENHANCE ACCLIMATION)
174
REMOVE PROSTHESIS FROM CENTRIFUGE
176
REMOVE PROSTHESIS FROM SLEEVE
178
DELIVER SEEDED PROSTHESIS FOR IMPLANTATION

FIG. 20

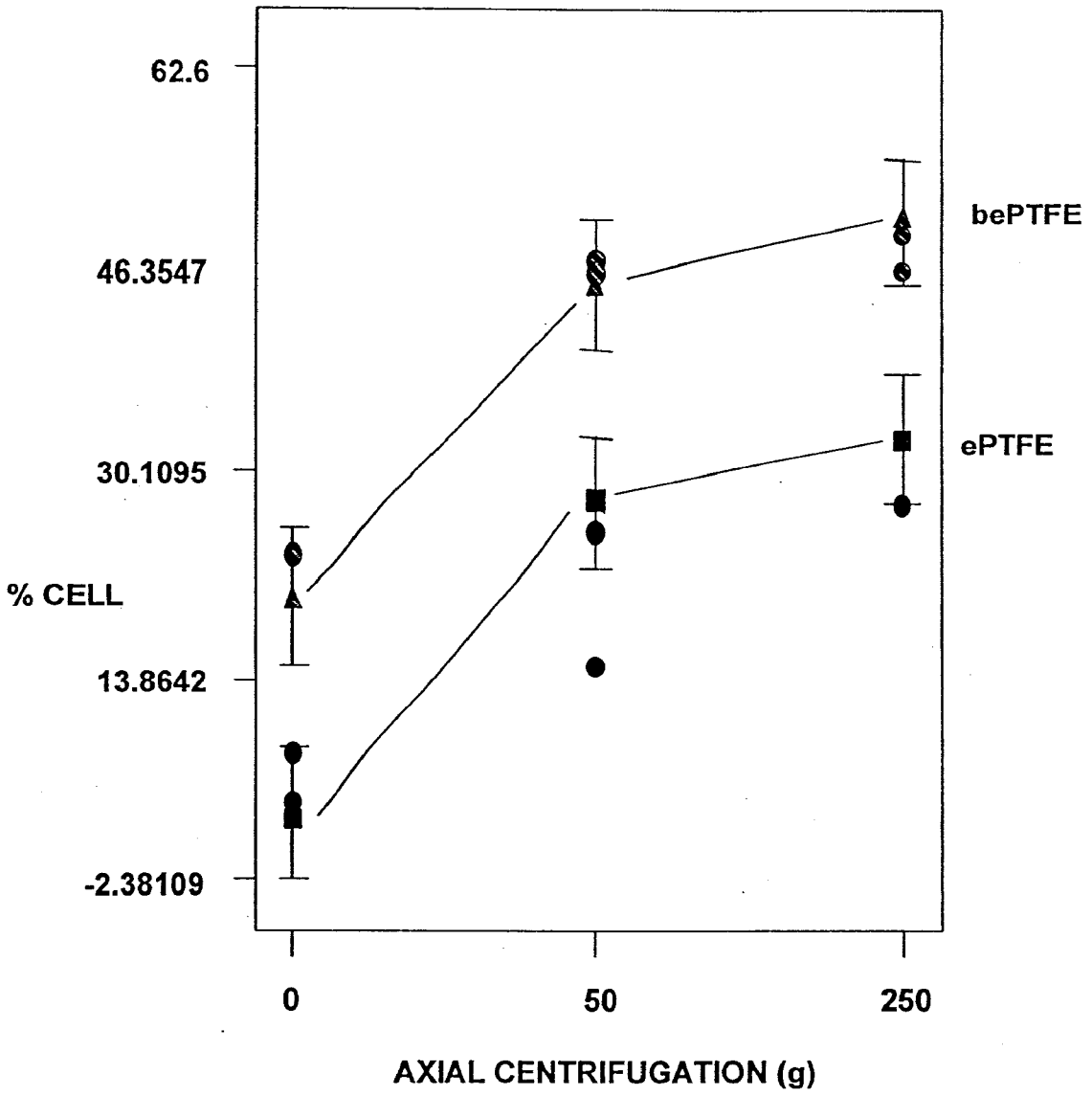

62.6
46.3547
30.1095
13.8642
-2.38109
% CELL
bePTFE
ePTFE
0
50
250
AXIAL CENTRIFUGATION (g)

FIG. 21

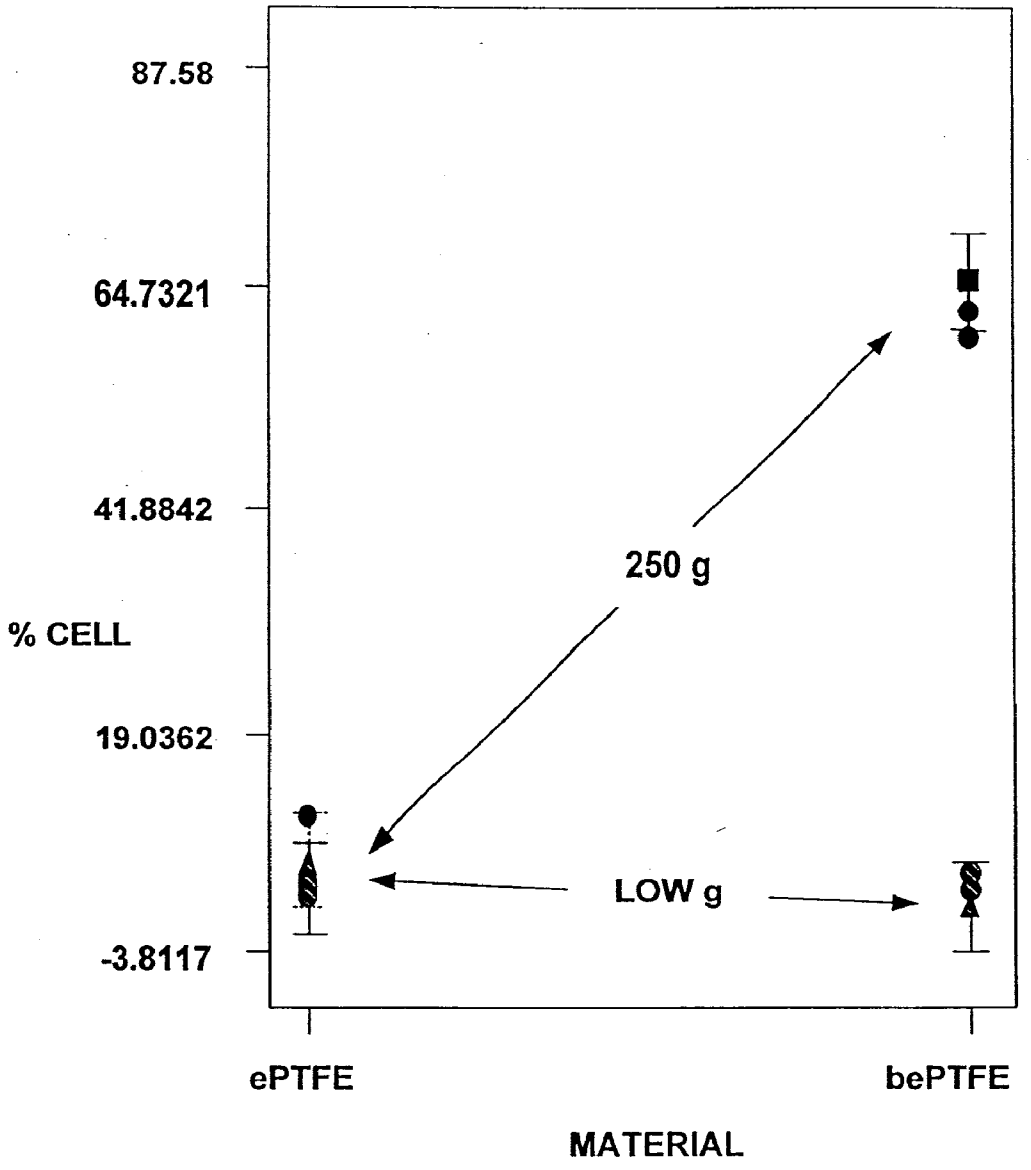

87.58
64.7321
41.8842
% CELL
19.0362
-3.8117
250 g
LOW g
ePTFE
bePTFE
MATERIAL

FIG. 22

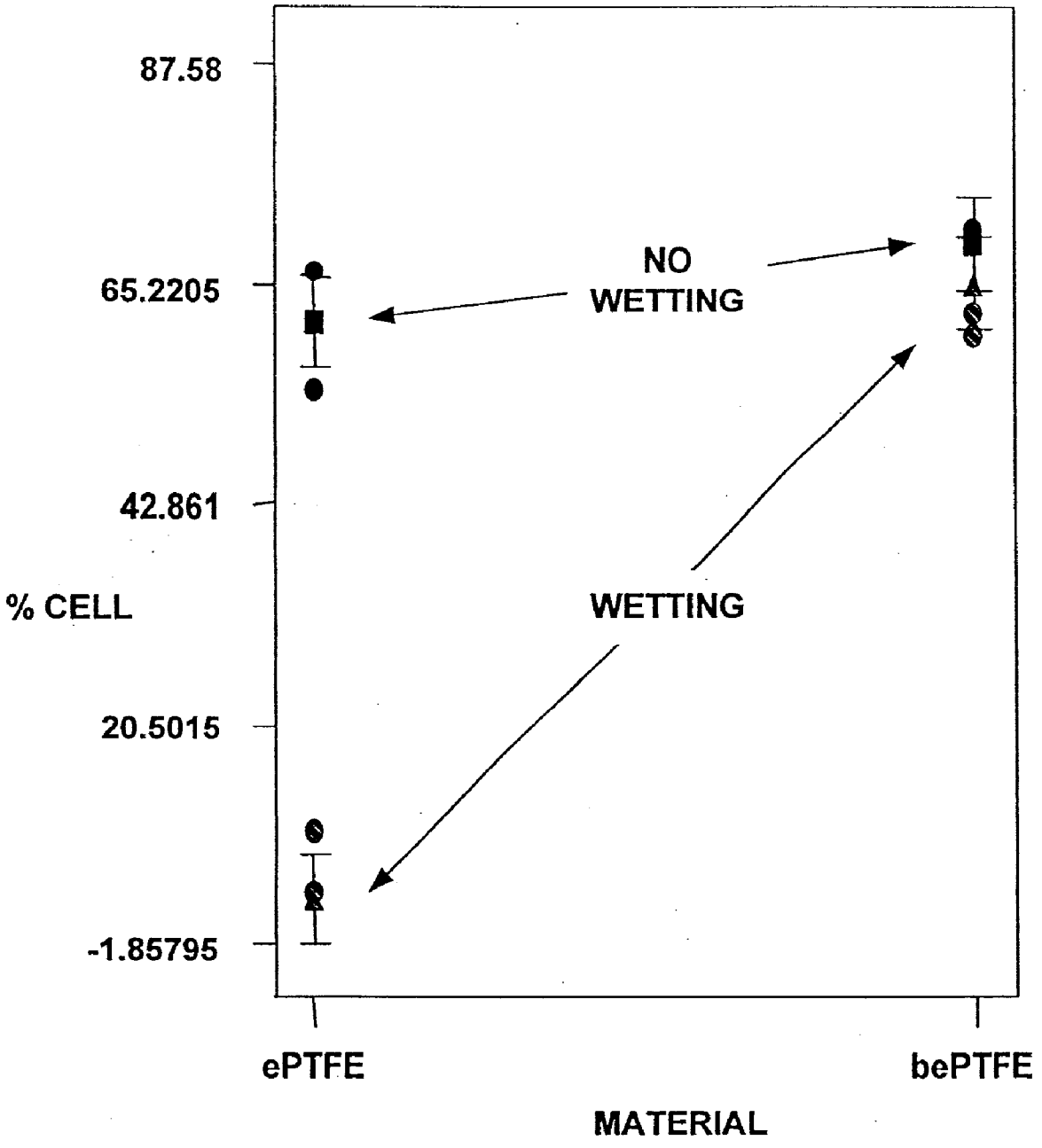

87.58
65.2205
42.861
% CELL
20.5015
-1.85795
NO
WETTING
WETTING
ePTFE
bePTFE
MATERIAL

FIG. 23

	MATERIAL	INCUBATION	% CELL RETENTION
A	ePTFE	0	28.0
	bePTFE	0	61.6
B	ePTFE	1 HOUR/STATIC	45.0
	bePTFE	1 HOUR/STATIC	55.4
C	ePTFE	1 HOUR/1 g	43.8
	bePTFE	1 HOUR/1 g	62.0
D	ePTFE	5 HOURS/STATIC	26.5
	bePTFE	5 HOURS/STATIC	44.4

FIG. 24

	MATERIAL	INCUBATION	FLOW	% CELL RETENTION
A	ePTFE	0	0	36.0
	bePTFE	0	0	54.7
B	ePTFE	1 HOUR	0	38.8
	bePTFE	1 HOUR	0	65.0
C	ePTFE	0	1 HOUR	25.1
	bePTFE	0	1 HOUR	44.9
D	ePTFE	1 HOUR	1 HOUR	24.3
	bePTFE	1 HOUR	1 HOUR	75.4

FIG. 25

SEEDING IMPLANTABLE MEDICAL DEVICES WITH CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. patent application Ser. No. 11/071,597 filed on Mar. 2, 2005, and entitled "Seeding Implantable Medical Devices with Cells", the content of which is hereby incorporated herein in its entirety.

FIELD OF THE INVENTION

[0002] The field of the invention relates to materials and devices implantable in a living human or animal body, such as materials and devices used in vascular prostheses.

BACKGROUND OF THE INVENTION

[0003] Some patients develop conditions that can be corrected with implantable medical devices such as mechanical and bioprosthetic heart valves, coronary stents, stent grafts, abdominal aortic aneurysm (AAA) grafts and other devices. Conditions that affect blood flow through the vessels of the body, for example, may be treated with vascular grafts, in which a surgeon applies the graft to supplant the damaged vascular tissue. Coronary artery disease, peripheral vascular disease and venipuncture for treatment of end stage renal disease are examples of conditions in which vascular flow is affected, and which can be addressed with surgical grafts.

[0004] Vascular grafts may be autologous, i.e., the graft may be taken from the patient for transplantation at another site. In some cases, however, an autologous graft may not be feasible, and a synthetic vascular graft may be employed instead. A synthetic vascular graft is a tube-shaped prosthesis made of a biocompatible material such as expanded polytetrafluoroethylene (ePTFE). The synthetic vascular graft includes a lumen through which blood flows.

[0005] In a vessel, the intima is the layer closest to the lumen where blood flows. It is made up mainly of a monolayer of endothelial cells attached to a basement membrane and matrix molecules. The endothelial cells are specialized cells that line the lumen of blood vessels, and play several roles. Endothelial cells secrete vasoactive substances, for example, and secrete substances that stimulate new vessel growth and promote or inhibit contraction and sometimes proliferation of smooth muscle cells in vessel walls in response to hemodynamic demands. Endothelial cells are also influential in formation and dissolution of thrombus, which is a precipitate of blood components that can restrict blood flow through the vessel lumen.

[0006] In humans, implanted vascular grafts typically heal by formation of an acellular psuedo-intima without large-scale outgrowth of the native endothelial cell lining at the point of anastomosis. It has been discovered that it is highly beneficial for a synthetic vascular graft to include a layer of endothelial cells in the lumen, to prevent thrombosis and to suppress abnormal smooth muscle cell proliferation that could lead to stenosis or narrowing of the vessel. To promote the formation of a homogeneous, dense and confluent layer of endothelial cells inside the synthetic vascular graft, techniques have been developed for "endothelial cell seeding" of vascular grafts. In general, this "seeding" or deposition of cells involves harvesting autologous endothelial cells and transplanting the harvested cells to the lumen of the synthetic vascular graft.

SUMMARY OF THE INVENTION

[0007] In general, the invention is relates to devices and methods that are useful for seeding implantable medical devices with cells, such as mechanical and bioprosthetic heart valves, coronary stents, stent grafts and AAA grafts. For purposes of describing the invention, however, the discussion will focus upon the seeding of a vascular prosthesis. The devices are configured to be implanted in a living body, i.e., a human or animal body.

[0008] Various methods for preparation of an implantable medical device to enhance endothelial cell seeding are described. Some of the methods involve creation of recesses in the luminal surface of implantable medical device, such as a vascular prosthesis, that can receive endothelial cells. When the implantable medical device is constructed of a material such as expanded polytetrafluoroethylene (ePTFE), the recesses may be created by physical processing of the microstructures of the material. The recesses support and shelter endothelial cells deposited on the lumen and reduce the risk of the cells being washed away. When the endothelial cells wash away, the vessel is less likely to endothelialize, and is at greater risk of developing complications, such as thrombosis and stenosis.

[0009] The invention describes methods for seeding the luminal surface of an implantable medical device with axial centrifugation. Cells are introduced in suspension into the lumen of the device, and the device is subjected to centrifugation around a longitudinal axis defined by the lumen. Axial centrifugation causes the cells to concentrate toward the luminal surface. Shortly after axial centrifugation, the seeded device can be presented for implantation in a patient. Because cells concentrate toward the luminal surface, the cells are more likely to coat the luminal surface, and are more likely to inhabit the sheltering recesses.

[0010] The methods described herein for cell seeding of an implantable medical device can be performed directly in the operating room. While the patient is undergoing surgery, the cells may be introduced into the lumen of the prosthesis, and the prosthesis seeded by subjection to axial centrifugation. Also described are methods to prepare the implantable medical device to receive the cells, as well as to protect of the implantable medical device from hazards associated with handling.

[0011] Also described herein is an apparatus to facilitate cell seeding of an implantable medical device that can be coupled to a typical tabletop centrifuge to perform axial centrifugation.

[0012] In one embodiment, the invention relates to a method for seeding an implantable medical device with cells. The method comprises introducing cells into a lumen of the implantable medical device adapted to be implanted in a living body, i.e., a human or animal body. The lumen includes a luminal surface that includes ePTFE. The method also includes applying centrifugation to the device to rotate the device around a longitudinal axis defined by the lumen. The method also describes placing the device in a protective sleeve prior to introducing the cells. The method further describes placing the device, with or without the protective

sleeve, in a tube with an open end prior to introducing the cells, and sealing the open end of the tube with a plug after introducing the cells.

[0013] In another embodiment, the invention relates to a method for seeding an implantable medical device with cells. The method comprises introducing cells into a lumen of the implantable medical device adapted to be implanted in a human or animal body. The lumen includes a luminal surface having recesses defined by nodes lifted from the surface. The method also includes applying axial centrifugation to the device.

[0014] In an additional embodiment, the invention relates to an apparatus comprising an adapter, a tube and a plug. The adapter is configured to mate with a rotor of a centrifuge proximate to an axis of rotation of the rotor. The adapter includes a chamber that extends in the direction of the axis. The tube is configured to receive an implantable medical device, and is further configured to be received in the chamber. The tube has an open end. The plug is configured to seal the open end of the tube.

[0015] In the case of an implantable medical device, such as a vascular prosthesis, manufactured and seeded as described, fewer endothelial cells will be washed away when the prosthesis is implanted, thereby benefiting the patient. Also, various embodiments of the invention take advantage of physical properties of ePTFE, a material that has a proven track record in implantable medical devices. The invention improves ePTFE without adversely affecting the favorable features of ePTFE, such as biocompatibility, physical properties and ease of handling and suturing.

[0016] In addition, the invention also makes a "one-stage procedure" feasible, in which endothelial cells can be harvested, a prosthesis can be seeded with the harvested cells, and the seeded device can be presented for implantation in a single surgical operation. Seeding with axial centrifugation can be an efficient way to deploy cells rapidly and evenly on the luminal surface.

[0017] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. **1** is a perspective view of a vascular prosthesis.

[0019] FIG. **2** is a perspective view of a tool assembly for processing a vascular prosthesis.

[0020] FIG. **3** is a scanning electron microscope (SEM) image of expanded polytetrafluoroethylene (ePTFE) material prior to processing.

[0021] FIG. **4** is an SEM image of ePTFE material after processing.

[0022] FIG. **5** is an SEM image of ePTFE material after processing, shown in cross-section and at an oblique angle.

[0023] FIG. **6** is an SEM image of ePTFE material after processing, seeded with endothelial cells.

[0024] FIG. **7** is a diagram illustrating the structure of ePTFE material.

[0025] FIGS. **8-10** are diagrams illustrating exemplary techniques for rubbing ePTFE material with a tool.

[0026] FIG. **11** is a flow diagram illustrating a technique for processing a vascular prosthesis.

[0027] FIG. **12** is a flow diagram illustrating an implantation technique.

[0028] FIG. **13** is a perspective diagram illustrating axial centrifugation of a vascular prosthesis.

[0029] FIG. **14** is a perspective diagram illustrating the effect of axial centrifugation of a vascular prosthesis upon cells in suspension.

[0030] FIG. **15** is a perspective diagram illustrating axial centrifugation of a vascular prosthesis with a protective sleeve.

[0031] FIG. **16** is a cross-sectional exploded view of an exemplary centrifugation apparatus.

[0032] FIG. **17** is a cross-sectional assembled view of the exemplary centrifugation apparatus depicted in FIG. **16**.

[0033] FIG. **18** is a perspective exploded view of the exemplary centrifugation apparatus depicted in FIGS. **16** and **17**.

[0034] FIG. **19** is a perspective assembled view of the exemplary centrifugation apparatus depicted in FIGS. **16**, **17** and **18**.

[0035] FIG. **20** is a flow diagram illustrating a cell seeding technique.

[0036] FIG. **21** is a graph of exemplary data illustrating relationships among centrifugation, cell retention and device material.

[0037] FIG. **22** is a graph of exemplary data illustrating cell retention for device materials following lower g and higher g axial centrifugation.

[0038] FIG. **23** is a graph of exemplary data illustrating cell retention for device materials as a function of wetting prior to axial centrifugation.

[0039] FIG. **24** is a table showing data collected pertaining to devices seeded with axial centrifugation, including devices with an incubation period after centrifugation.

[0040] FIG. **25** is a table showing data collected pertaining to devices seeded with axial centrifugation, including devices with an incubation period and flow acclimatization after centrifugation.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0041] FIG. **1** is a diagram of an implantable medical device, in particular, a vascular prosthesis **10**. For purposes of describing the invention, the discussion below focuses upon the seeding of vascular prosthesis **10** with cells. The invention is not limited to this particular implantable medical device, however. Each of these implantable medical devices can be configured to be implanted in a living body, i.e., a human or animal body.

[0042] Prosthesis **10** is a generally tube-shaped structure that includes a lumen **12** through which a fluid can flow. In a typical application, vascular prosthesis **10** supplants a blood vessel, and the fluid that flows through lumen **12** is blood. A luminal surface **14** of vascular prosthesis **10** comes in contact with the blood.

[0043] The geometry of luminal surface **14** of vascular prosthesis **10** defines a "luminal direction," which is along the longitudinal axis of the tubular prosthesis. Although fluid may physically flow through lumen **12** forward or backward along the luminal direction, fluid generally flows predominantly in one direction in an implanted environment. It is therefore useful to define a "flow direction" which represents a particular direction of fluid flow. In FIGS. **1** and **3-6**, arrow **16** identifies the flow direction. Flow direction **16** is coincident with the luminal direction, but is directed in a single direction. Fluid moving in flow direction **16** may be consid-

ered as moving "forward," and fluid moving opposite flow direction **16** may be considered as moving "backward."

[0044] FIG. **2** is a diagram of an exemplary tool assembly **20** that processes vascular prosthesis **10** by rubbing vascular prosthesis **10**. "Rubbing" comprises any process that includes moving a tool with pressure relative to vascular prosthesis **10**, such as by scraping, scoring, abrading, brushing, chafing, scratching or scuffing.

[0045] As shown in FIG. **2**, vascular prosthesis **10** has been everted, i.e., vascular prosthesis **10** has been turned "inside out" to facilitate processing with tool assembly **20**. Vascular prosthesis **10** has been mounted on a rotatable supporting mandrel **22**, which may be free to rotate as shown by directional arrow **24**.

[0046] A tool **26** rubs luminal surface **14**. In exemplary tool assembly **20**, tool **26** is mounted on a rotating shaft **28** that rotates as shown by directional arrow **30**. When tool **26** is brought in contact with luminal surface **14** and rotated, tool **26** rubs against luminal surface **14**. Mandrel **22** or shaft **28** or both further have freedom to move in a transverse direction, as shown by directional arrow **32**.

[0047] By rotating tool **26** and moving tool **26** and prosthesis **10** transversely to one another, and by rotating mandrel **22**, tool **26** can be brought into contact with any point on luminal surface **14**. In this way, tool **26** can rub the entire luminal surface **14**. Although not essential for the invention, there are advantages to rubbing the entire luminal surface, as will be described below. In addition, mandrel **22** need not have a circular or rounded cross-section as shown in FIG. **2**, but may include one or more flat surfaces.

[0048] When vascular prosthesis **10** is constructed of a material such as expanded polytetrafluoroethylene (ePTFE), rubbing luminal surface **14** with tool **26** creates recesses in the microstructures of luminal surface **14**. In particular, rubbing luminal surface **14** lifts microscopic "nodes" from luminal surface **14**, forming recesses that can receive seeded autologous endothelial cells. As used herein, "endothelial cells" includes endothelial precursor or stem cells, as well as developed endothelial cells.

[0049] Tool **26** may be any of several tools. Tool **26** may be solid, such as a rotating drum of metal, plastic, rubber or ceramic. Tool **26** may also include a wheel brush with bristles. The bristles may be constructed of any material, including metal, plastic, rubber or ceramic. Through experimentation, it has been discovered that a wheel brush with metal bristles, such as brass or stainless steel bristles, can generate recesses in the luminal surface. A wheel brush with nylon bristles also is effective in generating recesses. A technique for rubbing a luminal surface of a vascular prosthesis with a tool will be described below.

[0050] FIG. **3** is an image of ePTFE material **40** taken by a scanning electron microscope (SEM). The image of FIG. **3** depicts ePTFE material **40** such as that found in a standard vascular graft such as that shown in FIG. **1**. In particular, the image of FIG. **3** depicts a microscopic view of the luminal surface of a prosthesis, i.e., the surface that may be in contact with a flowing bodily fluid, such as blood.

[0051] Two types of microstructures provide ePTFE material **40** with its strength and other physical properties, and these microstructures are evident on the luminal surface shown in FIG. **3**. In particular, ePTFE material **40** includes thin polytetrafluoroethylene (PTFE) fibrils **42** draped between the much thicker islands or "nodes" **44** of PTFE. The orientations of fibrils **42** and nodes **44** are substantially perpendicular to one another, and result from the manufacture of ePTFE.

[0052] In general, the manufacture of ePTFE includes preparation of a material that includes PTFE particles that have been fused together. At one stage in the manufacturing process, the material is stretched or "expanded." The expansion causes fibrils **42** to form in the direction of the expansion, giving ePTFE directionality. The degree of expansion also affects the internodal distance, i.e., the average distance between neighboring nodes in the direction of expansion. Internodal distances may be, for example on the order of about 30 to 90 micrometers. Reference numeral **46** shows a typical internodal distance.

[0053] In FIG. **3**, ePTFE material **40** has not yet been rubbed with a tool. For reference, FIG. **3** shows flow direction **16**. Flow direction **16** is substantially perpendicular to the orientation of nodes **44**, and substantially parallel to the orientation of fibrils **42**.

[0054] FIG. **4** is an image of ePTFE material **50** taken by an SEM. Material **50** has been subjected to preparation, thereby creating a plurality of recesses **52** in the luminal surface. As will be described below, rubbing the luminal surface with a tool generates recesses **52**. Recesses **52** can receive endothelial cells. Recesses **52** represent "grooves," "wells," "harbors," "pockets" or "hiding spaces" for the endothelial cells.

[0055] As shown in FIG. **4**, recesses **52** are oriented at least partially along the luminal direction. In particular, the recesses extend into the luminal surface, but extend at least partially in the direction opposite flow direction **16**. In other words, a fluid moving in flow direction **16** would generally flow over recesses **52**, rather than into recesses **52**.

[0056] As shown in FIG. **4**, rubbing the luminal surface affects the fibrils network visible in FIG. **3**. As a result of rubbing, many of the fibrils are disrupted, resulting in smooth, fibrilfree surfaces. This effect is generally restricted to the luminal surface, however. Fibrils beneath the luminal surface are largely intact, imparting strength and other physical properties to material **50**. In addition, fibrils may reside inside recesses **52**. It has been discovered through experimentation that the extent of smooth, fibril-free surfaces is generally a function of the extent of rubbing.

[0057] Viewed with an SEM, the luminal surface of material **50** resembles a series of overlapping layers. The layers separate from one another in a scale-like texture that resembles a "fish-scale" pattern, creating recesses that can harbor endothelial cells.

[0058] FIG. **5** is an image of ePTFE material **60** taken by an SEM that shows the structure of material **60** following preparation and creation of recesses **62**. FIG. **5** shows in part a cross section **64** of material **60**, i.e., material beneath the luminal surface. Although rubbing has affected the luminal surface, the material below the luminal surface maintains its structure. In a typical vascular prosthesis having a wall thickness of three-tenths to seven-tenths of a millimeter, rubbing would generally affect no more than five to ten percent of the thickness of the material.

[0059] FIG. **5** also provides an oblique view **66** of the luminal surface. As can be seen from oblique view **66**, recesses **62** are oriented at least partially along the luminal direction, and extend into the luminal surface at least partially in the direction opposite flow direction **16**.

[0060] FIG. **6** is an image of ePTFE material **70** taken by an SEM. Material **70** is similar to material **50** in FIG. **4**, and

material **60** in FIG. **5**, but material **70** includes recesses **72** in the luminal surface and endothelial cells **74** received in recesses **72**. As shown in FIG. **6**, a fluid moving in flow direction **16** would generally flow over recesses **72** and over cells **74**. As a result, a cell residing in a recess is subjected to less shear force from the fluid than a cell outside a recess, and is less likely to be exposed and washed away by the fluid.

[0061] In a conventional vascular prosthesis seeded with endothelial cells, the endothelial cells deposited on the lumen of the prosthesis tend to be washed away by the flow of blood. Even when the cells adhere to the luminal surface, the shear forces associated with fluid flow often overcome the adhesion and wash the endothelial cells away. When the endothelial cells are washed away, the vessel is less likely to endothelialize and is at greater risk of developing complications, such as thrombosis and stenosis.

[0062] In a vascular prosthesis with a luminal surface such as shown in FIG. **6**, however, shear forces may wash away fewer endothelial cells. Because endothelial cells **74** reside in recesses **72**, fluid flow along fluid direction **16** is less likely to dislodge and wash away endothelial cells **74** in recesses **72**. With time, endothelial cells **74** grow in situ, mature and colonize the luminal surface, with recesses **72** providing a foundation for growth and colonization. As result, the vascular prosthesis maintains a population of endothelial cells that help reduce the risk of complications.

[0063] In addition, rubbing results in smooth, fibril-free surfaces. Endothelial cells **74** typically adhere more efficiently to smooth nodal surfaces than to fibrils. Rubbing the luminal surface with a tool, in addition to creating recesses, also creates a more suitable surface for cell adhesion.

[0064] As noted above, the manufacture of ePTFE includes an expansion that imparts directionality to ePTFE. FIG. **7** is a diagram of an ePTFE sample **80** that illustrates the directionality of ePTFE material. In FIG. **7**, sample **80** includes nodes **82** and fibrils **84**. Arrow **86** identifies a direction that is substantially perpendicular to the orientation of nodes **82**, and substantially parallel to the orientation of fibrils **84**. FIGS. **8-10** are diagrams illustrating techniques for rubbing ePTFE sample **80** with a tool.

[0065] As shown in FIG. **8**, one technique for rubbing sample **80** includes rotational rubbing with a tool such as a wheel brush. Rotational rubbing may be accomplished using tool assembly **20** shown in FIG. **2** by bringing the circular face of tool **26**, rather than the side of tool **26**, into contact with prosthesis **10**. With rotational rubbing, the tool rubs the luminal surface in many directions **88** simultaneously. Some of the rubbing may be substantially parallel to the orientation of nodes **82**, and some may be substantially perpendicular to the orientation of nodes **82**.

[0066] FIG. **9**, illustrates another technique for rubbing, i.e., radial rubbing with a tool. Radial rubbing comprises rubbing the luminal surface of sample **80** in a direction **90** that is substantially parallel to the orientation of nodes **82**, and substantially perpendicular to the orientation of fibrils **84**. Rotational rubbing may be accomplished using tool assembly **20** shown in FIG. **2** by bringing the side of tool **26** into contact with prosthesis **10**, and orienting mandrel **22** and shaft **28** in the same direction.

[0067] A further technique, shown in FIG. **10**, includes transverse rubbing of sample **80** with a tool. Transverse rubbing comprises rubbing the luminal surface in a direction **92** that is substantially perpendicular to the orientation of nodes **82**, and substantially parallel to the orientation of fibrils **84**. FIG. **2** depicts tool assembly **20** rubbing vascular prosthesis **10** in a transverse direction.

[0068] Through experimentation, it has been discovered that transverse rubbing as depicted in FIG. **10**, is effective in lifting nodes from the luminal surface to define a plurality of recesses. Radial rubbing, as depicted in FIG. **9**, tends to disrupt fibrils **84** without lifting large numbers of nodes **82** to create recesses. Rotational rubbing, as depicted in FIG. **8**, tends to produce regions in which nodes are lifted, comparable to the effect of transverse rubbing, and regions in which nodes are not lifted, comparable to the effect of radial rubbing.

[0069] It is possible to rub sample **80** with a tool in multiple directions simultaneously. For example, it is possible to rub sample **80** in a direction that has a radial rubbing component and a transverse rubbing component. In general, the greater the transverse rubbing in relation to the radial rubbing, the more nodes are lifted and the more recesses are created. It is also possible to repeat rubbing of the same region of sample **80** in the same way or a different way. Repeat rubbing can further refine the structure of the formed recesses.

[0070] Translational rubbing disrupts fibrils **84** on the luminal surface, but also lifts or "plucks" nodes from the luminal surface, thereby creating recesses oriented at least partially along the luminal direction. There may be one or more mechanisms that cause the nodes to be lifted from the luminal surface. When the tool used to rub the luminal surface is a wheel brush with bristles, for example, the bristles may contact nodes and lift the nodes from the luminal surface by friction. The contact between the tool and the surface may also facilitate PTFE "smearing," in which PTFE structures spreads and merge with one another, generating recesses in the process.

[0071] FIG. **11** is a flow diagram illustrating a process for preparing a luminal surface of a vascular prosthesis. The process includes applying a tool to a site on the luminal surface (**100**) rubbing the luminal surface with the tool (**102**). The rubbing lifts nodes, thereby creating recesses oriented at least partially along the luminal direction.

[0072] Exemplary tool assembly **20** shown in FIG. **2** depicts vascular prosthesis **10** mounted on a rotatable supporting mandrel **22**, with tool **26** brought in contact with luminal surface **14** of vascular prosthesis **10**. Tool **26** rubs luminal surface **14** of vascular prosthesis **10** when rotating shaft **28** rotates. By rotating tool **26** and moving tool and prosthesis **10** transversely to one another, and by rotating supporting mandrel **22**, tool **26** can be brought into contact with any point on luminal surface **14**.

[0073] In some implementations, mandrel **22** includes one or more flat surfaces. When prosthesis **10** is mounted on such a mandrel, prosthesis **10** conforms to the shape of mandrel **22** and flattens. Mandrel **22** can rotate to bring a flat surface to bear, then cease rotation. Tool **26** can rub luminal surface **14** of vascular prosthesis **10** where surface **14** is flattened.

[0074] Accordingly, once a site on the luminal surface has been rubbed, the process includes determining whether other sites need to be rubbed as well (**104**). In some circumstance, the entire luminal surface of the prosthesis may be rubbed. In other circumstances, it may be desirable to seed endothelial cells at specified sites, and only these specified sites will be rubbed. These specified sites may form patterns, such as

longitudinal or radial patterns. By selection of specific sites for rubbing, it is possible to create "paths" for cell growth in situ.

[0075] If additional rubbing is indicated, the tool is applied to another site (**106**) and the process is continued (**102**). When tool **26** has completed rubbing, the prosthesis may be everted for implantation (**108**), if necessary. Eversion may also be performed before rubbing, to bring luminal surface **14** to bear. In some embodiments, an everted prosthesis may be rubbed again, thereby processing the abluminal surface as well as the luminal surface.

[0076] It is believed to be possible to rub a luminal surface without everting the prosthesis, e.g., by running a brush through the lumen one or more times. Accordingly, everting the prosthesis for processing is not essential to the invention. Even so, mounting the prosthesis on a supporting mandrel, as shown in FIG. **2**, may allow for very precise control of the rubbing.

[0077] In one embodiment of the invention, a **4** millimeter diameter ePTFE vascular graft was everted, placed over a mandrel attached to a tooling jig parallel to the rotational axis of a model lathe via an adjustable loading spring, and the tooling jig fixed to the tool stock of an EMCO Unimat PC model lathe. A wheel brush with densely packed nylon bristles (The Mill-Rose Company, Mentor Ohio, Catalog No. 71810, 1 inch (2.5 cm) diameter, 0.006 inch (150 micrometer) in diameter bristles) was secured in the chuck of a vertical milling head attached to the model lathe. The tool stock was positioned to place the everted graft in contact with the brush attached to the vertical milling head. Uniform translation of the graft across the brush was achieved by attaching the tool stock lead screw to either a 2-rpm or a 10-rpm synchronous motor. While the brush was rotated at speeds ranging from 350 to 2500 rpm, the graft was first passed in one direction across the brush at 0.075 inches (1.9 mm) per minute (2 rpm synchronous motor) or 0.375 inches (9.5 mm) per minute (10 rpm synchronous motor) with a contact force of 15 gram weight (0.033 lb). The graft was then passed a second time across the rotating brush in the opposite direction with a contact force of 55 gram weight (0.12 lb) over the same range of brush rotation and tool stock translation speeds. The ePTFE may have a wide range of average intemodal distances, e.g., from 10 to 200 micrometers between nodes, but good results were obtained with average intemodal distances in the range of 30 to 90 micrometers. Vascular grafts of ePTFE are available from a variety of manufacturers.

[0078] In one embodiment of the invention, a wheel brush with densely packed nylon bristles (Mill-rose No. 71810, 1 inch (2.5 cm) in diameter, each bristle about 0.006 inches (150 micrometers) in diameter) was rotated at 350 to 2500 revolutions per minute against a vascular prosthesis made of ePTFE. The prosthesis had been everted so that that luminal surface was more accessible. The brush was moved along the prosthesis transversely at 1100 to 6500 inches per minute (28 to 165 meters per second). Forces in the range of 30 to 100 grams weight (0.066 to 0.22 pounds) were applied between the brush and the luminal surface. The ePTFE may have a wide range of average intemodal distances, e.g., from 10 to 200 micrometers between nodes, but good results were obtained with average intemodal distances in the range of 30 to 90 micrometers. Vascular grafts of ePTFE are available from a variety of manufacturers.

[0079] Brushing as described above does not necessarily lift every node in the surface, nor does it necessarily lift all nodes to the same degree. It is not uncommon, however, for a node to be lifted from the surface by many times its normal height.

[0080] The process depicted in FIG. **11** is not necessarily restricted to vascular grafts. Implantable devices other than vascular grafts may include ePTFE, and may benefit from having surface recesses for harboring endothelial or other cells, such as cells that improve healing following implantation. Even if not seeded with cells, the implantable devices may realize benefits from having surfaces undergo a process such as that depicted in FIG. **11**. For example, the surfaces may improve healing or decrease fibrous capsule formation. Implantable devices that may include ePTFE, and that may benefit from having surface recesses may include, for example, implantable prostheses for plastic surgery, artificial ligaments, annuloplasty rings, vascular patches, tubes for neural cell growth, sheathed stents, cardiac assist devices, sensors, pacemaker leads, catheters, shunts, sutures and heart valve sewing rings. Such devices may be implantable on a permanent or a temporary basis.

[0081] In addition, when the vascular prosthesis or other implantable device is made from ePTFE, the invention is not limited to physical rubbing with a solid tool. It is believed that nodes may be lifted from the surface of ePTFE by application of a pressurized fluid, such as air or water, to a surface made of ePTFE. In other words, an air jet or water jet may supply sufficient friction to lift nodes so as to define a plurality of recesses. Rubbing or application of a pressurized fluid applies a force to the ePTFE, thereby lifting nodes to define recesses. These techniques are not exclusive of one another. For example, a tool may rub the surface of ePTFE when the surface is coated with a liquid.

[0082] FIG. **12** is a flow diagram showing a technique for preparation of a vascular prosthesis for implantation. FIG. **12** depicts a "one-stage procedure," i.e., a procedure for preparation of a vascular prosthesis during a single surgical operation.

[0083] The technique of FIG. **12** includes harvesting endothelial cells (**110**). In a typical operation to repair a damaged vessel with a prosthesis, a surgeon retrieves a source of endothelial cells from the patient before or during the procedure to repair the damaged vessel. A surgeon may, for example, retrieve an expendable subdermal vein that includes endothelial cells, and supply the vein to the medical staff for harvesting of the cells. Another harvesting technique involves taking endothelial cells from adipose tissue. While the staff harvests the cells and prepares the prosthesis, the surgeon may begin repairing the damaged vessel, e.g., obtaining access to the implantation site and preparing the site to receive the prosthesis.

[0084] The staff may harvest the cells (**110**) using any harvesting method. The cells may be separated form the supplied vein and placed in suspension. The staff seeds the prosthesis with harvested endothelial cells (**112**). The prosthesis is a device having a plurality of recesses sized to receive endothelial cells, with at least some of the recesses oriented at least partially along the luminal direction. The prosthesis will ordinarily have been brought into the operating room with the recesses already formed, and with the prosthesis ready for seeding. The prosthesis may also be premarked to indicate to the surgeon the intended direction of fluid flow through the lumen.

[0085] Any seeding method (**112**) may be used. For example, the fluid with suspended endothelial cells may be

introduced into the lumen of the prosthesis, and the prosthesis may be spun with a centrifuge to cause the cells to come in contact with the luminal surface and be received in the recesses. Techniques for seeding with a centrifuge will be discussed in detail below. Following seeding, the seeded prosthesis is supplied to the surgeon for implantation (**114**). Harvesting and seeding in this way can be accomplished quickly, typically in sixty minutes or less, and sometimes in fifteen minutes or less.

[0086] FIG. **13** is a schematic view of an implantable medical device being rotated with a centrifuge (not shown). Once again, the implantable medical device is vascular prosthesis **10**, but the invention is not limited this device. Vascular prosthesis **10** is rotated to seed luminal surface **14** with cells. Lumen **12** defines a longitudinal luminal axis **120**, which is substantially perpendicular to the orientation of nodes on luminal surface **14**, and substantially parallel to the orientation of fibrils on luminal surface **14**. As indicated by directional arrow **122**, the centrifuge rotates prosthesis **10** axially, i.e., centrifuge rotates prosthesis **10** around axis **120**.

[0087] Although FIG. **13** depicts a vascular prosthesis, other implantable medical devices also have lumens that define longitudinal luminal axes. Many such devices can be seeded using the apparatus and methods described herein. Such implantable medical devices include, but are not limited to, mechanical and bioprosthetic heart valves, coronary stents, stent grafts and AAA grafts. Each of these devices includes a lumen and a longitudinal axis defined by the lumen. For purposes of simplicity, however, the discussion will focus upon the seeding of a vascular prosthesis.

[0088] FIG. **14** illustrates the effect of axial centrifugation. Although not shown in FIG. **14**, the open ends of prosthesis **10** could be plugged to prevent leakage of material from prosthesis **10** during axial centrifugation. Cells in suspension introduced into lumen **12** are prevented from moving out of lumen **12** by rotating luminal surface **14**. The force exerted on the cells in suspension by rotating luminal surface **14** is a function of the speed of rotation, the radius measured from axis **120** to luminal surface **14**, and the mass of the cells. The cells have greater mass per unit volume than the suspension, so the cells tend to separate from the suspension and concentrate toward luminal surface **14**, as indicated by arrows **124**. The overall effect of axial centrifugation is to increase the concentration of cells on luminal surface **14**.

[0089] The centripetal acceleration of a centrifuge is typically expressed in terms of "g." 1 g is approximately equal to the acceleration due to gravity on the Earth's surface, 100 g is one hundred times 1 g, and so on. In a centrifuge that can apply axial or longitudinal or angular centrifugation, the centrifuge typically applies more g's with longitudinal and axial centrifugation than with angular centrifugation for a given angular velocity, because objects that receive longitudinal or angular centrifugation typically are further from the axis of rotation of the centrifuge rotor. Computation of g's is straightforward, because centripetal acceleration is a function of the distance of luminal surface **14** from the axis of centrifugation and the angular velocity of the centrifuge rotor.

[0090] Axial centrifugation of a 4 mm diameter prosthesis with a typical tabletop centrifuge can produce about 1,000 g, although this is not a maximum for all centrifuges. Increased concentration of cells on luminal surface **14** can be produced with accelerations from 1 g to 10,000 g, although higher g's increase the risk of damage to the cells. Through experimentation, it has been discovered that centrifugation at 50 g to 500 g for one to ten minutes produces comparable concentrations of cells on luminal surface **14**. In practice, centrifugation could involve applying between 1 g and 1,000 g, preferably 50 g to 500 g, and more preferably about 250 g. Centrifugation could be applied for any length of time, but usually less than one hour and preferably for one to ten minutes.

[0091] Any of several media can serve as suspensions for endothelial cells. The suspension can be a buffered salt solution, for example, or a physiological balanced electrolyte solution such as Plasma-Lyte® A, commercially available from Baxter International, Inc. As discussed below, the introduction of a suspension can be preceded by introduction of preparatory fluids.

[0092] FIG. **15** is a schematic view of a vascular prosthesis **10** being spun with a centrifuge (not shown) to seed luminal surface **14**. Unlike FIGS. **13** and **14**, vascular prosthesis **10** is inside a protective sleeve **126**. Sleeve **126** may be constructed from any of several metallic, glass or ceramic materials or polymers, and may further include reinforcing fillers or fibers. Sleeve **126** may also include one or more lubricants that make it easier for a person to put prosthesis **10** into or remove prosthesis **10** from sleeve **126**. An exemplary material for construction of sleeve **126** is polytetrafluoroethylene, which is more resistant to bending than ePTFE.

[0093] Sleeve **126** is configured to receive and protect prosthesis **10**, and is shaped accordingly. When prosthesis **10** is cylindrical, sleeve **126** may likewise be substantially cylindrical. FIG. **15** depicts sleeve **126** as having a substantially circular cross-section of constant diameter, but other configurations may also work well. For example, sleeve **126** can have a tapered shape. Sleeve **126** can be open at one end or open at both ends. In addition, the thickness of sleeve **126** need not be constant. Sleeve **126** can have a circular crosssection that is substantially oval or polygonal. One or more surfaces of sleeve **126** may be smooth, ridged, grooved, textured and the like.

[0094] Sleeve **126** can be useful in preparation of prosthesis **10** for seeding and in maintaining the seeded condition of prosthesis **10** following seeding, by reducing the likelihood of reintroduction of air onto luminal surface **14** or the bulk of the prosthesis material. When wet ePTFE, for example, is bent or kinked, then straightened, air enters the bulk of the material, and the material soaks up air.

[0095] In a dry prosthesis constructed from ePTFE as described above, air can be present in the bulk of the material and in the recesses and the spaces between nodes and fibrils of luminal surface **14**. To promote effective seeding of luminal surface **14**, it is desirable to remove these small pockets of air. A procedure called "wetting," in which a fluid is introduced into lumen **12**, can displace the air. Because ePTFE is generally hydrophobic, water makes a poor wetting agent for displacing the pockets of air. A more effective wetting agent is ethanol. Wetting can be accomplished by known methods, such as centrifuging the device with ethanol in the lumen or soaking the device in ethanol. Experimentation suggests that it makes little difference whether prosthesis **10** is subjected to centrifugation with ethanol or whether prosthesis **10** is soaked in ethanol. Both processes are about equally effective in displacing air.

[0096] Ethanol is a poor medium for cells, however, so prosthesis **10** can be wetted with a second agent that displaces the ethanol and provides a growth medium for the cells. A growth medium is any medium that maintains the cells in a viable state during seeding. Experimentation indicates that a

growth medium may also enhance cell retention after implantation. An example of a second agent that can provide a growth medium is PlasmaLyte® A, which can be followed by wetting with platelet-poor plasma. Introduction of these agents may be achieved by, for example, centrifugation, soaking or other wetting methods. After the platelet-poor plasma has been in contact with luminal surface **14** typically for one to sixty minutes, the suspension with cells can be introduced, and centrifugation can be performed to cause the cells to accumulate on the luminal surface.

[0097] The wetting agents described above are for purposes of illustration, and the invention is not limited to those wetting agents. A fluorosurfactant, Zonyl® FSO, commercially available from DuPont, is an example of another wetting agent. Another exemplary wetting agent is phosphatidylcholine, which has a common name of lecithin and which is widely available from a number of suppliers. Lecithin is a natural surfactant emulsifier. It may be possible to immerse a prosthesis in an organic solution containing lecithin and let the solvent evaporate, leaving behind a lecithin surfactant-like coating.

[0098] By applying one or more wetting agents, air in prosthesis **10** can be displaced. Once the air is displaced, luminal surface **14** can be conditioned for seeding without reintroducing air. It is possible, however, that air may be reintroduced into luminal surface **14** by handling of prosthesis **10**, making seeding less effective. In particular, compressing and stretching of prosthesis **10**, or bending of prosthesis **10**, can result in reintroduction of air.

[0099] Sleeve **126** provides protection against compressing, stretching and bending of prosthesis **10**, and thereby reduces the risk that handling will reintroduce air onto luminal surface **14** or the bulk of prosthesis **10**. Sleeve **126** fits snugly over prosthesis **10** and is substantially more rigid than prosthesis **10**. As prosthesis **10** is placed into or removed from a centrifuge, or is otherwise handled, sleeve **126** helps prosthesis **10** retain its shape. At the time of implantation, prosthesis **10** can be extracted from sleeve **126**, and sleeve **126** can be discarded. In some cases, prosthesis **10** can be maintained inside protective sleeve **126** during implantation, with sleeve **126** removed near the conclusion of implantation, e.g., just prior to or just after cross-clamp release. Protective sleeve **126** can reduce the risk or seeded cell loss during implantation, due to factors such as bending, drying or cell dislodgement.

[0100] FIGS. **16-19** are views of an illustrative apparatus for axial centrifugation of prosthesis **10**. FIG. **16** is an exploded cross-sectional view showing the components, FIG. **17** is a cross-sectional view showing the components assembled and ready for centrifugation, FIG. **18** is a perspective exploded view, and FIG. **19** is a perspective assembled view. The apparatus depicted in FIGS. **16-19** can be used with a tabletop or laboratory centrifuge (not shown), which can be set up in an operating room. An example of a centrifuge that can accommodate the apparatus shown in FIGS. **16-19** is an Eppendorf Model 5416B commercially available from Gerätebau EppendorfGmbH of Engledorf, Germany.

[0101] Rotor **130** is the element that is mechanically coupled to, and that is directly rotated by, the centrifuge. An exemplary rotor for the Eppendorf Model 5416B centrifuge is a fixed angle rotor for microcentrifuge tubes, Type 16 F 24-11, Part No. 22 63 220-5, commercially available from Gerätebau Eppendorf GmbH of Engledorf Germany. The invention is not limited to this particular centrifuge or rotor. Such a rotor may include angular receptacles to receive Eppendorf tubes or other items. During axial centrifugation, it may be desirable to fill the receptacles to reduce noise.

[0102] Rotor **130** may be configured to support longitudinal and axial centrifugation. Rotor **130** may be constructed from a durable material such as aluminum or other metal, and can be constructed to support rotation at a range of angular velocities. The rotor model mentioned above is rated to 15,000 rpm, but typical lab centrifuge may drive rotor **130** to at higher angular velocities, e.g., around 18,000 rpm. The axis of rotation **132** of rotor **130** passes through rotor spindle **134**.

[0103] As shown in FIG. **16**, rotor spindle **134** lacks elements to hold and support centrifugation of a prosthesis **10**. In addition, spindle **134** is too small to accommodate a prosthesis having a length of four to fifteen centimeters, which are typical lengths for small diameter vascular prostheses. Accordingly, an adapter **136** can hold and support prosthesis **10** during axial centrifugation of prosthesis **10**.

[0104] Adapter **136** is configured to mate with rotor **130**, fitting over spindle **134**. Consequently, adapter **136** is configured to mate with rotor **130** proximate to axis of rotation **132**, and as shown in FIGS. **16** and **18**, axis of rotation **132** passes through adapter **136**. In some embodiments of the invention, spindle **134** can be machined to better accommodate the mating. In the event rotor **130** had a configuration different from that shown in FIGS. **16-19**, adapter **136** may likewise have a different configuration, to mate with rotor **130**.

[0105] As shown in FIG. **16**, rotor **130** can be machined to include receptacles **138** and **140**, which correspond respectively to holes **142** and **144** on a base **136**A of adapter **136**. Fixation mechanisms **146** and **148**, such as screws, can be used to secure adapter **136** to rotor **130** via holes **142** and **144** and fixation mechanisms **146** and **148**. Although FIG. **16** depicts two sets of holes and fixation mechanisms, the invention supports any number of sets of holes and fixation mechanisms. FIG. **18**, for example, depicts an arrangement that could support four equidistant sets of holes and fixation mechanisms, with fixation mechanisms **146**, **147** and **148** being visible. In addition, adapter **136** may be secured to rotor **130** with materials or apparatus other than what is shown in FIGS. **16** and **18**, such as bolts, adhesive, clasps, locks, clamps, welds, and the like.

[0106] Adapter **136** may be constructed from a durable material such as aluminum or other metal, and can be constructed to support rotation up to the same angular velocity as rotor **130**. Adapter **136** can have any dimension. As depicted in FIG. **16**, adapter **136** can extend vertically about 6.73 cm. Base **136**A of adapter **136** can be substantially circular, with an outer diameter of about 5.19 cm. Upper portion **136**B of adapter **136** can be substantially circular, with an outer diameter of about 1.8 cm. For a longer prosthesis, a taller adapter with a wider base can be used. It is believed that a tall adapter could be used to accommodate a prosthesis up to 20 cm. Similarly, adapter **136** and various features thereof can be shortened, widened, narrowed or otherwise configured to receive a particular implantable medical device.

[0107] Adapter **136** includes a chamber **150** that can receive prosthesis **10**, as well as other apparatus, as discussed below. Chamber **150** depicted in FIG. **16** can be substantially cylindrical, with a length of about 4.85 cm, and a diameter of about 0.96 cm. The height of chamber **150** extends substantially lengthwise in the direction of axis **132**, and the radius of chamber **150** extends substantially perpendicularly from axis **132**. Chamber **150** need not be an exact cylinder, but may

include tapered or ridged walls, for example, or may be prism- shaped. Adapter **136** is configured such that chamber **150** defines a closed end **150**A and an open or openable end.

[0108] It may not be convenient to attach adapter **136** to rotor **130**, and remove adapter **136** from rotor **130**, with each centrifugation. As depicted in FIGS. **16** and **17**, adapter **136** is configured to be firmly affixed to rotor **130**.

[0109] A sealable container such as tube **152** can be used to aid insertion of prosthesis **10** into chamber **150** of adapter **136**, and to aid removal as well. When suspension and cells are introduced into lumen **12** of prosthesis **10**, tube **152** contains and reduces the risk of spillage of the suspension and cells. Tube **152** can offer the additional advantage of protecting prosthesis **10** from risks associated with handling, as described above.

[0110] Tube **152** can be substantially cylindrical and can be constructed from a durable material such as polycarbonate or metal. As shown in FIG. **16**, tube **152** has a closed end **152**A and an open end **152**B. The outer diameter of tube **152** can be about 0.95 cm, slightly smaller than the bore of chamber **150**. Tube **152** includes an inner chamber **154**, which is substantially cylindrical. Exemplary dimensions of tube inner chamber **154** are 4.6 cm in length, and 0.60 cm in diameter. Tube inner chamber **154** receives prosthesis **10**. Although not depicted in FIGS. **16** and **17**, tube inner chamber **154** can also receive prosthesis **10** inside protective sleeve **126**, as shown in FIG. **15**.

[0111] A sealing device, in the form of a plug **156**, is configured to seal prosthesis **10** inside tube **152**. Plug **156** can be formed from a durable material such as aluminum or other metal or polycarbonate or polymer or plastic. Plug **156** is configured to seal open end **152**B of tube **152**. As depicted in FIGS. **16** and **17**, most of the body **156**A of plug **156** is configured to seat inside tube inner chamber **154**, with a top lip portion **156**B remaining outside inner chamber **154**, thereby an access structure for ready removal of plug **156** from open end **152**B of tube **152**. The open end of tube **152** can be configured to receive plug **156**. When prosthesis **10** is placed inside tube **152** and plug **156** is seated in tube **152**, prosthesis **10** and any material placed inside prosthesis **10** are prevented from being ejected from tube **152** during centrifugation.

[0112] A cap **158** is configured to mate securely but removably to adapter **136**. Cap **158**, which can be formed from a durable material such as aluminum, can mate with adapter **136** in any several ways. For example, upper portion **136**B of adapter **136** can be machined to include screw threads **136**C, as shown in FIG. **18**, which mate with matching grooves (not shown) in cap **158**, allowing cap **158** to be "screwed on" to adapter **136**. The exterior surface of cap **158** can be provided with knurling **158**A or other structures that assist a firm grip, enabling a person to secure cap **158** to, and remove cap **158** from, adapter **136** without a tool. Cap **158** can be substantially cylindrical, with length of 0.64 cm and a diameter of 1.91 cm.

[0113] FIG. **17** provides an assembled cross-sectional view of the apparatus shown in an exploded view in FIG. **16**. As depicted in FIG. **17**, cap **158** is configured to bear against plug **156** when cap **158** is secured to adapter **136**. Cap **158** may include, for example, a gum rubber disk or O-ring that bears against plug **156**, holding plug **156** in place and preventing leakage. In this way, securing cap **158** to adapter **136** fully seats plug **156** in tube **152**.

[0114] When tube **152** is placed inside chamber **150** of adapter **136**, and cap **158** is secured to adapter **136**, tube **152** is prevented from being ejected from adapter **136** during centrifugation. As described above, plug **156** prevents prosthesis **10** and any material placed inside prosthesis **10** from being ejected from tube **152** during centrifugation. In this way, cap **136** and plug **156** cooperate to prevent prosthesis **10** and any material placed inside prosthesis **10** from being ejected during centrifugation. When the apparatus is assembled as shown in FIGS. **17** and **19**, axis of rotation **132** is substantially collinear with longitudinal axis **120** of prosthesis **10**.

[0115] The apparatus shown in FIGS. **16-19** is exemplary, and the invention is not limited to the apparatus shown. For example, the apparatus shown in FIGS. **16-19** may be unable to accommodate prostheses longer than a certain length or wider than a certain diameter. Adapter **136** and tube **152** can be elongated, shortened, widened or narrowed to accommodate implantable medical devices of other dimensions. It is believed that adapter **136** can be as short as desired, but should not be overly tall, so as not to introduce a rotational inertia that could affect smooth centrifugation. It is believed that adapter **136** could be extended to about 22 cm, enabling adapter **136** to accommodate a prosthesis about 20 cm long.

[0116] In addition, the apparatus shown in FIGS. **16-19** holds prosthesis **10** vertically during axial centrifugation, but an apparatus that holds prosthesis **10** horizontally or on a slant during axial centrifugation is also contemplated. Further, it is contemplated that "axial centrifugation" encompasses centrifugation that is substantially axial. In other words, it is contemplated that some degree of longitudinal centrifugation may occur. It is believed, however, that as the component of longitudinal centrifugation increases, the effectiveness of seeding decreases. The best results would be obtained with centrifugation that is mostly or entirely axial.

[0117] FIG. **20** is a flow diagram illustrating an exemplary seeding process. Optionally, prosthesis **10** can be prepared for implantation by insertion into a protective sleeve **126**, as shown in FIG. **15** (**160**). Sleeve **126** provides protection against difficulties associate with handling prosthesis **10**. In addition, prosthesis **10** and sleeve **126** can be loaded into an element such as tube **152**, which provides further protection.

[0118] Optionally, one or more wetting agents (**162**) can be introduced into lumen **12** of prosthesis **10** to displace air inside lumen **12**. Wetting may take place in several stages, with later wetting agents supplanting earlier wetting agents. One of the wetting agents can provide a growth medium (**164**) for the cells that will seed luminal surface **14** of prosthesis **10**. Cells in suspension can be introduced into lumen **12** (**166**). Any technique can be used to introduce the cells, but an exemplary technique calls for dispensing the cells in suspension with a pipette or a sterile syringe and needle. It may be advantageous to introduce the cells in suspension along luminal surface **14**, rather than in the center of lumen **12**. To reduce the risk of creating air pockets, it may be advantageous to begin filling prosthesis **10** from the bottom up to the top.

[0119] Prosthesis **10**, with cells in suspension, is loaded into the centrifuge (**168**). As described above in connection with FIGS. **16-19**, loading may include a series of procedures, such as sealing tube **152** with plug **156**, inserting tube **152** into adapter **136**, and securing cap **158**. Axial centrifugation is performed (**170**). Examples of duration and degree of centrifugation are discussed above. It is usually desirable to perform axial centrifugation (**170**) promptly after cells in suspension are introduced into lumen **12** (**166**), so that the cell suspension is more uniform during centrifugation. Some cen-

trifuges support a short period of agitation prior to centrifugation. Agitation can help make the cell suspension more uniform.

[0120] Following centrifugation, prosthesis **10** can undergo an optional period of incubation (**172**). Incubation allows the seeded cells time to develop focal adhesions with luminal surface **14**, which will reduce the risk of later cell dislodgment. Incubation periods may vary in duration, for example, from five minutes to two hours. A typical incubation period may be twenty minutes. During incubation (**172**), it is possible that prosthesis **10** may be static, i.e., allowed to sit idle in the centrifuge at room temperature without any intervention. It is also possible that prosthesis **10** may be subjected to additional processing. Prosthesis **10** may be rotated at a much lower angular velocities, for example, applying lower g's. Another example of further processing is subjecting prosthesis **10** to pulsatile fluid flow that mimics the flow of fluid in the patient's body, which may enhance the acclimatization of the cells.

[0121] Following centrifugation and any incubation period, prosthesis **10** is unloaded from the centrifuge (**174**). Prosthesis **10** can be removed from protective sleeve **126** (**176**) and delivered for implantation in the patient (**178**). In some circumstances, removal of sleeve **126** (**176**) need not precede implantation (**178**). It may be possible to maintain prosthesis **10** inside protective sleeve **126** during implantation. Near the conclusion of implantation, e.g., just prior to or just after cross-clamp release, sleeve **126** can be removed. By keeping sleeve **126** in place, the risk or seeded cell loss during implantation can be reduced.

[0122] Preparation and seeding of prosthesis **10** can be performed in a matter of minutes, and can be performed in the operating room. Operating room personnel can, for example, introduce cells in suspension into lumen of prosthesis **10** (**166**), load prosthesis **10** into the centrifuge (**168**), operate the centrifuge to apply axial centrifugation (**170**), remove prosthesis **10** from the centrifuge (**172**) and the protective sleeve (**174**), and deliver prosthesis **10** for implantation in the patient (**176**). It may also be possible to place prosthesis in a protective sleeve (**160**) in the operating room, wet luminal surface **14** (**162**), and apply a growth medium (**164**). It may further be possible to perform additional functions in the operating room that are not shown in FIG. **20**, such as harvesting endothelial cells or cutting a prosthesis to a customized length for a patient.

[0123] The invention facilitates a "one-stage procedure," in which a vascular prosthesis is prepared for implantation and is implanted during a single surgical operation. This "one-stage procedure" has significant advantages over a conventional "two-stage procedure" for preparation of a vascular prosthesis for implantation. The "two-stage procedure" involves two surgical operations, typically separated by a month or more. In the first operation, the surgeon retrieves a source of endothelial cells from the patient. The surgeon does not implant a prosthesis during this first surgical operation. The medical staff harvests the endothelial cells, and cultures the cells (i.e., grows the cells in vitro) to increase their numbers. Culturing typically takes several weeks. Thereafter, the patient undergoes a second surgical operation to implant a seeded prosthesis. The medical staff seeds the prosthesis, and waits for a period after seeding to allow the cells to adhere to the prosthesis. Seeding may also entail employing adhesion-promoting substances, such as fibrin glue, that promote adhesion. After the waiting period, the medical staff supplies the seeded prosthesis to the surgeon for implantation.

[0124] The "one-stage procedures" shown in FIGS. **12** and **18** have the patient make a single visit to the operating room. Harvesting, prosthesis preparation and implantation can be accomplished during this single visit. Axial centrifugation can efficiently cause the seeded cells to accumulate evenly on the luminal surface within a matter of minutes. A single surgical procedure significantly benefits the patient in terms of convenience, comfort and cost.

[0125] The "one-stage procedure" omits culturing. In general, the purpose of culturing is to grow enough endothelial cells to compensate for cell losses that occur due to in vivo or postimplant washing away, and to form a confluent monolayer in the lumen. In the one-stage procedure, axial centrifugation of a prosthesis formed from ePTFE as described above can result in less risk of cells washing away because the seeded cells are received in the luminal surface of the prosthesis.

[0126] The one-stage procedure also omits the waiting period that allows the cells to adhere to the prosthesis after seeding. Because the recesses receive the cells, the cells are protected from washing away and can improve adhesion in vivo. Adhesion-promoting substances may be unnecessary. Administration of anticoagulant drugs can control the thrombotic potential of the prosthesis until the seeded prosthesis can form a confluent endothelial cell lining in the lumen. In addition, the one-stage procedure permits cells to grow under physiological conditions of pressure and shear stress, which promotes the formation of a more dense and orientated endothelial tissue lining.

[0127] Besides making a one-stage procedure feasible, the invention may result in one or more other advantages. In the case of a vascular prosthesis, fewer endothelial cells will be washed away from a luminal surface that includes recesses. As a result, the prosthesis maintains a high population of endothelial cells and can grow a confluent layer of cells in a short time. The prosthesis may also support in situ growth. If cell recesses are formed on substantially less than the full luminal surface of the prosthesis and if the seeding procedure deposits seeded cells onto the regions with recesses, fewer harvested cells are needed to seed the prosthesis. The harvested cells can be concentrated into cell-rich regions on the luminal surface supportive of rapid cell growth. The surface regions with cell recesses can be contiguous or interconnected by cell recess-containing paths to support formation of an endothelialized luminal surface. The patient benefits from the presence and health of the endothelial cells.

[0128] Moreover, various embodiments of the invention take advantage of physical properties of ePTFE, a material that has a proven track record in implantable medical devices. This material is biocompatible, and handles and sutures well. The techniques described herein for forming recesses and seeding do not adversely affect the favorable features of ePTFE. At the same time, the techniques described herein for forming recesses and seeding offer protection for endothelial cells as well as surface area for endothelial cell outgrowth.

EXAMPLES

[0129] FIG. **21** is a graph of exemplary data, with error bars, illustrating cell retention (identified with "% cell") as a function of axial centrifugation upon test devices. The designation "ePTFE" refers to one or more test devices constructed from material that has not been processed by rubbing, as described

above. The designation "bePTFE" refers to one or more test devices constructed from material that has been processed as described above. In particular, bePTFE has been rubbed with a brush, and the acronym stands for "brushed ePTFE." Each test device was about four cm long and about four mm in diameter. Axial centrifugation, where applied, was applied for five minutes using an apparatus similar to that shown in FIGS. **16-19**. Other data sets under different conditions have resulted in differing percentages of cell retention. Differences in conditions can include, for example, employment of wetting agents and growth medium. The data depicted in FIG. **21** are representative, however, of experimental results generally.

[0130] The left side data points of FIG. **21** represent cell retention without axial centrifugation, the center data points represent cell retention with axial centrifugation at 50 g, and the right data points represent cell retention with axial centrifugation at 250 g. As the data in FIG. **21** show, the bePTFE device demonstrated greater cell retention than the ePTFE device at all levels of centrifugation. The data in FIG. **21** also show, however, that axial centrifugation improves the cell retention on both the bePTFE device and the ePTFE device, and that higher g' s results in higher cell retention.

[0131] The data in FIG. **21** further illustrate that, even though higher g's results in higher cell retention, there is a "leveling off," and that, above a level of g' s, less marginal benefit is obtained by using higher g's. Further data, not shown in FIG. **21**, confirm the leveling off. In other words, although axial centrifugation improves cell retention for both the bePTFE device and the ePTFE device, the benefit achieved by higher g's diminishes.

[0132] FIG. **22** is a graph of exemplary data further illustrating cell retention following lower g and higher g axial centrifugation. Data pertaining to the ePTFE device are on the left and data pertaining to the bePTFE device are on the right. Once again, other data sets under different conditions resulted in differing percentages of cell retention, but the data depicted in FIG. **22** are representative of experimental results generally.

[0133] The "low g" data points represent data collected as the test devices were rotated at one rpm, thereby imparting very little axial centrifugation effect. For both the bePTFE device and the ePTFE device, cell retention was modest to poor at low g. When 250 g were applied, however, both devices demonstrated improved cell retention. The improvement of cell retention in the bePTFE device was markedly superior to the improvement demonstrated by the ePTFE device.

[0134] Experiments with test devices that include patterns of recesses also support the data presented in FIG. **22**. Cells may adhere to both ePTFE sites and bePTFE sites, but generally prefer the bePTFE sites. Also, brushing may produce lines or grooves of raised nodes. Following seeding with axial centrifugation, cells generally prefer to populate proximate to the grooves. Consequently, axial centrifugation can be used to support seeding of endothelial cells at specified sites on a device.

[0135] FIG. **23** is a graph of exemplary data illustrating cell retention with and without wetting, followed by axial centrifugation at 250 g for five minutes. Once again, other data sets under different conditions resulted in differing percentages of cell retention, but the data depicted in FIG. **23** are representative of experimental results generally. In particular, similar results appear when different levels of axial centrifugation are applied, and when no axial centrifugation is applied.

[0136] The test devices were wetted as described above with a basal growth medium commercially available from Cambrex as product CC-3 156, with SingleQuot® Supplements and Growth Factor, product CC-4143. Measurements of cell retention were taken promptly after centrifugation.

[0137] The data in FIG. **23** show that wetting depresses cell retention, but that the depression is significantly less with a bePTFE device than with an ePTFE device. It is believed that wetting can, in the short term, impair the ability of the cells to form focal adhesions to the device material.

[0138] The data in FIG. **23** do not compel the conclusion that wetting is undesirable, however. In spite of the depression of cell retention, wetting usually brings about many benefits, as described above, that outweigh the depression of cell retention. Generally speaking, wetting results in faster development of endothelial cells in the patient, and faster healing. In addition, activities following centrifugation, such as allowing incubation, can improve the ability of the cells to form focal adhesions to the device material when the device has been pre-wetted.

[0139] FIGS. **24** and **25** are tables showing data collected pertaining to devices seeded with axial centrifugation. The data were obtained by experimentation upon test devices that included ePTFE and bePTFE. The reported percentages represent averages for two test devices for each material.

[0140] In FIG. **24**, the test devices were about four cm long and about four mm in internal diameter. None of the test devices received a growth medium. Following introduction of cells into the respective lumens, all test devices were subjected to 250 g axial centrifugation for five minutes, using an apparatus similar to that shown in FIGS. **16-19**.

[0141] The row denoted "A" in FIG. **24** shows the percentage of cells retained on the respective materials promptly after centrifugation. Rows "B" and "C" show the percentage of cells retained on the respective materials following one hour of incubation, in which the seeded device remained untouched in the centrifuge. Row "B" relates to incubation with no additional processing, and row "C" relates to incubation with axial centrifugation at a reduced rate, one g. Row "D" shows the percentage of cells retained on the respective materials following five hours of incubation, in which the seeded device remained untouched in the centrifuge.

[0142] As FIG. **24** shows, a bePTFE device had a higher percentage of cell retention than an unprocessed ePTFE device, for each of the post-centrifugation activities. An hour of incubation, with and without low-g centrifugation, substantially increased percentage of cell retention for the ePTFE device, but not for the bePTFE device. It is believed that incubation enables cells to form focal adhesions to the ePTFE device, resulting in fewer cell dislodgements during handling. A five-hour incubation period, however, results in a drop in cell retention for devices made of both materials. Perhaps due to the absence of platelet-poor plasma in the experiment, the cells expired in a matter of hours.

[0143] In FIG. **25**, the test devices were about four cm long and about four mm in internal diameter. All test devices were subjected to 250 g axial centrifugation for five minutes, using an apparatus similar to that shown in FIGS. **16-19**.

[0144] Once again, a ePTFE device had a higher percentage of cell retention than an unprocessed ePTFE device, for each of the post-centrifugation activities. Row "A" of FIG. **25**

shows the percentage of cells retained on the respective materials promptly after centrifugation, and the results are comparable to those in row "A" of FIG. **24**. Row "B" of FIG. **25** shows the percentage of cells retained on the respective materials after an hour of static incubation, and these results are comparable to those in row "B" of FIG. **24**. The differences between the percentages in FIGS. **24** and **25** are within the range of error. Row "C" shows the percentage of cells retained on the respective materials when the test devices were not allowed to stay in the centrifuge for an incubation period, but instead were subjected to a pulsatile fluid flow for one hour. Pulsatile flow mimics the flow of fluid in the patient's body through the pumping of a fluid such as Plasma-Lyte® with platelet-poor plasma intermittently through the device. Pulsatile flow was hypothesized to enhance the acclimatization of the cells to the device. The percentage of retained cells declined markedly, however, perhaps because the cells had not had time to form focal adhesions, and were dislodged by the fluid flow.

[0145] Row "D" shows the percentage of cells retained on the respective materials when the test devices were allowed to stay in the centrifuge for a one-hour incubation period, then were subjected to one hour of pulsatile fluid flow. This processing did not result in improvement for the ePTFE device, but resulted in marked improvement for the bePTFE device. These data suggest that, in some cases, it may be advantageous to let the cells incubate and acclimatize prior to implantation.

[0146] Various embodiments of the invention have been described. The invention is not limited to the particular embodiments described above. In particular, the invention is not limited to vascular prostheses that include ePTFE. Although many implantable devices use ePTFE, other biocompatible materials, such as woven or veloured Dacron, also may used to form vascular prostheses or other implantable medical devices. Some materials may be processed as described above to create recesses sized to receive endothelial cells and may be seeded with axial centrifugation. In addition, the materials may be subjected to preimplantation processing in addition to that described herein. For example, to improve wettability, a device may be subjected to one or more gases-such as air, oxygen, argon, or water vapor-under gas-plasma discharge conditions, or treated with chemicals such as a sodium naphthalene complex.

[0147] Moreover, the invention is not limited to use of any particular apparatus. There are many different kinds of apparatus that can be used to seed vascular prostheses or other implantable medical devices with centrifugation, and the invention is not limited to the particular illustrative apparatus described herein. Furthermore, the invention is not limited to the exemplary centrifugation times or speeds mentioned herein. These and other embodiments are within the scope of the following claims.

What is claimed is:

1. A method for seeding cells comprising:

introducing cells into a lumen of an implantable medical device adapted to be implanted in a living body, wherein the lumen comprises a luminal surface comprising expanded polytetrafluoroethylene and wherein the lumen defines a longitudinal axis; and

applying centrifugation to the implantable medical device to rotate the implantable medical device around the axis.

2. The method of claim **1**, further comprising placing the implantable medical device in a protective sleeve prior to introducing the cells.

3. The method of claim **1**, further comprising delivering the implantable medical device for implantation in the living body after centrifugation.

4. The method of claim **3**, further comprising incubating the implantable medical device after centrifugation and before delivering the implantable medical device for implantation in the living body.

5. The method of claim **4**, wherein the incubating occurs for about five minutes to about two hours.

6. The method of claim **1**, further comprising:

wetting the luminal surface with a wetting agent;

placing a growth medium on the luminal surface, the growth medium supplanting the wetting agent; and

introducing the cells after the growth medium is placed on the luminal surface.

7. The method of claim **1**, wherein applying centrifugation to the implantable medical device comprises applying centrifugation for about 250 g to about 500 g to the luminal surface for about one to about ten minutes.

8. The method of claim **1**, wherein applying centrifugation to the implantable medical device comprises applying between about 1 g and about 10,000 g.

9. The method of claim **1**, wherein the cells are endothelial cells.

10. The method of claim **1**, further comprising:

placing the implantable medical device in a tube prior to introducing the cells, the tube comprising at least one open end;

introducing the cells into the lumen through the open end of the tube; and

sealing the open end of the tube with a plug.

11. The method of claim **10**, further comprising loading the sealed tube into a centrifuge.

12. The method of claim **1**, wherein the implantable medical device comprises a vascular prosthesis.

13. The method of claim **1**, wherein the implantable medical device comprises a mechanical heart valve, a bioprosthetic heart valve, a coronary stent, a stent graft or an AAA graft.

14. The method of claim **1**, wherein the surface comprising expanded polytetrafluoroethylene comprises nodes formed of polytetrafluoroethylene, and wherein the surface includes recesses defined by nodes lifted from the surface.

15. A method for seeding cells comprising:

introducing cells into a lumen of an implantable medical device adapted to be implanted in a living body, wherein the lumen includes a luminal surface having recesses defined by nodes lifted from the surface, and wherein the lumen defines a longitudinal axis; and

applying centrifugation to the implantable medical device to rotate the implantable medical device around the axis.

16. The method of claim **15**, wherein the luminal surface comprises expanded polytetrafluoroethylene.

17. The method of claim **16**, wherein the surface comprising expanded polytetrafluoroethylene comprises nodes formed of polytetrafluoroethylene, and wherein the surface includes recesses defined by nodes lifted from the surface.

18. The method of claim **15**, further comprising placing the implantable medical device in a protective sleeve prior to introducing the cells.

19. The method of claim **15**, wherein applying centrifugation to the implantable medical device comprises applying

centrifugation for about 250 g to about 500 g to the luminal surface for about one to about ten minutes.

20. The method of claim **15**, wherein applying centrifugation to the implantable medical device comprises applying centrifugation for about 1 g to about 10,000 g.

21. The method of claim **15**, further comprising:

incubating the implantable medical device after centrifugation; and delivering the implantable medical device for implantation in the living body.

22. The method of claim **15**, wherein the implantable medical device comprises a vascular prosthesis.

23. The method of claim **15**, wherein the implantable medical device comprises a mechanical heart valve, a bioprosthetic heart valve, a coronary stent, a stent graft or an AAA graft.

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