Mini Review

Mock Circulatory Systems for Vascular Tissue Engineering

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A circulatory mock system which biomimicks mechanical pulsatile stress field in arterial circulatory milieu plays significant roles for vascular tissue-engineering in the aspects of compliance matching and tissue morphogenesis, both of which appear to enable to architect a vascular graft morphologically and functionally resembling to those of a native artery. In this mini-review, fundamental effects of circulatory hydrodynamic forces, that is tangential shear stress and circumferential cyclic strain, are briefly summarized, followed by summary of various types of existing circulatory systems, and promising roles of the circulatory mock system in vascular tissue-engineering are discussed.

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Introduction

An arterial blood vessel is a vital life-line as to maintain physiological homeostasis under pulsatile flow milieu, which is characteristic of biomechanical stress field exposing hydrodynamic shear stress to luminal vessel surface and cyclic circumferential distension to vessel wall. These represent major mechanical stimuli-driven modulators of their biological functions at vascular cell and tissue levels.

When diseased vessels are hardly repaired by conventional surgical operations, artificial grafts are replaced with them to maintain blood circulatory system. However, smaller the inner diameter of the artificial graft, higher occurrence of occlusion is. That is, thrombus formation at the early stage of the implantation and neointimal hyperplasia at the late stage are principal determinants for occlusion. In fact, a clinically available small-diameter (less than 6 mm in inner diameter) vascular graft has not been materialized despite of many years' endeavor and attempts. In addition to inherent nature of blood compatibility of artificial graft, discussions have been made over the years that compliance matching between native and artificial grafts, upon the incorporation of mechanical responses to dynamic arterial stress field into design criteria of scaffold, becomes a primary

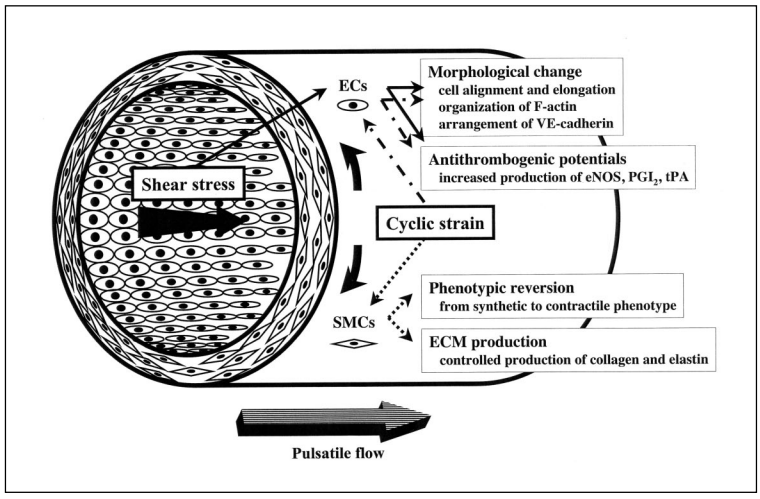

Fig.1 Mechano-biology of vascular cells under arterial circulatory milieu

factor determining the fate of implanted graft.

Studies on implantation of grafts into animals are time-, labor- and cost-consuming. Minimal try-and-error experiments may be realized by the use of an *ex vivo* arterial flow-simulator. In addition, in cardiovascular cell physiology, the effects of arterial flow dynamics on the cellular behaviors have been verified by an appropriate biosimulator. Further, enhanced tissue integrity of endothelium on engineered tissues must be" excised "*ex vivo* to avoid flow-induced cell and, or tissue detachment prior to direct exposure to arterial flow environment *in vivo*.

This mini-review focuses on the *ex vivo* realization of arterial circulation, by which pressure-diameter relationship (or compliance) of scaffold can be determined and pulsatile-induced morphogenesis of vascular cells and tissues can be simulated. Firstly, the significances of effects of pulsatile flow on vascular cells and compliance matching are summarized, followed by reviewing of various types of the circulatory apparatuses generating biomimicked mechanical forces, and present prototype of design of mechano-active artificial and tissue-engineered grafts.

Effects of Pulsatile Flow

Fig.1 shows mechano-biology under arterial circulatory system, in which hydrodynamic shear stress and pressure-dependent strain are major mechanical cues influencing tissue integrity and maintenance of vascular homeostasis.

1) Hydrodynamic shear stress-induced effects

Many previous studies demonstrated that endothelial cells (ECs) subjected to hydrodynamic shear stress undergo morphological changes which include their shapes from polygonal and cobblestone to elongated appearance with alignment parallel to the flow direction, and intracellular structural components such as cytoskeletal (F-actin) organization and cell-cell adhesive protein (VE-cadherin) arrangement[1,2]. Shear stress in a physiological range enhances production of antithrombogenic biological substances of ECs, such as endothelial nitric oxide synthase (eNOS), prostacyclin (PGI_2), tissue plasminogen activator (tPA)[3].

2) Cyclic strain-induced effects

In two-dimensional (2D) culture, both vascular cells [ECs and smooth muscle cells (SMCs)] subjected to cyclic strain demonstrate morphological changes to elongation and alignment perpendicular to the strain direction, which is due to " mechanical avoidance reaction "[4], whereas in three-dimensional (3D) culture, cells elongated and aligned parallel to the direction of distension, which is due to contact guidance of aligned extracellular matrices (ECM) which are induced by distension[5]. Similarly to shear stress, cyclic strain also increased production of antithrombogenic substances including eNOS, PGI_2, tPA from ECs[4,6]. Furthermore, cyclic strain induces phenotypic reversion from synthetic to contractile state of SMCs[4,7] as well as increased production of ECM including collagen and eleatin from SMCs and their supramolecular association[5,8]. These phenotypic and molecular events provide the basic rationales of making medial-like tissue using strain-loading bioreactor[9,10].

3) Synergic effects of shear stress and cyclic strain

Previous experimental circulatory apparatuses were designed to determine individual effect of shear stress or cyclic strain. For example, hydrodynamic shear-producing apparatuses used cone-plate or parallel-plate chamber[1-3], whereas distension- or strain-producing apparatuses used vacuum-induced substrate deformation technique, distension generated by an eccentric disk driven

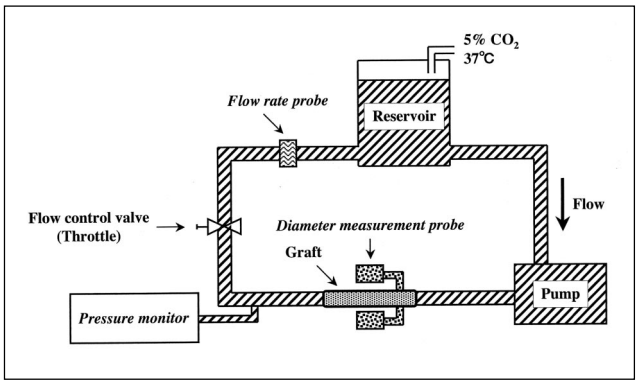

Fig.2 Basic components of pulsatile circulatory apparatus

Table 1 Pulsatile circulatory apparatuses

Operation and hydrodynamic parameters							Samples			Significances for tissue engineering	References
Type*	Pump	Pulsations	Fluid flow rate	Shear stress	Pressure	Strain	Graft (inner diameter)	Seeded cells	Response time	Flow-induced responses	
A	ventricle of artificial heart	60 bpm	NS	similar to femoral artery	NS	NS	ePTFE graft (6 mm)	HUVECs AHSVECs	48 hr	Reduction of cell loss by coating with fibrin glue	[12]
A	bellows pump	60 bpm	235/100 ml/min (mean; 150 ml/min)	2.15 dyn/cm^2	125/75 mmHg (mean; 100 mmHg)	2%	silicone tube (5 mm)	HSVECs HUVECs BAECs BASMCs	24 hr	Fabrication of new device producing biomimic pulsatile flow (fluid shear stress, wall strain and pressure)	[13]
A	steady flow pump + diaphragm pump	60 bpm	540 ml/min	8 dyn/cm^2	NS	10%	compliant tube (6 mm)	CAPE	24 hr	Synergic effect of shear and strain on ECs morphology and F-actin organization	[11, 14]
A	Cell-Max Quad pump	50-250 bpm	5-65 ml/min	0.9-1.4 dyn/cm^2 25.6-28.8 dyn/cm^2	NS	NS	Spun PU graft (1.5 mm)	BAECs	3 days 3 days	Improved cell adherence in response to *in vitro* shear stress	[15]
	Cell-Max Quad pump	NS	NS	1 dyn/cm^2 25 dyn/cm^2	NS	NS	Spun PU graft (1.5 mm)	RAECs	3 days 3 days	Increased antithrombogenic potential Reduced neointimal hyperplasia (*in vivo* study)	[16]
A	pulsatile pump + continuous pump	165 bpm (-)	NS 2-6 ml/min	NS 0.1-0.3 dyn/cm^2	NS NS	5% (-)	PGA tube (3 mm)	BASMCs BAECs	8 weeks 3 days	Fabrication of tissue-engineered medial-like scaffold Improved patency rate (*in vivo* study)	[9]
A	pulsatile pump	60 bpm	130 ml/min	NS	25 mmHg	5%	PLCL tube (4 mm)	RbSMCs	8 weeks	Fabrication of tissue-engineered medial-like scaffold	[10]
A	centrifugal pump	60 bpm	15-330 ml/min	0.3-5.8 dyn/cm^2	2.5/0.7-89.5/44.3 mmHg	1.4-7.2%	ELSP PLCL tube (2.3-2.5 mm)	(-)	NS	Dependence of compliance on wall thickness (Similar to native artery)	[17, 21]
B	roller pump + Tamari-Kaplitt pulser	50 bpm	300 ml/min	6-10 dyn/cm^2	systolic; 120 mmHg	NS	ePTFE graft (4 mm)	HUVECs	90 min	Optimal seeding density and preculture period confluent-seeded ECs for 24 hour-92% retention subconfluent-seeded ECs for 7 days-82% retention	[18]
B	roller pump + electromagnetic valve	120 bpm	11.3 ml/min	0.3 dyn/cm^2	(mean; 100 mmHg)	NS	Dog femoral artery *ex-vivo*	*ex-vivo*	180 min	PGI_2 production by shear stress normal flow (high shear); increased production abnormal flow (low shear); decreased production	[19]
B	centrifugal pump + variable outflow resistance	60 bpm	132 ml/min	NS	70/10-140/80 mmHg (mean; 30-100 mmHg)	NS	CPU tube (5 mm)	(-)	NS	Determination of dynamic compliance of CPU tube (Similar to human artery)	[20]

*Type A: "pump-induced flow-change type", Type B: "valve-induced resistance-change type"

Abbreviations: bpm, beats per minute; NS, not stated; ePTFE, expanded polytetrafluoroethylene; PU, polyurethane; PGA, polyglycolic acid; ELSP, electrospun; PLCL, poly(L-lactide-*co*-ε-caprolactone); CPU, poly(carbonate)-polyurethane; HUVECs, human umblical vein ECs; AHSVECs, adult human saphenous vein ECs; BAECs, bovine aortic ECs; BASMCs, bovine aortic SMCs; CAPE, calf pulmonary artery ECs; RAECs, rat aortic ECs; RbSMCs, rabbit aortic SMCs.

by a DC motor, speed motor-driven camshaft, and respirator[4-8]. However, a vessel wall is subjected to both shear stress and cyclic strain *in vivo*. Shear stress and cyclic strain have been advocated to have synergic effects on vascular cells in the meaning of morphological arrangement[11] and antithrombogenic (atheroprotective) benefits as described above. In reality closer to dynamic arterial circulation, one apparatus providing simultaneously hydrodynamic and wall distension effects on an artificial graft is mock circulatory system, as schematically shown in **Fig.2**. Such an apparatus producing biomimicked pulsatile flow provides a research tool for evaluation of mechano-active scaffold and tissue-engineered vascular graft (cellular function and tissue integrity).

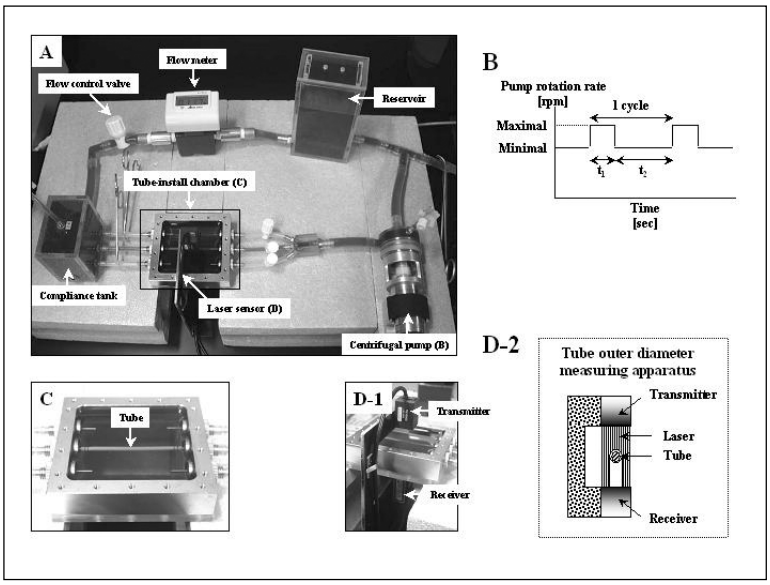

Fig.3 Pulsatile circulatory apparatus in our laboratory

A: Actual view of circulatory apparatus.

B: Schedule of periodic pump rotation rate generating pulsatile flow. The minimal rotation rate (duration; t_2 = 0.7 sec) was attenuated down to 70% of the maximal one (duration; t_1 = 0.3 sec), which produced physiologically simulated pulsatile flow at 1 Hz (60 beats per min).

C: Magnified image of tube-install chamber in photograph A. Electrospun PLCL tube was installed.

D: Laser sensor to determine outer diameter of installed tube.

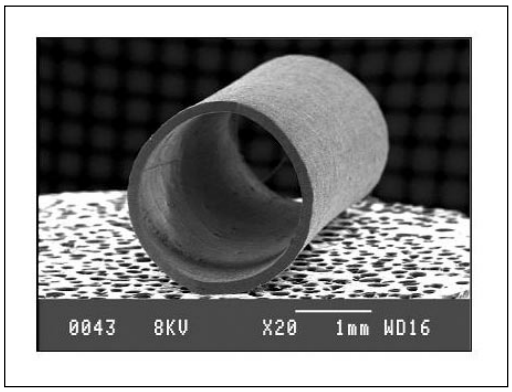

Fig.4 Scanning electron microscopy photograph of electrospun PLCL tube fabricated in our laboratory

Mock circulatory apparatus

1) Basic components of pulsatile circulatory apparatus

As shown in **Table 1**, several research groups including ours have designed mock circulatory pulsatile apparatuses[9-21]. As schematically shown in **Fig.2**, each apparatus is composed of quite similar basic components as follows: a pump, graft-connected tube, a flow control valve or throttle, and a reservoir, which correspond to the heart, artery, peripheral vessel resistance and vein *in vivo*, respectively, and all of them are connected to each other with elastic tubes to form a closed circuit. When the graft is inoculated or seeded with viable vascular cells, the circuit is filled with culture medium as a circulation fluid and maintained at constant temperature (37°C) and pH (7.4) with 5% CO_2. Some groups have added Dextran to the culture medium to increase the viscosity of the fluid, which is almost identical with that of blood[11,14,20]. To monitor and calculate the mechanical hydrodynamic forces (shear stress, cyclic strain and pressure) applied to the graft, measuring devices including a flow probe, diameter measurement probe and pressure monitor are equipped to this apparatus (described in **Fig.2** with Italic characters).

2) Principles to produce pulsatile flow

Reviewing the pulsatile circulatory apparatuses reported previously (**Table 1**), they appear to be divided into two major categorized groups according to the principles of generation of pulsatile flow. One group is“ pump-induced flow-change type ”(designated Type A) and the other one is“ valve-induced resistance-change type ”(Type B). In Type A, the pump, which biomimics the heart *in vivo*, produces alternately high and low fluid flow rates. These pumps include a ventricle of artificial heart[12], a bellows pump[13], a pulsatile pump[9,10,15,16] and combination of a roller pump with diaphragm pump or pulse generator[11,14]. Very recently, our laboratory has also designed an unique and simple circulatory apparatus classified as this Type A (**Fig.3A**), in which pulsatile flow is generated by the centrifugal pump, originally developed for ventricular assist device[21], periodically produced alternate minimal and maximal rotation rates as shown in **Fig.3B**[17].

On the other hand, resistance in the closed circuit determined by the degree of valve's opening is changed alternately to change steady flow produced by roller or centrifugal pump into pulsatile flow in type B[18-20]. The valve is in a more open state during so-called systolic phase while it is in a less open state during diastolic phase. For instance, Onohara et al[19] have succeeded to simulate various flow waveforms in human femoral arteries including the normal flow of healthy volunteers and abnormal flow of patients suffering from arteriosclerosis obliterans by chang-

ing the duration when the electromagnetic valve is open.

3) Applications of pulsatile circulation apparatus

Compliance matching between an elatomeric prosthetic graft and its host native artery is a principal issue for development of a small-diameter vascular graft. To incorporate compliance matching, a " compliant " graft should be designed and fabricated in materials, structure and fabrication aspects. The utilization of mock pulsatile circulation apparatus, therefore, enables to determine the mechanical responses including dynamic compliance of this graft. For instance, using our simulated circulatory apparatus (**Fig.3**), we have determined the dynamic compliance (defined as relative diameter change per one pulse) of the electrospun poly(L-lactide-*co*-ε-caprolactone) (PLCL) tube (**Fig.4**) under biomimicked stress field using an installed laser sensor (**Fig.3D**). Tai et al.[20] reported the similar study, in which the dynamic compliance of poly(carbonate)-polyurethane (CPU) based graft has been determined using a sophisticated, well-designed apparatus.

Not only compliance-matching but also antithrombogenic potential and tissue integrity are required to give the small-diameter vascular grafts to realize the clinical application of such grafts. To express native's nonthrombogenicity, a tissue-engineered approach in which a graft inner surface was covered with inherent nonthrombogenic liner, ECs, was devised and clinical trials have showed much higher patency rate than non ECs-seeded grafts. Several efforts to provide higher tissue integrity under hostile flow condition include pre-coating or immobilization of cell-adhesive substances, such as collagen, fibronectin, arginine-glycin-asparate (RGD) peptides, resulting in the formation of shear-resistant ECs layer prior to implantation. In addition, mechanical " preconditioning " concept has been introduced: ECs-seeded graft subjected to low shear stress under *in vitro* circulatory apparatus enables to obtain the morphologically and functionally biomimicked ECs layer to be shear resistant[15]. Thus, the intimal layer is transformed to a well-organized 2D tissue with high interaction strengths at both cell-substrate and cell-cell interfaces. In fact, it has also reported that such a " preconditioned " graft, implanted in the rat aorta, showed antithrombogenic potential at the early stage as well as inhibitory effect on neointimal hyperplasia at the latter stage[16], both of which are major roles for the long patency rate of small-diameter vascular grafts.

In addition to intimal tissue architecture, a medial tissue constructed from a biodegradable synthetic polymer, collagen and SMCs can be formed. Engineered grafts should withstand against periodically loaded physiological arterial pressure, which may induce rupture in the early stage and aneurysm formation in the latter stage. To avoid this problem, pulsatile circulatory apparatus or bioreactor may help regenerate highly integrated vascular tissue by ECM production from SMCs[5,8] and phenotypic reversion from synthetic to contractile state[4,7] (**Fig.1**). SMCs-inoculated biodegradable scaffold subjected to repeated cyclic strain obtains not only increased mechanical strength to resist arterial pressure but also contractive response to the contractive agents during the biodegradation of scaffold material[9,10]. The ECs-seeded on tissue-engineered biomimicked vascular medial-like scaffold showed promising patency rate *in vivo* study[9].

Conclusions

Using designed shear- and/or strain-producing apparatuses, the understanding of mechano-biology of vascular cells and tissue has been deepened, and the significances of mechanical hydrodynamic forces, both shear stress and cyclic strain, have been proved as to play crucial roles in the viewpoints of morphology, antithrombogenic potential, phenotypic reversion and ECM production. Circulatory apparatuses or bioreactors biomimicking *in vivo* milieu have been thus designed to obtain the synergic effects of these two forces, and provide solid basis of fabrication of more mechano-active and nonthrombogenic tissue-engineered vascular graft using autologous vascular, stem or progenitor cells. In addition to mechano-active scaffold design, the combination of strain-produced medial-like tissue and " preconditioning " of inimal-like tissue under the circulatory apparatus will realize the small-diameter vascular graft applicable in the clinical settings in future. In fact, two studies revealed that much higher patency and enhanced cell retention of 3 days- or 8 weeks-continuous loading of pulsatile flow *ex vivo* were reported as compared with those of non-loaded controls. Thus, the significant importance of realization of arterial flow circuit will have been verified by logical design of fabrication of engineered tissues, physiological study of vascular cell types enhanced patency of grafts.

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