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Chapter 6

Cardiovascular

Bioreactor system for tissue-engineered vascular construct

Although tissue-engineered great vessels and heart valves are currently undergoing clinical trials in some institutions [1, 2], their routine clinical application is still underway. One major problem thwarting their wide clinical usage is lack of physical endurance, which limits their application to cardiovascular structures exposed to high systemic pressure. Although durable scaffold materials have been designed to resist such high pressure *in vivo*, these materials remain subject to degradation over a longer period, resulting in unsatisfactory outcomes after implantation. It is well known that cardiovascular cells such as endothelial or smooth muscle cells are influenced by their extracellular environment, especially local fluid dynamics. Numerous scientists have reported the effect of shear stress or stretch stress on both endothelial and vascular smooth muscle cells [3–5]. Bioreactors for cardiovascular conduits are designed to utilize the effects of those physical strains on the cells to create more optimal tissue for grafting. Although a biomimetic *in vitro* environment is known to increase the endurance of tissue-engineered cardiovascular components [6], the optimal culture conditions including various pressure profiles are not known for a bioreactor. It was our aim to design a bioreactor system that can reproduce a wide range of pulsatile flows with a completely physiological pressure profile. To develop this novel bioreactor system, an intra-aortic balloon pumping (IABP) system was combined with an outflow valve and a compliance chamber to obtain both physiological systolic and diastolic pressures. The compliance chamber and the resistant clamps were designed to reproduce a physiological and relatively wide pressure waveform instead of the original peaky waveform generated by the power source. With a computed manipulation system, this novel bioreactor allows adjustment over a varying range of pressures, pulse rates, and intervals. In this study, we also demonstrated the morphology and the biochemical properties of the tissue-engineered products to illustrate the practicality of this novel bioreactor system. Details of the bioreactor system are presented below.

Bioreactor design

Our bioreactor housing and tubing are made of acryl and polyvinyl chloride (PVC), respectively. The bioreactor is small enough to fit inside a standard CO₂ incubator (Fig. 32), and the driving system is situated outside the incubator. The bioreactor consists of four chambers: a balloon chamber (1), a compliance chamber (2), a culture chamber (3), and a reservoir (4) (Fig. 33). The pressure generated by the IABP is conducted via the balloon (30 or 40 cm³), which is located inside the balloon chamber. The pulsatile flow is generated in the compliance chamber through a one-way outflow valve. The compliance chamber and the culture chamber are connected by a PVC tube with a clamp that controls the fluid flow and pressure. The tissue-engineered products are fabricated inside the culture chamber, which is connected to the reservoir via a PVC tube. A resistance clamp is located in the middle of the connection tube to regulate the afterload. The air

filter on the reservoir regulates the amount of CO₂ in the atmosphere. Fluids recirculate back to the balloon chamber via one-way valve from the reservoir. This system is sterilized with ethylene oxide. Conditions in the culture chamber are monitored by a pressure meter and a flow meter, and are recorded by a computerized analyzer.

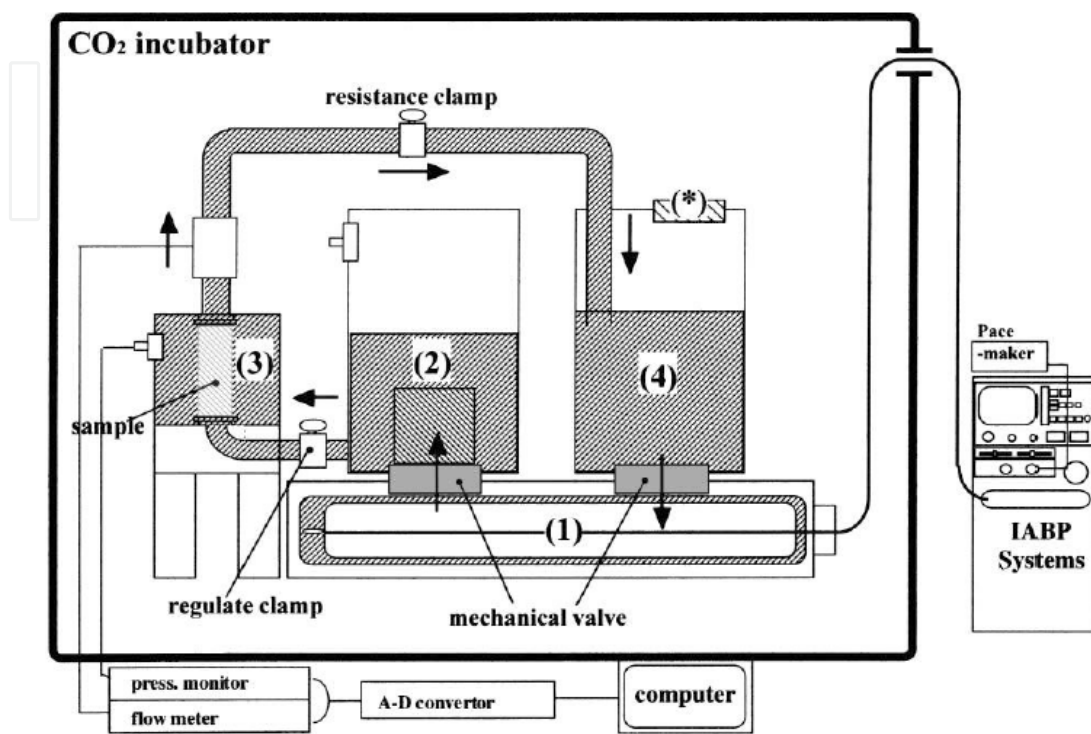


Fig. 32. Schema of the pulse-duplicating bioreactor system. The bioreactor is small enough to fit inside a standard CO₂ incubator, and the driving system is situated outside the incubator. The basal part of this bioreactor is the balloon chamber (1). Control of inflow to and outflow from the balloon chamber is by a mechanical valve. The compliance chamber (2) buffers the pumping flow and pressure. The engineered tissue sample is located in the culture chamber (3). A regulating clamp is placed between (2) and (3). The next chamber is the reservoir equipped with an air filter for a CO₂ incubator (4). The air filter (*) regulates the amount of CO₂ in the atmosphere. A resistance clamp between (3) and (4) regulates the afterload. Conditions in the culture chamber are monitored by a pressure meter and a flow meter, and are recorded by a computerized analyzer (From Narita et al. 2004. Reprinted with permission).

Bioreactor function analyses

After confirming the capability of this bioreactor system for flow, pressure, and frequency, we tried to evaluate the actual physical strains for the culture cells. First, the shear stress level (γ) in the scaffold was calculated as

$$\gamma = \mu \times du/dr = 4\mu Q/\pi r^3$$

where μ is the viscosity of the culture medium, du/dr is the velocity gradient, Q is the flow rate of the bioreactor, and r is the radius of the scaffold under the physiological or peaky waveform pattern of pulsatile flow (with a mean flow rate of 500 ml/min and 40 mmHg of systolic pressure). Next, we estimated the uniaxial cyclic strain under pulsatile flow by

measuring the diameter change of the scaffold under various pressure conditions, using a specially designed system. The system consisted of a digital video camera, a pressure monitor, a flow meter, and an analyzing computer. Under various conditions, the outside diameter of the scaffold, which was mounted in the culture chamber, was recorded with a digital video camera. The images were downloaded to a computer and the diameter was measured with NIH Image software. With this system, the actual stretch of the scaffold (only the direction perpendicular to the axis of the scaffold) could be estimated as a change in the scaffold radius as follows:

$$\partial = 100 \times (\pi D_{\max} - \pi D_{\min}) / \pi D_{\min}$$

where ∂ is the percentage of radial diameter change (which is equivalent to the change of scaffold length around the axis), D_{\max} is the maximum diameter, and D_{\min} is the minimum diameter of the scaffold. The sensitivity of the system was enough to detect a difference of more than 0.2%.

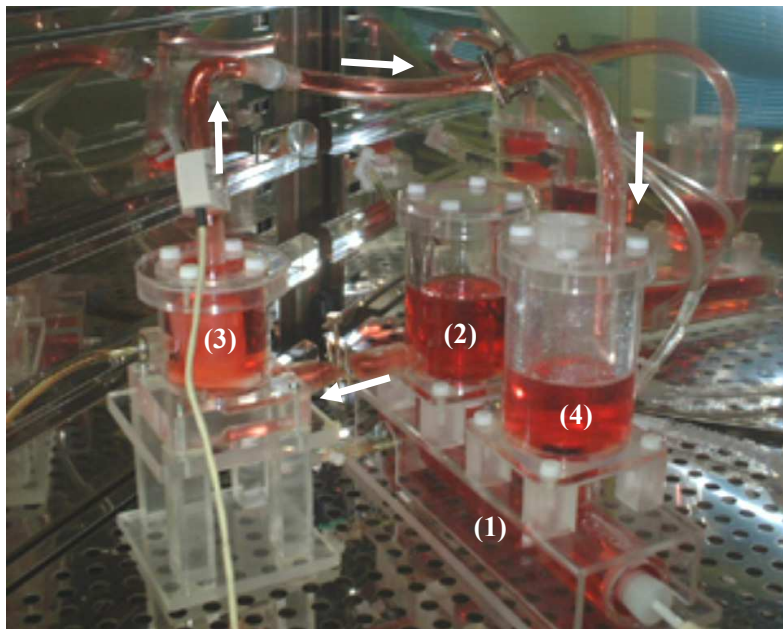


Fig. 33. Experimental setting of the pulse-duplicating bioreactor. The bioreactor consists of four chambers: a balloon chamber (1), compliance chamber (2), culture chamber (3), and reservoir (4). Pulsatile flow is generated in the compliance chamber (2) through a one-way outflow valve. The compliance chamber (2) and the culture chamber (3) are connected by a PVC tube with a clamp that controls the fluid flow and pressure. Tissue-engineered products are fabricated inside the culture chamber (3), which is connected to the reservoir (4) via a PVC tube. Fluids recirculate back to the balloon chamber (1) via a one-way valve from the reservoir (4) (From Narita et al. 2004. Reprinted with permission).

The combination of an outflow valve, compliance chamber, and resistant clamps together with a balloon pumping system was able to successfully reproduce both physiological systolic and diastolic pressures. The compliance chamber was especially effective in transforming the original peaky pressure waveform into a physiological pressure profile. The tissues, cultured under a physiological pressure waveform with pulsatile flow,

presented widely distributed cells in close contact with each other. They also showed significantly higher cell numbers, total protein content, and proteoglycan-glycosaminoglycan content than cultured tissues under a peaky pressure wave or under static conditions (Fig. 34). This new bioreactor system is suitable for evaluating a favorable environment for tissue-engineered cardiovascular components.

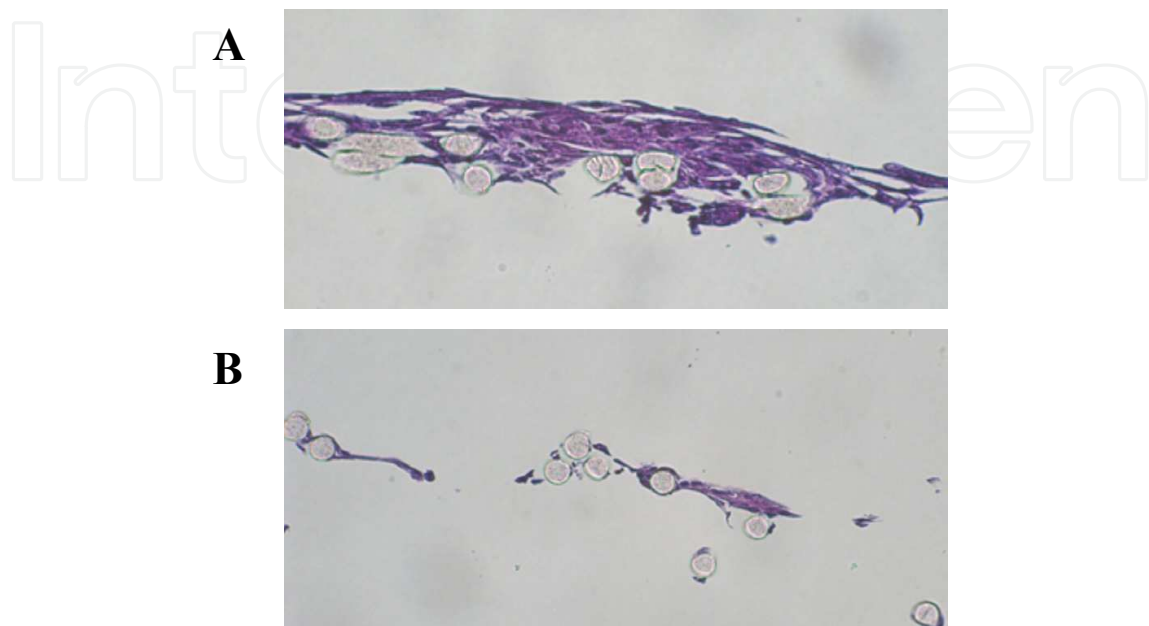


Fig. 34. Bright-field photomicrographs showing H-E staining of newly formed tissues under dynamic and static conditions. A: The tissues in group D-w were exposed to pressure with physiological wide-shaped waveforms for 7 days. B: Tissue in group S was maintained under static conditions. Note that cell density was clearly higher under dynamic conditions than under static conditions (Original magnification $\times 100$) (From Narita et al. 2004. Reprinted with permission).

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Tissue engineering, which aims at regenerating new tissues, as well as substituting lost organs by making use of autogenic or allogenic cells in combination with biomaterials, is an emerging biomedical engineering field. There are several driving forces that presently make tissue engineering very challenging and important: 1) the limitations in biological functions of current artificial tissues and organs made from man-made materials alone, 2) the shortage of donor tissue and organs for organs transplantation, 3) recent remarkable advances in regeneration mechanisms made by molecular biologists, as well as 4) achievements in modern biotechnology for large-scale tissue culture and growth factor production.

This book was edited by collecting all the achievement performed in the laboratory of oral and maxillofacial surgery and it brings together the specific experiences of the scientific community in these experiences of our scientific community in this field as well as the clinical experiences of the most renowned experts in the fields from all over Nagoya University. The editors are especially proud of bringing together the leading biologists and material scientists together with dentist, plastic surgeons, cardiovascular surgery and doctors of all specialties from all department of the medical school of Nagoya University. Taken together, this unique collection of world-wide expert achievement and experiences represents the current spectrum of possibilities in tissue engineered substitution.

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