# New Pulsatile Bioreactor for Fabrication of Tissue-Engineered Patches

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**Abstract: To date, one approach to tissue engineering has been to develop *in vitro* conditions to ultimately fabricate functional cardiovascular structures prior to final implantation. In our current experiment, we developed a new pulsatile flow system that provides biochemical and biomechanical signals to regulate autologous patch-tissue development *in vitro*. The newly developed patch bioreactor is made of Plexiglas and is completely transparent (Mediport Kardiotechnik, Berlin). The bioreactor is connected to an air-driven respirator pump, and the cell culture medium continuously circulates through a closed-loop system. We thus developed a closed-loop, perfused bioreactor for long-term patch-tissue conditioning, which combines continuous, pulsatile perfusion and mechanical stimulation by periodically stretching the tissue-engineered patch constructs. By adjusting the stroke volume, the stroke rate, and the inspiration/expiration time of the ventilator, it allows various pulsatile flows and different levels of pressure. The whole system is a highly isolated cell culture setting, which provides a high level of sterility, gas supply, and fits into a standard humidified incubator. The bioreactor can be sterilized by ethylene oxide and assembled with a standard screwdriver. Our newly developed bioreactor provides optimal biomechanical and biodynamical stimuli for controlled tissue development and *in vitro* conditioning of an autologous tissue-engineered patch.** © 2001 John Wiley & Sons, Inc. J Biomed Mater Res (Appl Biomater) 58: 401–405, 2001

## INTRODUCTION

Tissue engineering represents a new and promising concept to create functional and viable tissue from autologous cells for surgical application. In our laboratory, we are focusing on tissue engineering of cardiovascular structures using biodegradable polymer matrices as a scaffold for tissue development until the cells produce their own matrix. Autologous cells are seeded onto the polymer scaffold and form viable tissue while the polymer degrades. This *in vitro* created construct could potentially then be implanted, integrated into the native tissue, and utilize biological mechanisms for repair, remodeling, and growth. Although the feasibility of *in vitro* and *in vivo* formation of cardiovascular tissue has been demonstrated in previous experiments, the fabrication of viable tissue *in vitro* remains a significant problem for tissue engineering of autologous cardiovascular structures.[1–3]

In our current experiment, we focused on developing optimal *in vitro* conditions to ultimately fabricate autologous patch tissue for use in cardiovascular surgery. Our intent was to develop a device that provides dynamic cell culture conditions and directs new tissue development *in vitro*. This strategy has worked well for vascular and heart valve tissues.[4–6] However, we developed a new, pulsatile cell culture bioreactor, which provides physiological flow and a wide range of biomechanical stresses so that a viable and surgically feasible neo-patch tissue could be formed prior to implantation.

## METHODS AND RESULTS

### Bioreactor Design

The newly developed patch bioreactor is made of Plexiglas (Acryl/Mediport Kardiotechnik, Berlin) and is completely transparent, which is important in order to observe the tissue development during *in vitro* conditioning. The outer diameter is 150 mm and the height is 205 mm. The bioreactor consists of three different chambers: the air chamber (I) and two cell culture medium chambers (II and III). Chamber I is separated

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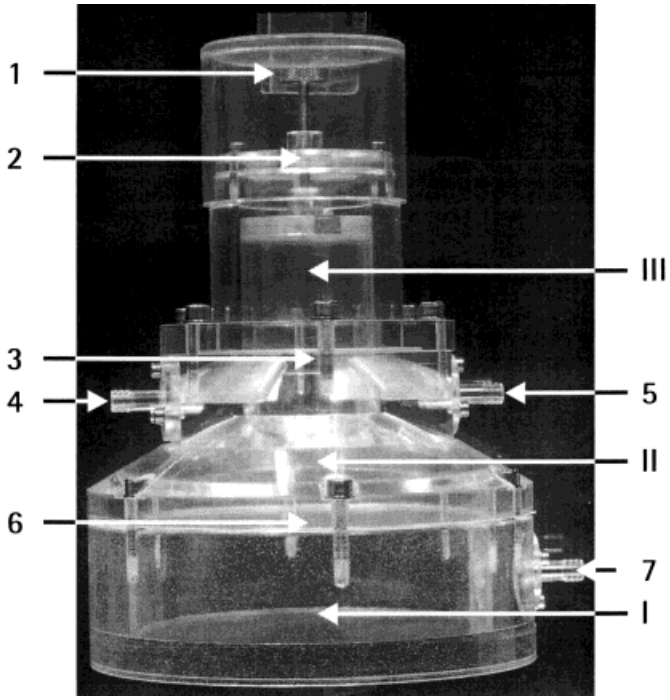



**Figure 1.** Pulsatile bioreactor.

from chamber II by a silicon diaphragm (6) (diameter 150 mm; thickness 0.5 mm; Mediport Kardiotechnik, Berlin) and connected to an air-driven respirator pump (7) with a single silicone tubing. The patch construct (3) divides chamber II from chamber III and is surrounded by cell culture medium that is continuously recirculated through a closed loop, which is connected to a reservoir filled with cell culture medium (total volume, 600 mL). Consequently, the patch constructs function as a diaphragm between chamber II and chamber III, which can be tightly fixed by pressing a Plexiglas cylinder (2) on to the outer edge of the patch (patch diameters ranging from 40–45 mm). The cell culture medium inlet (4) is connected to chamber II and is pointed directly at the patch. The medium outlet (5) is on the opposite side of the medium inlet and is connected to the cell culture medium reservoir through a silicone tube (Fig. 1). The whole system can be sterilized by ethylene oxide and assembled with a standard screwdriver.

### Bioreactor Function

The patch bioreactor has been developed as a closed loop perfused bioreactor. Therefore, the cell culture medium chamber II is connected to a separate medium reservoir via a valved inlet and a valved outlet tube. By pumping air into the air chamber I, the silicone diaphragm is lifted up and presses the cell culture medium against the patch construct, which is arched into chamber III. In parallel, the cell culture medium in chamber II is pumped through the valved outlet tube into the reservoir. In a second phase, the air in chamber I is sucked up by the respirator and the silicone diaphragm becomes arched into chamber I. Consequently, the cell culture medium is aspirated through the valved inlet tubing from the reservoir and is pointed directly at the patch construct, which is arched into chamber II. In this conditioning phase, the surface shear stress ($\tau_w$) we calculated ranged from 0.2–10 dyne/cm$^2$, which occurred at the center of the patch construct. The varying shear stress rates are related to different flow rates, which can be adjusted while conditioning the tissue-engineered patch construct.

In a preliminary bioreactor experiment, using a polymeric patch scaffold (polyhydroxyalcanoid; Tepha Inc., MA) seeded with vascular cells, the histologic examination showed a confluent cell layer on the polymer. Hematoxylin and eosin stain showed the cells grew into pores and formed viable connective tissue between the inside and the outside of the patch construct (Fig. 2).

We thus developed a closed-loop perfused bioreactor for long-term patch-tissue conditioning, which combines continuous, pulsatile perfusion and mechanical stimulation by periodically stretching the tissue-engineered patch construct [Fig. 3(a)–(b)].

### Experimental Setting

The described bioreactor is placed into a standard humidified incubator at 37 °C and 5% $CO_2$ (Forma Scientific Inc., USA) and connected to a reservoir (Trypsinizing Flask 355757; Wheaton Inc., USA) filled with cell culture medium. The whole system is air-driven by a simple respirator (dual-phase control ventilator, Harvard Apparatus Inc., USA), which is placed outside the incubator and connected to the air chamber of the bioreactor by a single silicon tube (Fig. 4). By adjusting the stroke volume, the stroke rate and the inspiration/expiration time of the ventilator, various pulsatile flows and biomechanical stresses were established. The flow rates ranged from 60 mL/min to 2.5 L/min and pressures from 5 mmHg to 250 mmHg [Fig. 5(a)–(b)].

## DISCUSSION

The use of patches in cardiac surgery and particularly in congenital cardiac surgery is a widely accepted surgical tech-

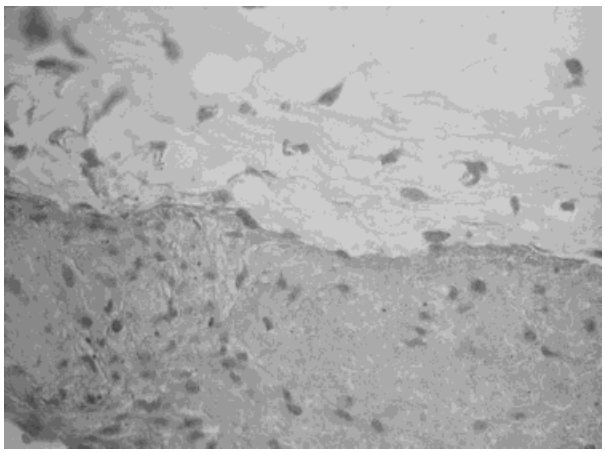


**Figure 2.** Hematoxylin and eosin staining of a tissue-engineered patch after 4 days of incubation in the newly developed patch bioreactor, demonstrating cell ingrowth into the pores of the polymeric scaffold and the formation of multiple cell layers on the flow-exposed side of the patch construct.

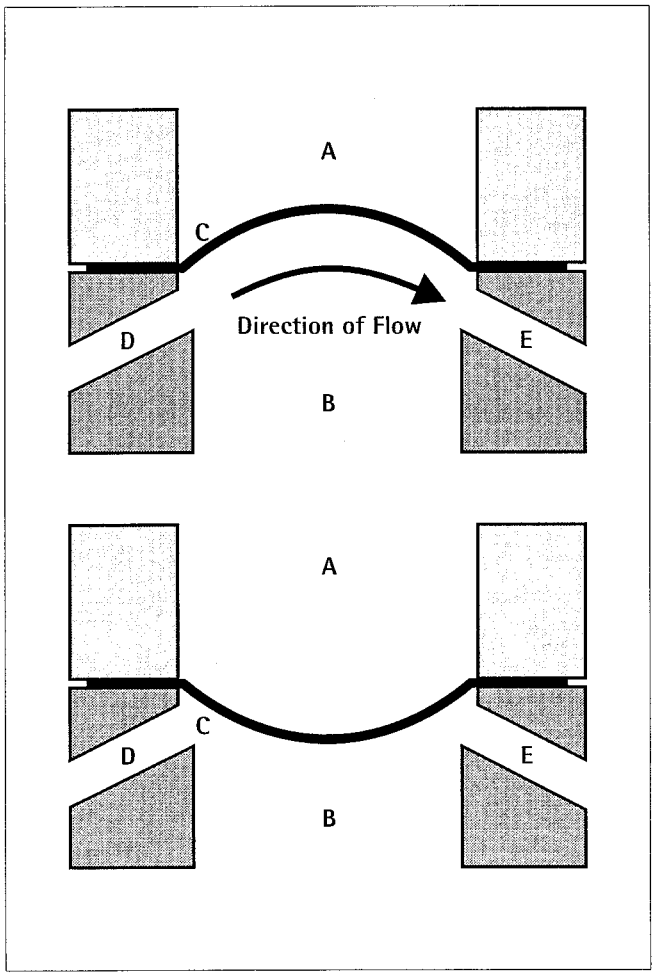



**Figure 3.** (a)–(b) Periodical stretching and flow exposure of the tissue-engineered patch.

nique for repair or reconstruction of cardiovascular structures. Currently, the choice of patch material is limited to prosthetic materials (e.g., Dacron) or glutaraldehyde-fixed pericardium. Both materials are used in numerous surgical procedures for patch closure or reconstruction of cardiovascular tissues with the appropriate results. Beside this, each of the patch materials used has certain limitations, such as aneurysm formation in patch aortoplasty, inelasticity of the prosthetic materials, and an increased risk of hemolysis induced by contact of the blood with the prosthetic materials.[7–9]

To overcome these limitations, our laboratory started focusing on the use of tissue-engineering techniques to create an autologous and viable patch construct for cardiovascular surgery. One approach in tissue engineering is to establish optimal *in vitro* conditions to guide tissue development and to create cell-polymer constructs with a high degree of maturity prior to implantation. The ideal *in vitro* conditions for the formation of such a tissue construct are not known, but it is widely confirmed that a dynamic tissue environment stimulates extracellular matrix formation that might lead to appropriate biochemical and biomechanical properties in tissue-engineered constructs prior to potential implantation.[10,11] For this reason, we started developing a new pulsatile bioreactor for the fabrication of tissue-engineered patches.

In our experiment, we developed a device that provides physical signals comparable to those that a patch is exposed to under *in vivo* conditions, including flow and pressure. On reviewing the literature, we noted that the combination of flow-induced and biomechanically induced stress might be a significant factor for appropriate tissue development *in vitro*.[12,13] Using this concept, Niklason et al. were able to fabricate solid vascular tissue comparable to a native vessel.[14] However, in our experimental setting, the patch construct was stretched by arching into one of the cell culture chambers and, at the same time, the cellular surfaces were continuously exposed to varying rates of pulsatile flow. We combined flow and biomechanical stress in order to provide physical signals to induce extracellular matrix formation and to ultimately form reliable patch tissue prior to *in vivo* implantation.

We present a technical description of a pulsatile bioreactor for the fabrication of a tissue engineered patch construct, which has not been described previously. The newly developed bioreactor is a compact system, which can easily be placed in a standard humidified incubator. This is important with regard to conditioning a patch construct under optimal cell culture conditions and additionally to minimize the risk of potential microbiological contamination. The materials used in the bioreactor (Plexiglas, silicone, and stainless steel screws) are very robust and heat resistant so that the whole system can be sterilized in a standard ethylenoxide gas sterilization process (6% EO/ 94% $CO_2$, 37 °C, Vanguard, Berlin). Furthermore, the bioreactor design and the operating principles have been kept simple, so that the perfused tubes and chambers are easy and safe to reassemble under sterile

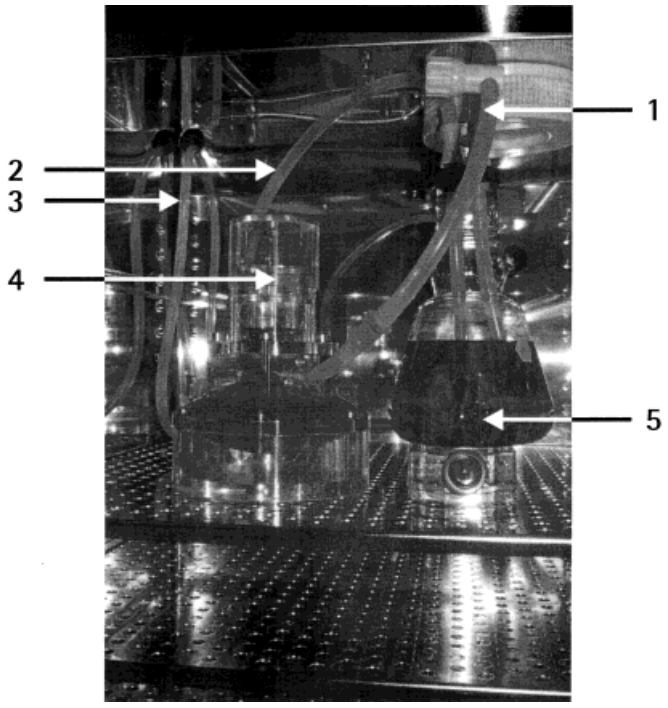
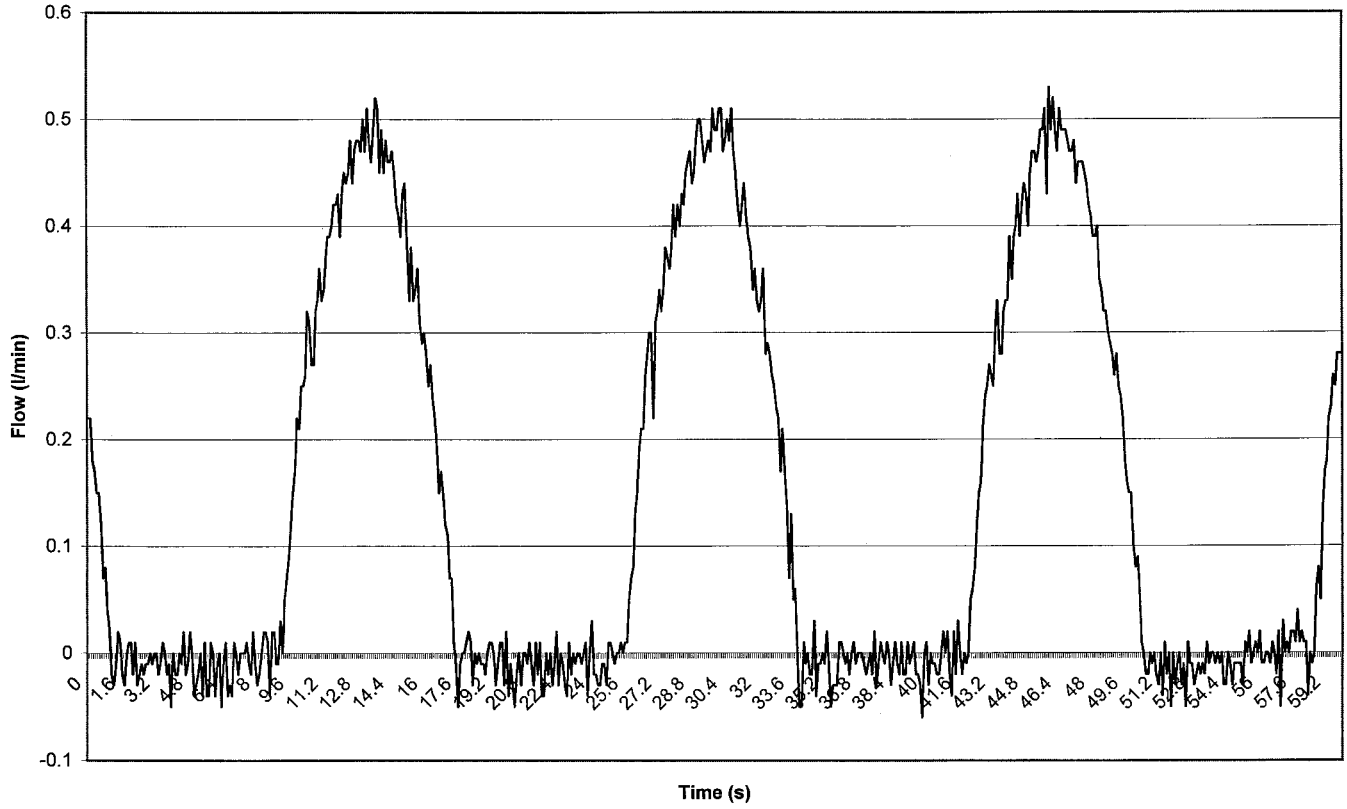



**Figure 4.** Experimental setting inside a standard incubator: (1) valved outlet tubing; (2) valved inlet tubing; (3) single silicon tube connecting the air chamber of the bioreactor and the respirator outside the incubator; (4) bioreactor; (5) reservoir filled with cell culture medium.



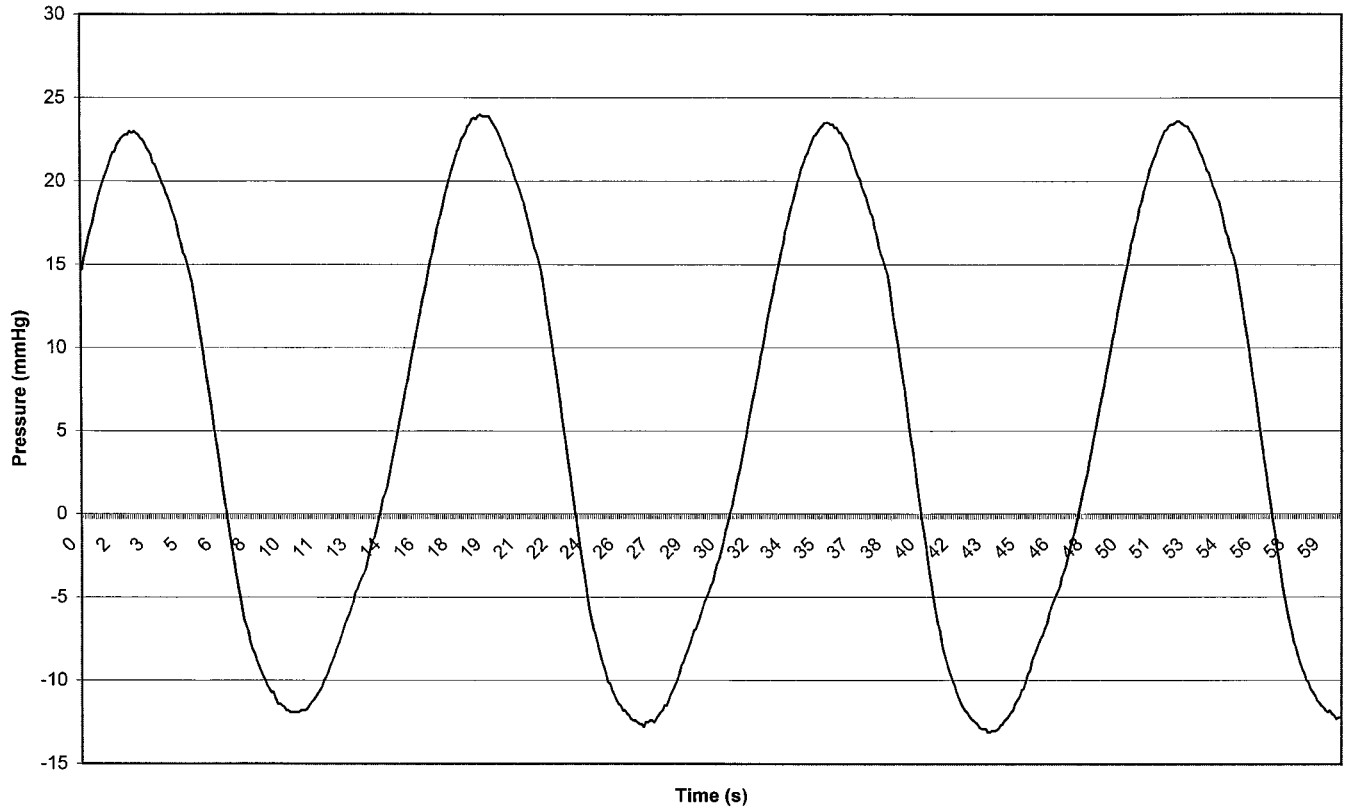




**Figure 5.** (a)–(b) Example of a flow and pressure curve during patch conditioning.

conditions in a standard cell-culture safety cabinet. Additionally, with the newly developed bioreactor, it is possible to adjust sub- and supraphysiological flow and pressure conditions to pretest the functional and mechanical requirements for a tissue-engineered patch construct prior to final implantation.

The whole system is highly transparent, which is important to observe the newly developed tissue and to detect potential contamination. In a preliminary bioreactor experiment, we demonstrated the feasibility of the approach and showed that the bioreactor can support the growth of vascular cells and ultimately the development of a viable tissue-engineered patch construct.

In general, the main function of our bioreactor is to provide optimal biomechanical and biodynamic stimuli for controlled patch-tissue development *in vitro.* Currently, our bioreactor design seems to offer theoretical advantages for *in vitro* conditioning of a tissue-engineered patch. Further advances in bioreactor technology, specific growth conditions, and polymer chemistry are likely to have a major impact on the development of mature tissue prior to implantation in the cardiovascular system.

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