CARDIAC TISSUE ENGINEERING

GORDANA VUNJAK–NOVAKOVIĆ[1]
MILICA RADIŠIĆ[1]
BOJANA OBRADOVIĆ[2]

[1]Massachusetts Institute of Technology, Cambridge MA 02139, USA

[2]Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia and Montenegro

The restoration of normal cardiac function can potentially be achieved by functional tissue constructs grown in vitro by using differentiated or progenitor cells, biomaterial scaffolds and bioreactors. Engineered tissues can also serve as high–fidelity models for basic studies of cells and tissues, in response to gene alterations, drugs, hypoxia, or physical stimuli.

Tissue engineering combines the principles of biology, engineering and medicine to create functional, biologically based grafts capable of repairing native tissues following a congenital deformity, disease or trauma. Cardiac tissue engineering has been motivated by the high incidence and mortality of heart disease, and by the minimal ability of cardiac muscle to regenerate after myocardial infarction. Engineered constructs may serve as grafts for myocardial repair, if they are thick and compact, contain a high density of metabolically active cells, and contract synchronously in response to electrical stimulation. Engineered tissues of such a high fidelity can also serve as models for basic studies of cell function and tissue development, and responses to gene alterations, drugs, hypoxia, and physical stimuli.

In vivo, cell differentiation and tissue assembly are directed by multiple factors acting in concert and according to specific spatial and temporal sequences. *In vitro*, cell function can be modulated by the same factors known to play a role during normal embryogenesis. Biophysical regulation of cultured cells can be achieved by an integrated use of biomaterial scaffolds and bioreactors, as shown in Fig. 1. A "biomimetic" approach to tissue engineering involves the in vitro creation of functional tissues by an integrated use of: (i) cells that can be selected, expanded, and transfected to express the genes of interest, (ii) biomaterial scaffolds that serve as a structural and logistic template for tissue development and biodegrade at a controlled rate, and (iii) bioreactors that provide environmental conditions necessary for tissue development.

Scaffold seeding is the first step of the bioreactor cultivation of engineered tissues. Seeding requirements include (i) high cell yield (to maximize cell utilization), (ii) high kinetic rate of cell attachment (to minimize the time in suspension), (iii) high and spatially uniform distribution of attached cells (to promote rapid and spatially uniform tissue assembly) (1,2), and (iv)

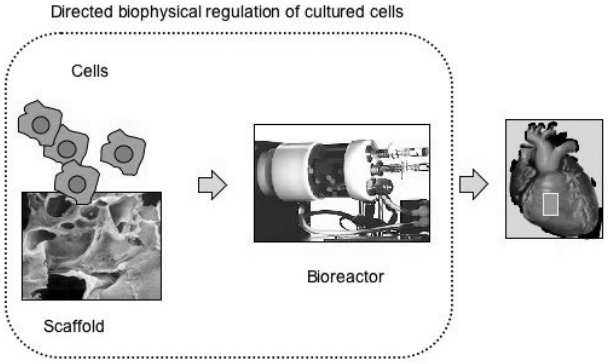

Figure 1. Tissue engineering paradigm. The regulatory factors of cell differentiation and tissue assembly in vivo can be utilized in vitro to engineer functional tissues by an integrated use of isolated cells, biomaterial scaffolds and bioreactors. The cells themselves (either differentiated or progenitor/stem cells seeded onto a scaffold and cultured in a bioreactor) carry out the process of tissue formation, in response to regulatory signals. The scaffold provides a structural, mechanical and logistic template for cell attachment and tissue formation. The bioreactor provides the environmental conditions and regulatory signals (biochemical and physical) that induce, enhance or at least support the development of functional tissue constructs.

immediate establishment of interstitial flow to prevent damage and death of cardiac myocytes due to hypoxia. For engineered cardiac muscle, cell densities that need to be established on seeded scaffolds are ≥ 100 million cells/cm^3 of scaffold volume. In particular, cardiac myocytes require efficient supply of nutrients and oxygen at all times during seeding and cultivation, in order to maintain their viability and function. The technique of seeding that was specifically developed for cardiac myocytes involves: (a) rapid inoculation of cardiac myocytes into collagen sponges using Matrigel® as a cell delivery vehicle, and (b) transfer of inoculated scaffolds into perfused cartridges with immediate establishment of the interstitial flow of culture medium. In this system, cells are "locked" into the scaffold during a short (10 min) gelation period, and evenly distributed by medium perfusion. Forward–reverse flow was used to further increase the rate and spatial uniformity of cell attachment [1].

Author address: Gordana Vunjak–Novaković, Massachusetts Institute of Technology, Cambridge MA 02139, USA

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Bioreactor cultivation of cardiac tissue constructs has been done routinely in static flasks, mixed flasks, rotating vessels and perfused cartridges [3–5]. The hydrodynamic environment of a bioreactor is important as it can affect cell function in at least two ways: via associated effects on mass transport between the tissue and culture medium, and by direct physical stimulation of the cells. In static flasks, the cultured tissues are fixed in place and exposed to static medium, such that the mass transport between the tissue and culture medium is governed by molecular diffusion, and there is no hydrodynamic shear acting at the cells. In mixed flasks, tissues are fixed in place and exposed to well mixed medium. The flow conditions were characterized as turbulent, with the associated hydrodynamic shear that was below the level causing cell death or damage, but sufficient to affect the function of cells at construct surfaces [3]. Mass transport between the tissue and culture medium is enhanced by convection, whereas the transport within the tissue remains governed by molecular diffusion like in static flasks. In rotating vessels, cultured tissues are dynamically suspended in the rotating flow without external fixation. The flow conditions were characterized as dynamic and laminar, with tissue constructs settling in a tumble–slide regime associated with fluctuations in fluid pressure, velocity and shear [3]. Mass transport between the tissue and culture medium is enhanced by dynamic laminar convection, a flow regime that is stimulatory to the cells, whereas the transport within the tissue remains governed by molecular diffusion. In perfused cartridges, cultured tissues are perfused with culture medium, at interstitial velocities comparable to those of blood flow in native tissues. Medium flow is within the laminar regime, and can be either steady or pulsatile. Mass transport is enhanced by interstitial flow of medium in conjunction with external gas and medium exchange [6–8].

Cardiac tissue is normally vascularized, with capillaries spaced at 20 μm distances, an indicator of the high oxygen demand [9–10]. Therefore, tissue engineering of 1–5 mm thick, functional cardiac constructs critically depends on our ability to seed the cells at a high and spatially uniform initial density, and to enhance the supply of oxygen to maintain their viability and function. To engineer thick, compact, functional cardiac constructs, we developed a "biomimetic" culture system that can mimic the convective–diffusive oxygen transport present *in vivo* and maintain oxygen supply to the cells at all times during the *in vitro* cultivation. Physiologic densities of viable cardiac myocytes were achieved by rapid cell inoculation into a porous scaffold using Matrigel® followed by the establishment of the perfusion of culture medium through the construct immediately after gel hardening (15 min) [1,2]. Non–perfused constructs had most cells located in the 100–200 μm thick layer at the top surface, and only a small number of cells penetrated the entire construct depth (Fig. 2, left). Constructs seeded in perfusion exhibited high and spatially uniform cell density throughout the construct volume (Fig. 2, right).

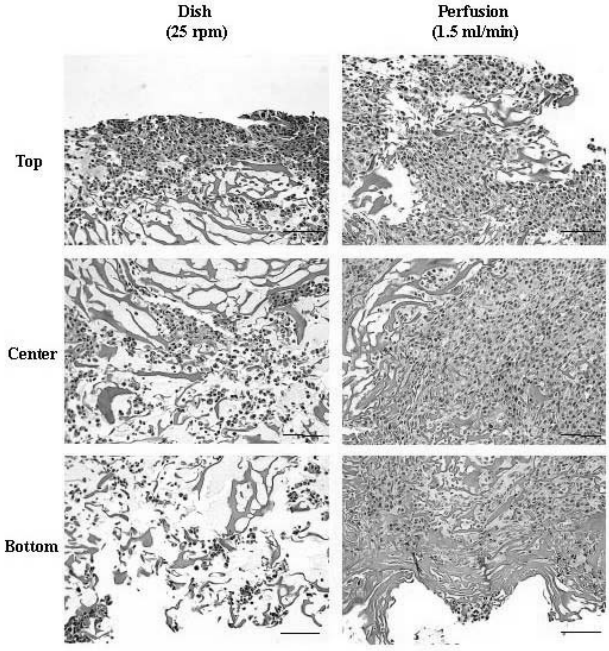

Figure 2. Effects of perfusion on cardiac cell distribution. Cross–sections of constructs inoculated with 2 million cells and transferred either into dishes (25 rpm, left) or into perfused cartridges (1.5 ml/min, right). The top, center and bottom areas of a 650 μm wide strip extending from one construct surface to the orther are shown. Scale bar 100 μm [8].

To provide convective–diffusive oxygen supply to the cells, interstitial medium flow was maintained for 7 days during cultivation; constructs seeded and cultivated in dishes served as a control. Throughout the cultivation, the number of live cells in perfused constructs was significantly higher than in dish–grown constructs [8]. Notably, the number of live cells in dish–grown constructs decreased rapidly during the first day of culture and continued to decrease between days 1 and 7. In contrast, live cell numbers in perfused constructs were constant during day 1, and decreased only slowly with time in culture. Cell viability was significantly higher in perfused than in dish–grown constructs at all time points. Importantly, the final cell viability in perfused constructs (81.6±3.7%) was not significantly different than the viability of the freshly isolated cells (83.8±2.0) and it was markedly higher than the cell viability in dish–grown constructs (47.4±7.8%). The molar ratio of lactate produced to glucose consumed (L/G) was ~1 for perfused constructs throughout the duration of culture, indicating aerobic cell metabolism. In dishes, L/G increased progressively from 1 to 2, indicating a transient to anaerobic cell metabolism. Cell damage was assessed by monitoring the activity of lactate dehydrogenase (LDH) in culture

Table 1. Effects of perfusion on contractile properties of 7–day constructs. Excitation threshold (ET) was measured at a stimulation frequency of 60 bpm. Maximum capture rate (MCR) was measured at voltages equivalent to 150% and 200% ET. Data were collected before the treatment with palmitoleic acid (before PA) and after the washout (after PA); constructs could not be induced to contract in the solution containing PA. Data are expressed as Ave ± SE. p values were calculated by one–way ANOVA in conjunction with Tukey's test for pair–wise multiple comparisons. (n = 2–6). Differences were considered statistically significant if p < 0.05 [8].

	Neonatal rat ventricle	Perfused construct (7 days @ 0.5 ml/min)	Dish–grown construct (7 days @ 25 rpm)
ET (V)			
Before PA*	1.6±0.1	3.3±0.2*	4.5±0.4*[&]
After PA**	1.5±0.1	3.5±0.1*	4.4±0.1*[&]
MCR at 150%ET (bpm)			
Before PA*	413±7	420±30	502±32
After PA**	465±15	415±35	378±31[§]
MCR at 200% ET (bpm)			
Before PA*	427±40	415±45	523±14
After PA**	427±58	435±45	380±31[§]

*significant difference between constructs and neonatal rat ventricles
[&]significant difference between perfused and dish–grown constructs
[§]significant difference before and after PA treatment

medium. At all time points, the levels of LDH were significantly lower in perfusion than in dish cultures, indicating that medium perfusion reduced cell damage.

Spontaneous contractions were observed in some constructs early in culture (dish–grown constructs 2–3 days after seeding), and ceased after approximately 5 days of cultivation, indicating the maturation of engineered tissue. In response to electrical stimulation (e.g., at 5V and 60 bpm), all constructs were reproducibly induced to contract synchronously. However, in perfused constructs the contraction frequency was constant, whereas in dish–grown constructs the contraction frequency spontaneously increased every 1–2 min and the contraction pattern appeared arrhythmic. Overall, medium perfusion during cell seeding and construct cultivation markedly improved the contractile behavior of engineered cardiac constructs (Table 1).

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