ELSEVIER

Biomaterials 24 (2003) 89–95

Biomaterials

www.elsevier.com/locate/biomaterials

An in vitro model of pericardial tissue healing

Douglas C. Marchion, Jean C. Pfau, Patricia A. Weber, Albert C. Grobe, Carlos M.G. Duran*, David T. Cheung

The International Heart Institute of Montana Foundation, 554 West Broadway, 59802-4008, Missoula, MT, USA

Received 8 August 2001; received in revised form 20 March 2002; accepted 21 June 2002

Abstract

Introduction: A previous study in our laboratory showed that a flap of fresh autologous pericardium bisecting the aorta of sheep retracted and became fibrotic. Histologic analyses suggested that activated cells within the pericardium contributed to the retraction of the implant. Here we report the development of an in vitro model to investigate the effects of serum on cellular proliferation and cell-mediated tissue contraction.

Methods: Sections of living and ethanol-treated sheep pericardium were incubated with 0.5%, 5%, 10%, 20%, and 50% serum in medium for up to 8 days and evaluated for cellular proliferation and tissue contraction. These serum-stimulated events were further evaluated in the presence of Mitomycin C, Cytochalasin B and D, Aphidicolin, AraC, and Cycloheximide.

Results: Cellular proliferation and cell-mediated tissue contraction were induced by serum in a dose-dependent manner. Expression of PCNA was suppressed in the presence of Cytochalasin B, Cytochalasin D, Aphidicolin, and AraC. Tissue contraction was prevented by Cycloheximide. Mitomycin C inhibited both proliferation and tissue contraction. Ethanol-treated tissue, which was absent of living cells, did not respond to stimulation with serum.

Conclusions: An in vitro model was developed to study the responses of cells within pericardial tissues to stimulation by serum. In this model, serum induced cellular proliferation and tissue contraction. Different chemical inhibitors independently modulated these serum-stimulated events. Pre-existing cells within pericardial tissues might respond to stimulus through differential pathways. This model may help to develop methods to make autologous pericardium a clinically useful biomaterial.

Keywords: Pericardium; In vitro *model*; Contraction; Proliferation; Serum

1. Introduction

Since the early days of cardiovascular surgery, autologous pericardium has been used as a repair biomaterial [1,2]. Its accessibility within the operative field and the ease of obtaining large sheaths of pliable material made it an obvious choice to the surgeon. Autologous pericardium was first used as a patch to close atrial septal defects and to enlarge the right ventricular outflow tract [3–5]. Valve-bearing conduits were also constructed and used in the low-pressure right side of the heart [6,7]. Although it healed well to the surrounding tissues and therefore solved the clinical problem, thickening and retraction of the pericardium was a constant finding. When used as a large patch to redirect blood flow within the atria, reoperation was often needed because of retraction [8]. However, autologous pericardium became dilated and even aneurysmatic when used in a high-pressure environment such as a large ventricular septal defect, the right outflow tract in the presence of pulmonary hypertension, or in the aorta [9]. In practice, the clinical use of autologous pericardium has been abandoned; but, recently, the need for a biomaterial in heart valve repair has rekindled interest. Its experimental use for leaflet enlargement and mitral valve chordae replacement still shows progressive fibrosis and severe retraction [2,10]. Despite these negative findings and in the absence of a satisfactory biomaterial with the accessibility, low cost, non-immunogenicity, and freedom from donor-derived pathogens, autologous pericardium remains an

*Corresponding author. Tel.: +1-406-329-5668; fax: +1-406-329-5880.

E-mail address: duran@saintpatrick.org (C.M.G. Duran).

0142-9612/02/$ - see front matter
PII: S0142-9612(02)00255-7

attractive alternative provided its retraction and dilatation response can be controlled.

To better understand this issue, our laboratory investigated healing reactions of vital autologous pericardium implanted as a flap that bisected the descending aorta of sheep to mimic a cardiac valve leaflet [11]. In this in vivo model, the pericardial flap became fibrotic and was characterized by cellular accumulation and tissue retraction. Histologic analyses of recovered implants indicated that activation of cells endogenous to the pericardium contributed to the detrimental healing outcomes of the flap implants.

The current study focuses on understanding the activation of cells within cultured pericardial tissues to gain insight into the development of fibrosis observed in the vital autologous pericardial flap. Although in vivo models of fibrosis would best represent actual clinical situations, these models do not allow for the isolation of the variables that may be responsible for the observed healing outcomes. Our approach was the development of an in vitro model using living pericardial tissue that simulated healing responses associated with the fibrotic pericardial flap in vivo.

Recently, an in vitro wound-healing model composed of native tissue was developed. It was theorized that native tissue models would maintain the natural morphology of the in situ cell population thereby providing an environment closer to the in vivo conditions. Kratz [12] reported in vitro incision and burn wound-healing models using viable human skin explants. These models allowed for the interaction of cells native to the tissue. Therefore, the cells displayed natural morphology and proliferative activity after 14 days of incubation. Furthermore, each of these models showed distinctive healing characteristics that were similar to clinical cases of incision and burn wounds.

Here, we describe an in vitro native tissue culture model that allows for selective manipulation of environmental conditions using living pericardial tissue. Within this model, the mechanism by which cellular activation contributed to pericardial tissue contraction was studied. Pericardial tissues contracted and the cells in situ to the tissue proliferated when stimulated with serum. In contrast, pericardial tissues that were killed by a 5 min wash with 50% ethanol did not contract. Living pericardium has been studied under in vitro tissue culture conditions of load-bearing stress for the evaluation of collagen and elastin synthesis [13] but, to our knowledge, this is the first report of in vitro healing responses relating to pericardium. The information gained from this study may impact the clinical use of pericardium as a cardiovascular biomaterial and for heart valve reconstruction, in particular.

2. Methods

2.1. Pericardial tissues

Parietal pericardium was dissected from normal sheep under sterile conditions. Adherent loose tissue and the mesothelium were removed by rubbing the tissue with sterile gauze. The tissue, between 1 and 2 mm thick, was cut into approximately 1 cm^2 sections and immersed in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Gaithersburg, MD, USA) containing 0.5% fetal bovine serum (FBS) and incubated for 48 h at 37°C, 5% CO_2 prior to use. In a preliminary study, pericardial tissue sections exhibited varying cellular activation states in relation to proliferative activity. For this reason, tissue sections were cultured for 48 h in serum-deprived conditions. This serum deprivation was designed to induce cells within the pericardium into a resting state promoting cell cycle synchronization and allowing comparisons to be made between stimulated and unstimulated samples [14].

2.2. Stimulation of pericardial tissue contraction

Serum-deprived pericardial tissue sections ($n = 4$) were placed in Costar 24-well tissue culture plates (Corning Inc., Corning, NY, USA) and incubated as free-floating sections with DMEM (1 ml) containing 0.5%, 5%, 10%, 20%, and 50% FBS. Media were purchased at $1\times$ concentration and mixed with the appropriate volume of FBS to achieve the desired percentage of FBS with the exception of 50% FBS, which was mixed 1:1 with $2\times$ DMEM to insure an appropriate buffering capacity of the medium. Tissue sections were incubated with the above media for a maximum of 14 days with the media being replaced every other day. Serum-induced tissue contraction was evaluated visually by tissue curling. Tissue sections killed by a 5 min wash in 50% ethanol [11] were used as a control.

2.3. Inhibition of pericardial tissue contraction

Various inhibitors were added to DMEM containing 20% FBS (DMEM/20% FBS) for up to 14 days to determine the ability of these compounds to prevent pericardial tissue contraction. Inhibitors included Mitomycin C, an inhibitor of nucleic acid synthesis; Aphidicolin and AraC, specific inhibitors of DNA synthesis; Cytochalasin B and D, which prevent contractile actin filament formation; Cycloheximide, an inhibitor of protein synthesis; and Prostaglandin E_2 (PGE_2) an activator of adenylate cyclase. All chemicals were acquired from Sigma Chemical Co., St. Louis, MO, USA.

To determine the optimal concentration for use in the pericardial tissue contraction assay, increasing concentrations of chemical inhibitors in DMEM/20% were incubated on cell monolayers ($n = 3$) isolated from fresh pericardium for 14 days and used at the highest concentration that was not cytotoxic. For all inhibitors this concentration was 1 μg/ml. Toxicity was defined as the concentration of inhibitor that induced cellular rounding and/or loss of cellular adhesion from the culture plate.

2.4. Stimulation of proliferation

Vital pericardial tissue sections ($n = 4$) were incubated in Costar 24-well culture plates and incubated as free-floating sections in DMEM containing 0.5%, 5%, 10%, 20%, or 50% FBS. The carbonate level in the medium was adjusted in order to compensate for the differing volumes of serum. Because the DMEM used in this experiment was high glucose (4500 mg/l), glucose-free RPMI was also used to determine if excess energy contributed to the expression of proliferating cell nuclear antigen (PCNA, Sigma Chemical Co., St. Louis, MO, USA). The medium was changed daily for 4 days and the sections were fixed in Histo-Choice (Amresco Inc., Solon, OH, USA), dehydrated, and embedded. Immunohistologic analyses using an antibody specific to PCNA were performed on 5 μm sections. The percentage of PCNA expression was calculated from replicate tissue samples counting 500 cells per replicate in random fields.

2.5. Inhibition of cellular proliferation

Pericardial tissue sections ($n = 4$) were transferred to Costar 24-well tissue culture plates and incubated as free-floating sections in DMEM/20% FBS, with inhibitors of proliferation and protein synthesis. Mitomycin C, Cytochalasin B, Cytochalasin D, Aphidicolin, or AraC were used at a concentration of 1 μg/ml and tested for toxicity as described. The media were replaced every day for 4 days and samples fixed in Histo-Choice, dehydrated, and embedded. Proliferation was assessed through the expression of PCNA. The percentage of PCNA expression was calculated from replicate samples counting 500 cells per section in random fields and compared to cellular PCNA expression in pericardial tissue sections maintained in DMEM/20% FBS.

2.6. Histology/immunohistochemistry

Specimens were fixed in Histo-Choice for a minimum of 12 h. The sections were dehydrated and embedded in PolyFin (Polysciences, Inc., Warrington, PA, USA) wax for sectioning. Wax blocks were sectioned at 5 μm and collected on poly-L-lysine coated slides and stained for PCNA. Sections were deparaffinized and treated with 3% H_2O_2 for 5 min to remove endogenous peroxidases. The sections were blocked with 0.05% Tween-20 (Sigma Chemical Co., St. Louis, MO, USA) in phosphate buffered saline (PBS-T) for 30 min and incubated with an appropriate concentration of primary antibody in PBS-T for 30 min. Sections were rinsed with PBS-T and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody in PBS-T for 30 min. Antibody incubations were performed in a moist chamber at room temperature. Sections were rinsed with PBS-T and 100 μl of DAB substrate (Sigma Fast DAB, Sigma Chemical Co., St. Louis, MO, USA) was added to each section for development. For some antibody markers, such as PCNA where amplification was needed for optimal visualization, the LSAB-2 strepavidin-biotin amplification kit (DAKO, Carpenteria, CA, USA) replaced the secondary antibody. Development was performed using the ImmunoPure metal enhanced DAB substrate kit (Pierce, Rockford, IL, USA). The development was stopped with water and the slides counterstained with H&E. Negative controls included using an irrelevant primary antibody or replacing the secondary antibody with PBS-T. A positive control, such as a tissue known to express the marker in question, was used for every immunohistochemical stain.

3. Results

3.1. Stimulation of pericardial tissue contraction

Cell-mediated contraction of pericardial tissue was observed as early as day 6 in 20% FBS (Fig. 1, top

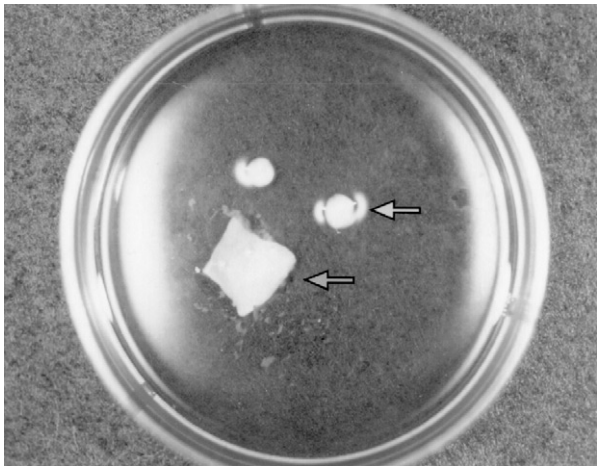

Fig. 1. Contraction of living versus killed pericardial tissues in vitro. Pericardial tissue sections incubated with DMEM/20% FBS contracted between day 6 and 8 (top arrow). Control tissues, killed by washing in ethanol or freezing (bottom arrow), and living tissues incubated with 0.5% FBS did not contract.

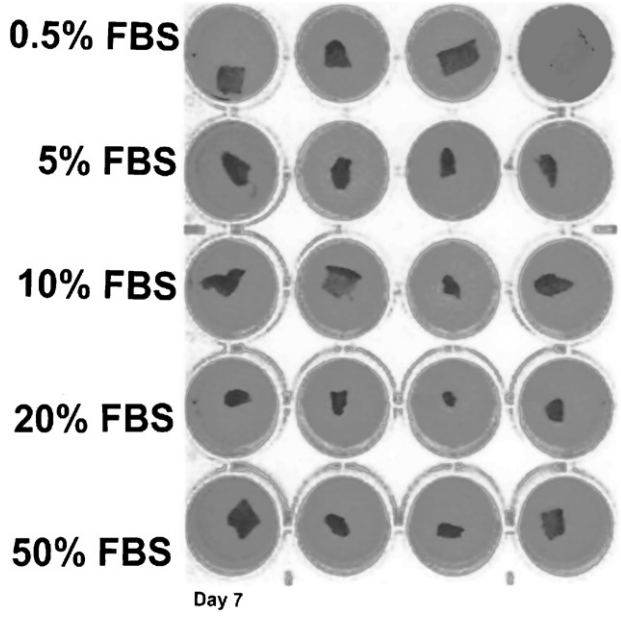

Fig. 2. Dose-dependent tissue contraction in response to serum. Pericardial tissues ($n = 4$ per group, 1 cm^2) were incubated with DMEM containing 0.5%, 5%, 10%, 20%, or 50% FBS for 7 days at 37°C, 5% CO_2. The tissues contracted in a dose-dependent manner in response to serum with a peak at 20% FBS. Tissues incubated with DMEM containing 0.5% FBS did not contract.

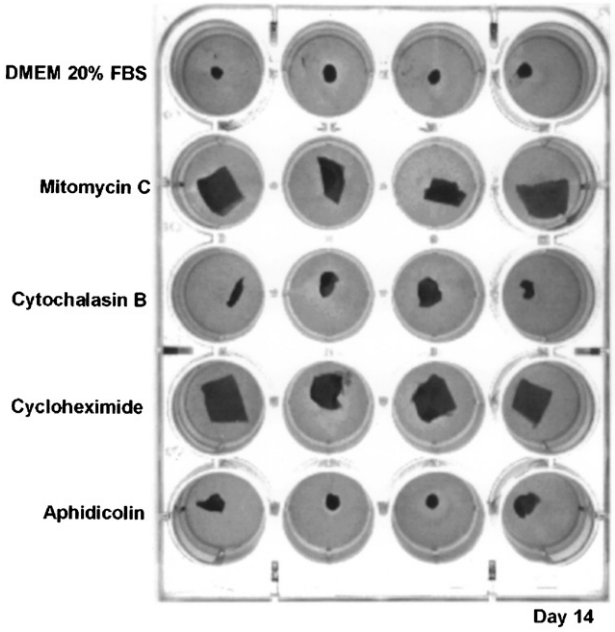

Fig. 3. Tissue contraction in the presence of inhibitors. Pericardial tissue sections (1 cm^2, $n = 4$) were incubated for 14 days at 37°C, 5% CO_2 with DMEM/20% FBS (1 ml) in the presence of inhibitors. Inhibitors included Mitomycin C (1 μg/ml); an inhibitor of nucleic acid synthesis, Cytochalasin B (1 μg/ml); an inhibitor of f-actin polymerization, Cycloheximide (1 μg/ml); a protein synthesis inhibitor, and Aphidicolin (1 μg/ml); an inhibitor of DNA synthesis. Mitomycin C and Cycloheximide effectively prevented serum-stimulated tissue contraction whereas Cytochalasin B and Aphidicolin did not.

arrow). Ethanol-treated pericardium did not contract (bottom arrow). Fig. 2 shows a dose-dependent contraction response of pericardial tissue to increasing percentages of FBS ($n = 4$ per treatment) at day 7. All tissues incubated with 20% FBS contracted by day 6, and by day 7 three of four samples showed moderate contraction and one exhibited maximal contraction (third well from left). Tissues maintained with 5%, 10%, and 50% FBS had just begun to curl at day 6. By day 7, 5% FBS showed a mild degree of contraction in four of four wells while three of four samples exposed to 10% FBS exhibited mild curling and one moderate curling (third well from left). The 50% FBS group showed mild contraction in two of four samples and moderate contraction in the remaining two samples. In contrast, tissues incubated with 0.5% FBS did not contract.

3.2. Inhibition of tissue contraction

Pericardial tissue sections ($n = 4$) incubated for 14 days with DMEM/20% FBS in the presence of proliferative and protein synthesis inhibitors showed that tissue contraction was effectively prevented by 1 μg/ml of Mitomycin C or 1 μg/ml Cycloheximide (Fig. 3). In contrast, Aphidicolin and Cytochalasin B did not prevent tissue contraction. Inhibitors Cytochalasin D, AraC, and PGE_2 also did not prevent tissue contraction (data not shown).

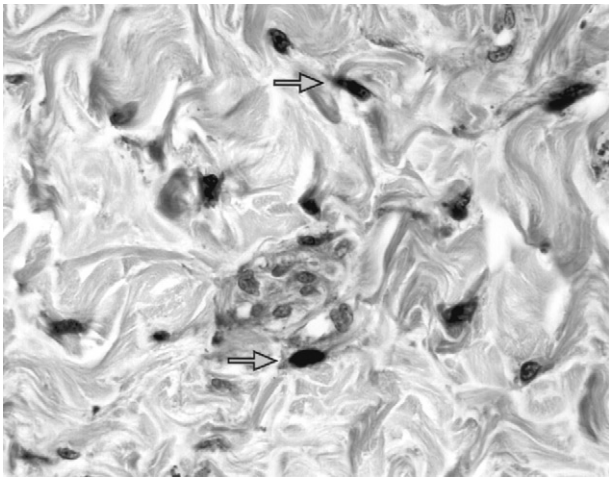

Fig. 4. Immunohistologic staining for PCNA indicating dividing cells. Antibody recognition of PCNA was visualized by DAB staining resulting in a brown precipitate (arrows). Tissue sections were counterstained with Hematoxylin, which stains nuclei blue, and Eosin, which stains collagen pink. 400 ×.

3.3. Stimulation of proliferation

Fetal bovine serum stimulated the proliferation of cells within pericardial tissues, which was demonstrated by PCNA expression (Fig. 4, arrows). In order to determine the optimal conditions for cellular

proliferation, a preliminary experiment was performed where daily tissue samples were harvested and assayed for PCNA expression. Based on this data, tissue incubation in subsequent experiments was terminated on 4 days of incubation.

The percentage of cells expressing PCNA increased in a dose-dependent manner to the percentage of FBS present in the medium (Fig. 5). Expression of PCNA increased from 19% to 61% when the FBS was increased from 0.5% to 50%. Changes in glucose concentration of the medium did not alter PCNA expression, because glucose-free RPMI 1640 medium showed similar results as DMEM medium containing the same concentration of FBS.

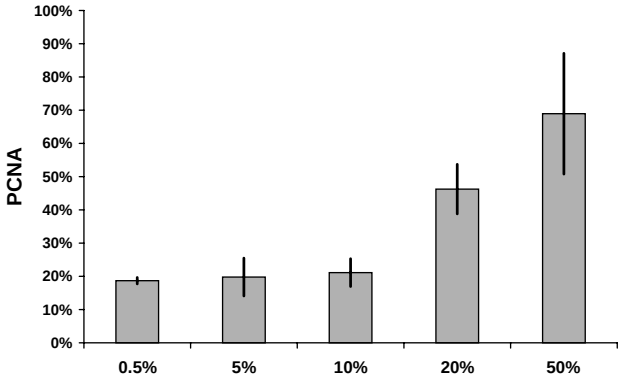

Fig. 5. Serum-stimulated PCNA expression. Percent cellular expression of proliferating cell nuclear antigen (PCNA) increased in a dose-dependent manner to increasing serum concentration in DMEM. Pericardial tissues ($n = 4$) were incubated with 1 ml DMEM containing 0.5%, 5%, 10%, 20%, or 50% serum for 4 days at 37°C, 5% CO_2. Tissues were embedded in wax and assayed immunohistologically for the expression of PCNA. Error bars indicate ranges from replicate tissue samples counting 500 cells per replicate in random fields.

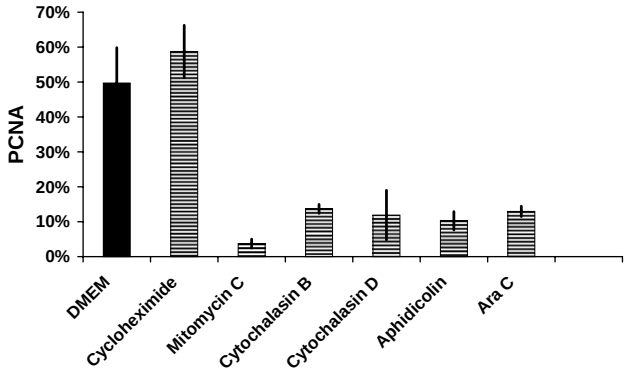

Fig. 6. Chemical inhibition of cellular proliferation. Cellular proliferation, evaluated by the expression of PCNA, was investigated in the presence of inhibitors. Pericardial tissues were incubated for 4 days at 37°C, 5% CO_2 with 1 ml DMEM/20% FBS containing 1 μg/ml Mitomycin C, Cycloheximide, Cytochalasin B, Cytochalasin D, Aphidicolin, or AraC ($n = 4$). Tissues were embedded in wax, sectioned, and assayed for the expression of PCNA by immunohistologic techniques. Error bars indicate standard deviation from replicate tissue samples counting 500 cells per replicate in random fields.

3.4. Inhibition of cellular proliferation

Fig. 6 shows that Cytochalasin B, Cytochalasin D, Aphidicolin, and AraC reduced cellular PCNA expression within pericardial tissue sections when compared to the section incubated with DMEM/20% FBS only. These same inhibitors did not prevent tissue contraction (Fig. 3). In contrast, Cycloheximide, which effectively prevented serum-stimulated tissue contraction, did not reduce cellular expression of PCNA (Fig. 7). Mitomycin C inhibited serum-stimulated tissue contraction and reduced cellular PCNA expression. Cytotoxicity was not observed with any inhibitor used in this experiment.

4. Discussion

Fibrosis and retraction are the most common clinical outcomes when vital autologous pericardium is used to repair or reconstruct heart valve leaflets. Previously, we simulated these detrimental healing outcomes using pericardium fashioned as a flap in the descending aorta model in sheep. Within this in vivo model, vital autologous pericardium retracted and became fibrotic [11]. Histologic and immunohistologic analyses of recovered implants suggested that activated cells within the implant contributed to the fibrotic outcome of the flap. However, this in vivo model was limited in its ability to determine if the cells from the host or the cells intrinsic to the fresh implant became activated, and it could not determine how these cells became activated or the mechanism by which the fibrotic reactions developed. Furthermore, the techniques used in this study

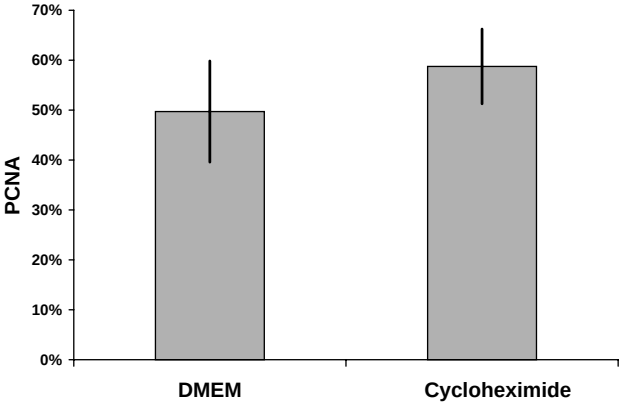

Fig. 7. Percent PCNA expression within cultured pericardium in the presence of the protein synthesis inhibitor Cycloheximide. Pericardial tissues were incubated with DMEM/20% FBS with 1 μg/ml Cycloheximide for 4 days at 37°C, 5% CO_2. The expression of PCNA was detected immunohistologically and visualized by DAB development resulting in a brown precipitate. Error bars indicate standard deviation from replicate tissue samples counting 500 cells per replicate in random fields. There were no significant differences in PCNA expression within tissues incubated with and without the presence of Cycloheximide.

included immunohistochemistry and, therefore, were limited in the ability to differentiate between smooth muscle cells and myofibroblasts as well as fibroblasts and myofibroblasts.

In this report, we designed an in vitro model to simulate the healing responses of the vital fibrotic pericardial flap implant in vivo. Although in vivo models would best represent clinical situations, in vitro models allow the investigation of complex biologic processes in an isolated environment so that the conditions can be selectively manipulated. A popular model for studying matrix retraction and its relation to wound healing uses fibroblasts populated in type I collagen gels (FPCG). These gels are composed of low-density extracellular matrices that do not reflect those of cardiovascular implants, where organized, complex matrices are concerned.

In our in vitro model of tissue contraction, living pericardium composed of native cells and extracellular matrix (with the exception of the mesothelium) displayed healing characteristics similar to those observed in the vital autologous pericardial flap in vivo. We demonstrated that cells in cultured pericardium, similar to those in implanted autologous pericardium in the blood stream in vivo, expressed the proliferative marker PCNA and contracted the tissue when exposed to serum. Although these tissues were stimulated by serum in a simple diffusion model, differences in tissue thickness did not affect cellular activation in relation to PCNA expression. In fact, expression of PCNA was not limited to areas of tissue near the surface but was observed throughout the tissue.

Tissue contraction was dependent on living cells within the pericardium because ethanol-treated tissue devoid of living cells did not contract in vivo or in vitro. Tissue contraction in vitro was induced by serum because living tissue incubated in low serum or without serum did not contract. It should be noted that this model was limited in identifying the cell type(s) responsible for tissue contraction. Pericardial tissue is known to contain mesothelial cells, fibroblasts, smooth muscle cells, and pericytes [15]. It is well known that fibroblast transition into myofibroblasts is an important step in the closure and healing of skin wounds [16,17]. In this study, immunohistochemical techniques failed to identify cells expressing smooth muscle cell α actin, a known myofibroblast and smooth muscle cell marker. Therefore, an alternative mechanism of tissue contraction was obviously involved. Furthermore, the source of serum was not a factor. In this experiment, similar behavior was observed when pericardial tissues were incubated with normal sheep serum, sheep serum acquired 1 h post surgery, and also with FBS (data not shown). Serum-stimulated tissue contraction required de novo protein synthesis because the addition of Cycloheximide to the media prevented contraction. We also demonstrated that tissue contraction and cellular proliferation were independent events. Inhibition of cellular proliferation did not prevent tissue contraction and the protein synthesis inhibitor, Cycloheximide, did not affect PCNA expression.

Observations made in this study show consistency with results reported using FPCG. It is well known that serum stimulation augments FPCG gel contraction [18,19], and it was recently reported that contraction of these gels may be prevented by Cycloheximide. In this study, Miki et al. [20] suggested that the ability of Cycloheximide to prevent FPCG contraction was linked to the prevention of f-actin expression. This observation was supported by previous studies, which indicated that an organized cytoskeleton was required for FPCG contraction to occur [18,21]. In addition, Lee et al. [21] reported that Cytochalasin, an inhibitor of f-actin polymerization, suppressed fibroblast-mediated gel contraction.

There were discrepancies in the results obtained using our living tissue model with those acquired using FPCGs. In this study, Cytochalasin B and Cytochalasin D did not prevent cultured pericardial tissue contraction. In addition, Skold et al. [22] reported the prevention of FPCG contraction with the addition of PGE_2 (10^{-7} M) to the culture medium. In our model, pericardial tissues contracted in the presence of PGE_2 even when concentrations as high as 1 μg/ml were used (data not shown). The reasons for these discrepancies is not known but may be related to the nature and density of our matrix. In this in vitro study, serum-stimulated pericardial tissue contraction was prevented by chemical inhibition of protein synthesis. Mitomycin C and Cycloheximide effectively prevented tissue contraction by global inhibition of protein expression.

In summary, the results of this study showed that the ability of cells in the pericardium to proliferate and contract the tissue is consistent with the suggestion that cells in the implant are required for the development of fibrosis, because ethanol-killed tissue did not retract or become fibrotic in vivo. Furthermore, this in vitro study showed serum stimulation was also required for tissue contraction to occur. These data suggest that cellular activation significantly contributed to the detrimental healing outcomes of the pericardial flap. One possible reason for the activation of these cells was a change in microenvironment. Pericardium is a sack that contains fluid and surrounds and cushions the heart. Pericardium does not normally contract and is compartmentalized in the aspect that it is removed from direct contact with high concentrations of serum in the blood stream. In our in vivo sheep model as well as the in vitro model report here, the pericardium was exposed to high concentrations of serum. It is reasonable to believe that an increase in exposure to the stimulatory components of serum activated cells within pericardium to contract the

tissue and proliferate. Further experimentation exploring inhibition of specific protein expression may indicate mechanisms by which tissue contraction occurs.

Acknowledgements

We would like to thank Julianne Hoy for her outstanding histology expertise and technical support.

References

[1] Duran CMG, Gometza B. New uses of pericardium for valve surgery. In: D' Alessandro LC, editor. Heart Surgery. Rome: Casa Editrice Scientifica Internazionale, 1993. p. 69–82.
[2] Frater RW, Bodnar E. Towards understanding the pericardium as valve substitute (editorial). J Heart Valve Dis 1992;1:213–5.
[3] Mohri H, Barnes RW, Rittenhouse EA, Reichenbach DD, Dillard DH, Merendino KA. Fate of autologous pericardium and dacron fabric used as substitutes for total atrial septum in growing animals. J Thorac Cardiovasc Surg 1970;59:501–11.
[4] Hawe A, Rastelli GC, Ritter DG, DuShane JW, McGoon DC. Management of the right ventricular outflow tract in severe tetralogy of Fallot. J Thorac Cardiovasc Surg 1970;60:131–43.
[5] Hjelms E, Pohlner P, Barratt-Boyes BG, Gavin JB. Study of autologous pericardial patch-grafts in the right ventricular outflow tracts in growing and adult dogs. J Thorac Cardiovasc Surg 1981;81:120–3.
[6] Rendina EA, Venuta F, De Giacomo T, Vizza DC, Ricci C. Reconstruction of the pulmonary artery by a conduit of autologous pericardium. J Thorac Cardiovasc Surg 1995;110: 867–8.
[7] Schlichter AJ, Kreutzer C, Mayorquim RC, Simon JL, Vazquez H, Roman MI, Kreutzer GO. Long-term follow-up of autologous pericardial valved conduits. Ann Thorac Surg 1996;62:155–60.
[8] Parenzan L, Locatelli G, Alfieri O, Villani M, Invernizzi G. The Senning operation for transposition of the great arteries. J Thorac Cardiovasc Surg 1978;76:305–11.
[9] Piehler JM, Danielson GK, Pluth JR, Orszulak TA, Puga FJ, Schaff HV, Edwards WD, Shub C. Enlargement of the aortic root or anulus with autogenous pericardial patch during aortic valve replacement. Long-term follow-up. J Thorac Cardiovasc Surg 1983;86:350–8.
[10] Bortolotti U, Gallo JI, Gabbay S, Factor SM, Sisto D, Frater RW. Replacement of mitral valve chordae with autologous pericardium in dogs. Thorac Cardiovasc Surg 1984;32:15–7.
[11] Cheung DT, Choo SJ, Grobe AC, Marchion DC, Luo HH, Pang DC, Favara BE, Oury JH, Duran CM. Behavior of vital and killed autologous pericardium in the descending aorta of sheep. J Thorac Cardiovasc Surg 1999;118:998–1005.
[12] Kratz G. Modeling of wound healing processes in human skin using tissue culture. Microsc Res Tech 1998;42:345–50.
[13] Naimark WA, Lee JM. A computer-controlled system for mechanostimulation of tissues in culture: a preliminary study of protein synthesis in the pericardium. Biomed Sci Instrum 1999; 35:397–402.
[14] Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, Lee JCF, Trent JM, Staudt LM, Hudson Jr J, Boguski MS, Lashkari D, Shalon D, Botstein D, Brown PO. The transcriptional program in the response of human fibroblasts to serum (see comments). Science 1999;283:83–7.
[15] Taylor PM, Allen SP, Yacoub MH. Phenotypic and functional characterization of interstitial cells from human heart valves, pericardium and skin. J Heart Valve Dis 2000;9:150–8.
[16] Moulin V. Growth factors in skin wound healing. Eur J Cell Biol 1995;68:1–7.
[17] Gabbiani G. Evolution and clinical implications of the myofibroblast concept. Cardiovasc Res 1998;38:545–8.
[18] Tomasek JJ, Haaksma CJ, Eddy RJ, Vaughan MB. Fibroblast contraction occurs on release of tension in attached collagen lattices: dependency on an organized actin cytoskeleton and serum. Anat Rec 1992;232:359–68.
[19] Borderie VM, Mourra N, Laroche L. Influence of fetal calf serum, fibroblast growth factors, 0and hepatocyte growth factor on three-dimensional cultures of human keratocytes in collagen gel matrix. Graefes Arch Clin Exp Ophthalmol 1999;237:861–9.
[20] Miki H, Mio T, Nagai S, Hoshino Y, Tsutsumi T, Mikuniya T, Izumi T. Glucocorticoid-induced contractility and F-actin content of human lung fibroblasts in three-dimensional culture. Am J Physiol Lung Cell Mol Physiol 2000;278:L13.
[21] Lee YR, Oshita Y, Tsuboi R, Ogawa H. Combination of insulin-like growth factor (IGF)-I and IGF-binding protein-1 promotes fibroblast-embedded collagen gel contraction. Endocrinology 1996;137:5278–83.
[22] Skold CM, Liu XD, Zhu YK, Umino T, Takigawa K, Ohkuni Y, Ertl RF, Spurzem JR, Romberger DJ, Brattsand R, Rennard SI. Glucocorticoids augment fibroblast-mediated contraction of collagen gels by inhibition of endogenous PGE production. Proc Assoc Am Physicians 1999;111:249–58.