Transforming Growth Factor-β-Induced Transition of Fibroblasts: A Model for Myofibroblast Procurement in Tissue Valve Engineering

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Background and aim of the study: **The selection of a suitable cell type for scaffold seeding, its isolation and adequate expansion in vitro remains a major challenge in tissue valve engineering. The study aim was to establish a model for efficient procurement of myofibroblasts for in-vitro seeding using fibroblasts as progenitor cells.**

Methods: **Dermal and arterial mesenchymal cells from human (hDMC1.1 and hAMC1.1) and sheep (sDMC1.1 and sAMC1.1) were isolated by sequential culture. Cell isolates were characterized by stringent criteria based on morphology, immunocytochemistry using antibodies to vimentin, cytokeratin, prolyl 4-hydroxylase, smooth muscle α-actin (α-SMA) and smooth muscle myosin, and by Western blotting for α-SMA and N-cadherin. Cultures with less than 10-20% α-SMA-positive cells were considered to be fibroblastic. Cells were subsequently transdifferentiated with the cytokine transforming growth factor-β1 (TGF-β1) during five days, and then evaluated morphotypically, by immunocytochemistry, and by Western blotting. The metabolic and functional properties of TGF-β1-treated and untreated colonies were compared by measuring the expression of extracellular proteins (collagen type 1 and tenascin-C) and by a collagen matrix contraction assay.**

Results: **TGF-β1 successfully transformed both human and sheep fibroblasts to metabolically active and functional myofibroblasts based on stringent criteria for myofibroblast characterization. α-SMA positivity of 100% was obtained in all cases (hDMC1.1, hAMC1.1, sDMC1.1, and sAMC1.1) after transformation compared to less than 50% in the non-transformed state (hAMC1.1, 17%; hDMC1.1, 10%; sAMC1.1, 43%; and sDMC1.1, 30%). This observation was further supported by increased contractility and an up-regulation of extracellular protein production in transdifferentiated cells.**

Conclusion: **Untreated arterial cell isolates were, at best, less than 50% α-SMA-positive. By allowing procurement of high densities of myofibroblasts in a relatively short time, the model was seen to be a potentially useful tool in tissue valve engineering, at least in investigations using autologous cells in the sheep model.**

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Intense research into the tissue engineering of heart valves has resulted from the limitations of currently available heart valve prostheses, including both mechanical and bioprosthetic heart valves (1). Whilst mechanical valves are thrombogenic and require long-term anticoagulation therapy with its associated risks (2-5), bioprosthetic devices (derived from xenogenic and human tissue) are more biocompatible but less durable. Once manufactured, bioprosthetic valves are no longer biologically active, and their cells are unable to replenish or remodel the extracellular matrix. Consequently, these valves are prone to deterioration (6,7). Moreover, all currently available prosthetic valves lack the ability to grow. A tissue-engineered valve developed using viable cells, with its potential to grow and maintain the extracellular matrix, offers the potential to overcome these limitations. However, many challenges remain in tissue valve engineering, including the selection of a suitable cell type, its isolation, separation and adequate expansion in vitro for use in seeding biological and synthetic scaffolds.

The histological structure of human and porcine cardiac valve leaflets has been well documented (8-11). Each valve leaflet is composed predominantly of structural (collagen and elastin) and cellular (interstitial and

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endothelial cells) elements that organize to form three distinct layers: (i) the ventricularis; (ii) the spongiosa; and (iii) the fibrosa. The cellular and structural elements are surrounded by proteoglycans, which are most predominant in the spongiosa layer. Recent interest in the tissue engineering of cardiac valves has led to more thorough investigations of valve leaflet mesenchymal cells. The latter has been reported as comprising a heterogeneous phenotypic and functional fibroblastic cell population. Moreover, it has been suggested that myofibroblasts represent the major phenotype of valvular interstitial cells (9,12). In addition to contractile properties, cardiac valve interstitial cells have also been shown to possess secretory properties (8,11).

The study aim was to characterize arterial and dermal mesenchymal cells from human and sheep using stringent morphological and immunocytochemical criteria. The feasibility of obtaining higher densities of myofibroblasts for cell seeding in tissue valve engineering by treating fibroblasts from these sources with the cytokine transforming growth factor-β1 (TGF- β1) was investigated. The seeding of higher densities of myofibroblasts could yield more densely populated matrices which are more representative of the native aortic valve.

Materials and methods

Cell culture

Arterial and dermal mesenchymal cell lines were established by using fresh tissue fragments from human pulmonary and sheep carotid arteries, human neonatal foreskin and sheep subcutaneous tissue. Fresh tissue fragments were maintained for 30 min in Dulbecco's Modified Eagle's Medium (DMEM; Sigma, St. Louis, MI, USA) supplemented with 10-fold the normal concentration of antibiotics (fungizone 25 µg/ml, penicillin 100 µg/ml, streptomycin 1 mg/ml). The core was then cut into discs and placed in a 6-well tissue plate. An aliquot (100 µl) of pure fetal bovine serum (FBS; Sigma) was placed on top of the explants to promote adhesion to the substrate. After 24 h, a 3-ml aliquot of DMEM supplemented with 10% FBS and antimycotics and antibiotics (fungizone 2.5 µg/ml, penicillin 100 µg/ml, streptomycin 100 µg/ml) was added to each well. The explants were incubated at 37°C in 10% CO_2 with a change of medium every 48 h, or as necessary. Mesenchymal cells migrated out of the explants and attached to the tissue culture dish. When the cells had reached confluence, the explants were removed and the cells passaged. Confluent flasks split at 1 in 3 became confluent within 7 days. Cells were passaged using 0.25% trypsin in 1 mM EDTA and used

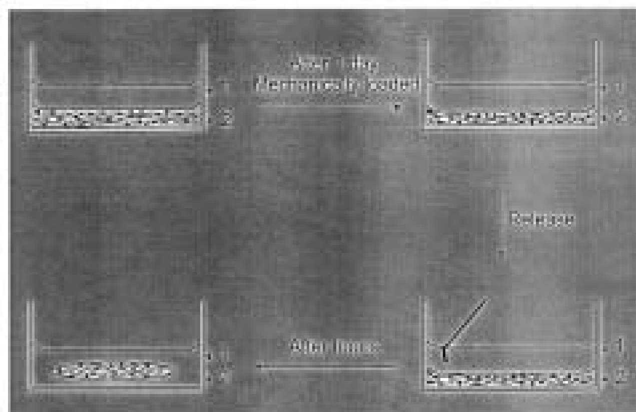


Figure 1: Schematic representation of the collagen contraction assay. (Reproduced with permission from Grinnel et al., The Journal of Cell Biology, 1994;124:401; © Rockefeller University Press.)

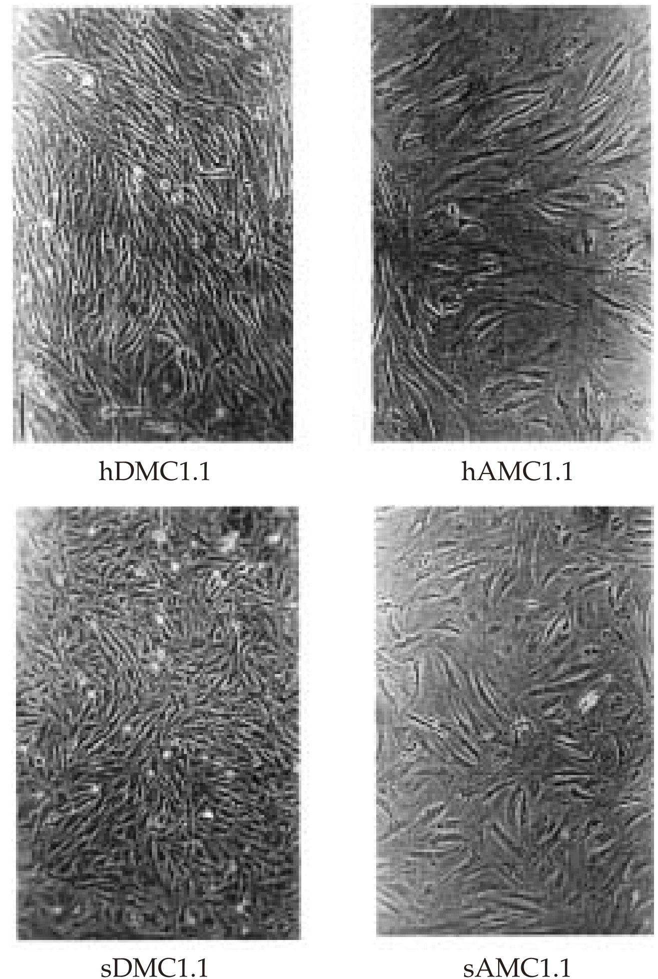


Figure 2: Confluent cultures on plastic substrate. Human arterial (hAMC.1.), human dermal (hDMC.1.1), sheep arterial (sAMC.1.1) and sheep dermal mesenchymal cells (sDMC.1.1) show spindle-shaped cells with slender processes.

for experiments from passages 1 to 3. The following primary cultures were obtained: human arterial mesenchymal cells (hAMC1.1 to 1.7), sheep arterial mesenchymal cells (sAMC1.1 to 1.4), human dermal mesenchymal cells (hDMC1.1 and 2.1) and sheep dermal mesenchymal cells (sDMC1.1 to 1.6). (The first number in the acronym indicates the source and the second number is the identification of the primary culture.)

Antibodies and reagents

The following primary antibodies were used: anti-smooth muscle α-actin (α-SMA) (clone 1A4), anti-tenascin-C (TNC) (clone BC-24), anti-pancytokeratin (clone C-11), anti-vimentin, anti-collagen type I (clone col 1) and anti-tubulin (clone B-5-1-2) (all from Sigma), and anti-human prolyl 4-hydroxylase (DAKO, Glostrup, Denmark). Secondary antibodies coupled to horseradish peroxidase and antibodies coupled to fluorescein or biotin were obtained from Amersham Pharmacia (Buckinghamshire, UK). Recombinant TGF-β1 was from R&D systems (Minneapolis, MN, USA), and collagen type I from Upstate Biotechnology (Lake Placid, NY, USA).

Protein analysis

Cultured mesenchymal cells (0.5-1.0 × 10^5 cells per coverslip) were plated onto sterile coverslips, placed in 24-well plates containing DMEM supplemented with 10% FBS, and left for one day. Cells were then washed with serum-free DMEM and treated for one week with serum-free DMEM supplemented with TGF-β1 (1 ng/ml) or vehicle alone. Cells were then fixed and permeabilized by immersing the coverslips in ice-cold methanol for 10 min. Coverslips were washed in Tris-buffered saline (TBS) and blocked with TBS + 5% bovine serum albumin (BSA) for 30 min at room temperature. Primary antibodies, recognizing α-SMA (1/500), vimentin (1/50), cytokeratin (1/50), prolyl 4-hydroxylase (1/100) and TNC (1/100), were diluted in TBS and applied to coverslips for 1 h at room temperature. Coverslips were rewashed in TBS and consequently incubated with secondary antibodies and 4′,6-diamino-2-phenylindol (DAPI) for 1 h at room temperature. Coverslips were washed in TBS, dehy-

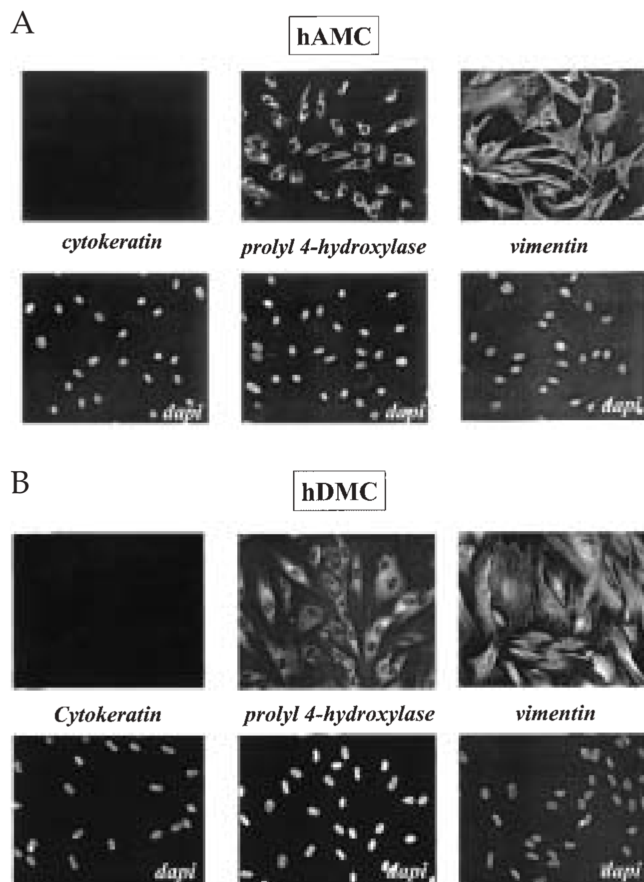


Figure 3: A, B) Immunocytochemistry of isolated cells. Human arterial and dermal mesenchymal cells (hAMC.1.1 and hDMC.1.1) are 100% positive for prolyl 4-hydroxylase and vimentin, but negative for cytokeratin and smooth muscle myosin.

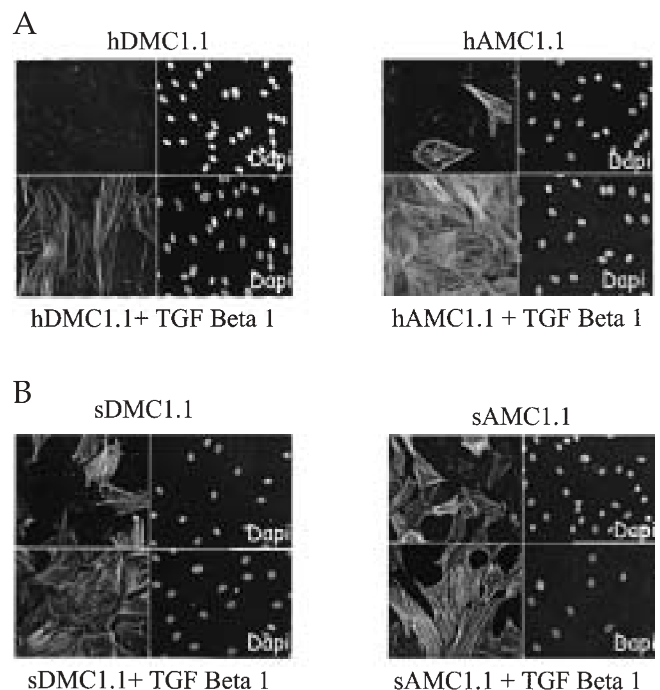


Figure 4: A, B) Effect of TGF-β1 on smooth muscle α-actin (α-SMA). TGF-β1 treatment resulted in the up-regulation of α-SMA in human arterial (hAMC.1.1), human dermal (hDMC.1.1), sheep arterial (sAMC.1.1) and sheep dermal (sDMC.1.1) mesenchymal cells compared to untreated colonies. In all cases, TGF-β1 treatment resulted in an up-regulation of α-SMA.

drated and mounted on glass microscope slides. Samples were viewed using fluorescence microscopy (Dialux 20; Leitz, Wetzlar, Germany) and photographed using a Leitz Orthomat E camera system. The number of positively stained cells for each antigen was assessed quantitatively and expressed as a percentage. No significant staining was observed if coverslips were incubated with secondary antibody only.

Western blotting

Western blotting was performed as previously described (13). Briefly, cells were lysed with Laemmli buffer, after which equal amounts of protein were boiled and separated using SDS-PAGE. Proteins were transferred onto Hybond membrane (Amersham). To perform dot-blot analyses, cells were lysed with Laemmli buffer, and equal amounts of native protein were spotted onto Hybond membrane and dried. For immunostaining, blots were quenched with 3% BSA in phosphate-buffered saline (PBS) containing 0.5% Tween-20. The membranes were incubated with primary antibody, followed by three 5-min washes and incubation with horseradish peroxidase-conjugated secondary antibodies. Specific antibody binding was detected using an enhanced chemiluminescence system (Amersham). Scanning densitometry was performed by means of the Quantity One program (Bio-Rad; Hercules, CA, USA).

Collagen contraction assay

Mesenchymal cells cultured in 25 cm^2 plastic Falcon flasks were treated for one week with serum-free DMEM supplemented with TGF-β1 (1 ng/ml) or vehicle alone. Refreshments were performed on day 3 and day 5. Thereafter, cells were trypsinized and counted. Plates (24-well) were filled with 300 µl of neutralized type I collagen mixed with 2.5×10^5 treated mesenchymal cells in suspension. Immediately after polymerization, 500 µl DMEM containing 10% BSA was added to each well, and the gels were mechanically loaded by incubation at 37°C during 24 h. To initiate contraction, the gels were detached with a spatula and gentle shaking of the culture wells until they floated in the medium (Fig. 1). Collagen gel cultures of both TGF-β1-pretreated and non-treated cells were maintained in the culture medium without TGF-β1 during the experimental period. The diameters of the gels were measured at each time point to calculate the percentage contraction using the formula: [diameter time 0 - mean diameter time X)/diameter time 0], expressed as percentage, wherein X represents the number of hours after TGF-β1 treatment.

Results

Morphology of cell isolates

Confluent cultures of hDMC1.1, hAMC1.1, sDMC1.1 and sAMC1.1 after seven days are illustrated in Figure 2. Morphotypically, cultures from both sheep and human sources were composed of spindle-shaped cells with slender processes consistent with mesenchymal fibroblastic colonies. In all experiments, cells from low-passage culture (less than five) were used.

Human mesenchymal cells (hAMC1.1 and hDMC1.1) were further characterized by immunocytochemical staining for vimentin, prolyl 4-hydroxylase,

Table I: Immunocytochemistry and Western blotting of isolated human arterial (hAMC.1), human dermal (hDMC1.1), sheep arterial (sAMC1.1) and sheep dermal (sDMC1.1) mesenchymal cells.

Cell line		Marker						
Tissue of Origin	Acronym	Immunocytochemistry					Western blot	
		Vimentin	Cytokeratin	Prolyl 4-hydroxylase	Smooth muscle myosin	α-SMA	N-cadherin	α-SMA
Human pulmonary artery	hAMC1.1	+	-	+	-	17%+	+	+
Sheep carotid artery	sAMC1.1	ND	ND	ND	ND	43%+	+	+
Human foreskin	hDMC1.1	+	-	+	-	10%+	+	+
Sheep dermis	sDMC1.1	ND	ND	ND	ND	30%+	+	+

ND: Not detected; α-SMA: Smooth muscle α-actin.

smooth muscle myosin and cytokeratin. Human arterial mesenchymal cell cultures were negative for cytokeratin and smooth muscle myosin, but 100% positive for prolyl 4-hydroxylase and vimentin (Table I; Fig. 3). Sheep arterial and dermal mesenchymal cells were not stained for the above-mentioned markers, as antibodies that cross-react with these sheep proteins are not available. All isolated primary cell lines were positive for N-cadherin. After characterization, cultures were stored frozen, and thawed as required for further investigation.

Morphotype, protein synthesis and collagen contraction of transdifferentiated cells

Thawed cell lines were analyzed comparatively before and after treatment with TGF-β1 for five days.

The effect of TGF-β1 on α-SMA expression in hAMC.1.1, hDMC.1.1, sAMC.1.1 and sDMC.1.1 is shown in Figure 4. In all cases, TGF-β1 resulted in an up-regulation of α-SMA expression with pronounced stress fibers in mesenchymal cells. The number of cells in comparative colonies was the same, as evidenced by nuclei staining using DAPI. TGF-β1-treated cells were 100% positive for α-SMA compared to untreated cells (Table II).

These findings were confirmed by Western blotting, which showed an increase in α-SMA after TGF-β treatment in all cell lines (Fig. 5).

Expression of the extracellular proteins; collagen type 1 and TNC

Collagen type-1 and TNC expression were both up-regulated in TGF-β-treated cells.

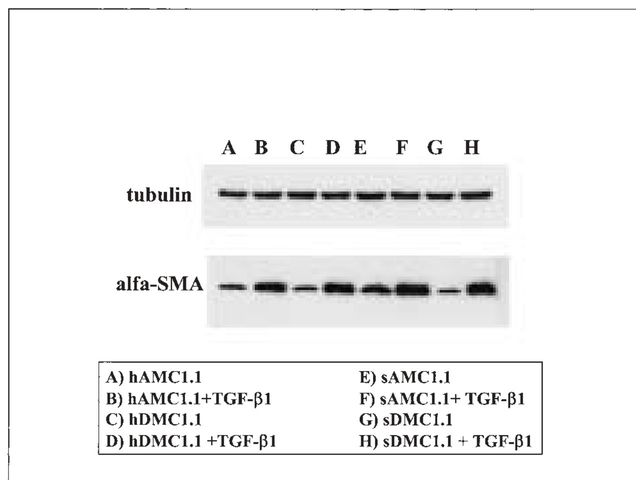


Figure 5: Western blotting. The up-regulation of α-SMA expression in TGF-β1-treated human arterial (hAMC.1.1), human dermal (hDMC.1.1), sheep arterial (sAMC.1.1) and sheep dermal (sDMC.1.1) mesenchymal cells. Tubulin staining confirmed the spotting of equal amounts of cells.

Table II: Smooth muscle α-actin (α-SMA) expression in human arterial (hAMC1.1), human dermal (hDMC1.1), sheep arterial (sAMC1.1) and sheep dermal (sDMC1.1) mesenchymal cells with and without TGF-β1 treatment.

Cell type	α-SMA expression (%)
hDMC1.1	10
hDMC1.1 + TGF-β1	85
hAMC1.1	17
hAMC1.1 + TGF-β1	100
sDMC1.1	30
sDMC1.1 + TGF-β1	100
sAMC1.1	43
sAMC1.1 + TGF-β1	100

Collagen production was monitored using two techniques: (i) indirectly, by investigating the enzyme prolyl 4-hydroxylase which is essential for the maturation of collagen type 1; and (ii) directly, by performing a dot-blot assay on the total cell lysate. Dot-blot analysis showed increased collagen production in TGF-β-treated human mesenchymal cells (Fig. 6). An irrelevant antibody IgG showed no significant staining, and tubulin staining confirmed a fixed protein concentration in analyzed blots. These results showed a concomitant increase in prolyl 4-hydroxylase staining, and suggested an increased collagen synthesis in hAMC.1.1 and hDMC.1.1 (Fig. 3B; Table II).

Immunocytochemical analysis of TNC in hAMC.1.1 and hDMC.1.1 is illustrated in Figure 7. In both cases, TGF-β1 resulted in an up-regulation of TNC.

Collagen contraction assay

The rate and extent of collagen contraction in both sheep and human mesenchymal cells treated with

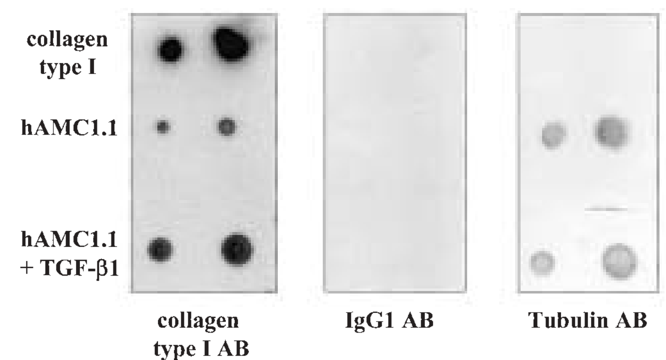


Figure 6: Dot-blot analysis, showing an increase in collagen expression in human mesenchymal cells after TGF-β1 treatment, as illustrated for human arterial mesenchymal cells (hAMC.1.1).

TGF-β1 is shown in Figure 8. Both the rate and extent of collagen contraction was increased in the transdifferentiated cells. Indeed, transdifferentiated arterial cells contracted more rapidly and to a greater extent than did transdifferentiated dermal cells.

Discussion

The role of fibroblasts in the maintenance of heart valve extracellular matrix has been extensively reported (14-16). In addition to structural proteins, valve interstitial cells have been shown to express growth factors, cytokines and chemokines. More recently, Taylor and co-workers elegantly illustrated the expression of the fibroblastic cell markers prolyl 4-hydroxylase, β-tubulin and vimentin by some valve interstitial cells (9).

It has also been shown that, in addition to expressing markers similar to skeletal, cardiac and smooth muscle cells, many valve interstitial cells express α-SMA, which is characteristic of myofibroblasts. Fibroblastic cells expressing α-SMA were also shown to be associated with contractile properties and rapid remodeling of the extracellular matrix (17,18).

Several authors have reported on the use of interstitial cells to seed biological and synthetic matrices in tissue valve engineering (19-23). Particularly encouraging were the investigations of Shinoka, Breuer and co-workers, who used mesenchymal cells from sheep carotid arteries. These authors reported the tissue engineering of complete pulmonary leaflets from autologous sheep mesenchymal cells seeded onto biodegradable polyglycolic acid scaffolds and implanted into juvenile sheep for up to 24 weeks. In the above-mentioned reports, cells on tissue-engineered constructs were evaluated phenotypically and functionally after placement either in sheep or in a hemodynamic culture chamber. The demonstration of myofibroblasts (α-SMA) in the latter cell populations has generally been taken as evidence of the constructs' potential to mimic the native aortic valve. Although native valves are predominantly composed of myofibroblasts, the same cannot be said for mesenchymal cell isolates, as discussed below.

The stimulatory effects of TGF-β on mesenchymal cells are known. The isoforms TGF-β1, TGF-β2 and TGF-β3 belong to the TGF-β superfamily of cell-cell signaling proteins, the pleiotropic effects of which are mediated from cell membrane to nucleus by type I and type II serine/threonine kinase receptors and their downstream effectors known as Smad proteins (24,25).

In the present study, the morphotypes of cells on a plastic substrate were in keeping with that of fibroblastic mesenchymal cells, and were considered as such in this study. Isolated cells expressed vimentin (the intermediary filament of mesenchymal cells) and prolyl 4-hydroxylase, an enzyme that synthesizes hydroxyproline in collagen synthesis (26).

At the same time, these cultures were negative for smooth muscle heavy chain polypeptide (200 kDa) of

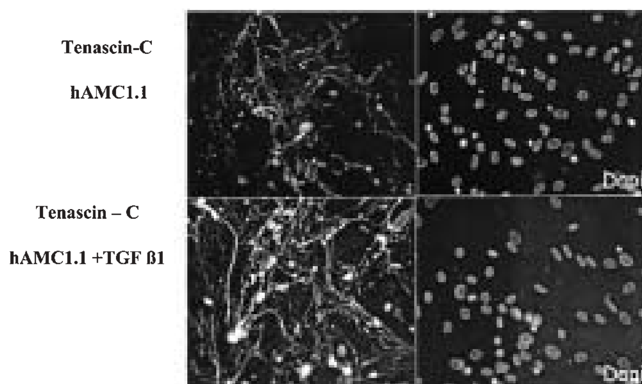
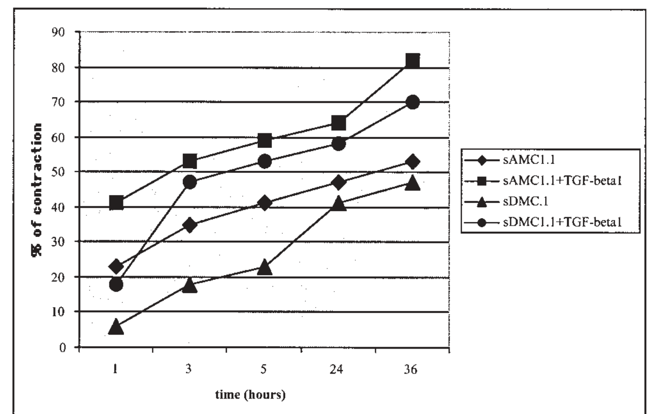


Figure 7: Immunocytochemistry of extracellular proteins. Tenascin-C was up-regulated in TGF-β1-treated human mesenchymal cells, as shown for human arterial mesenchymal cells (hAMC.1.1).



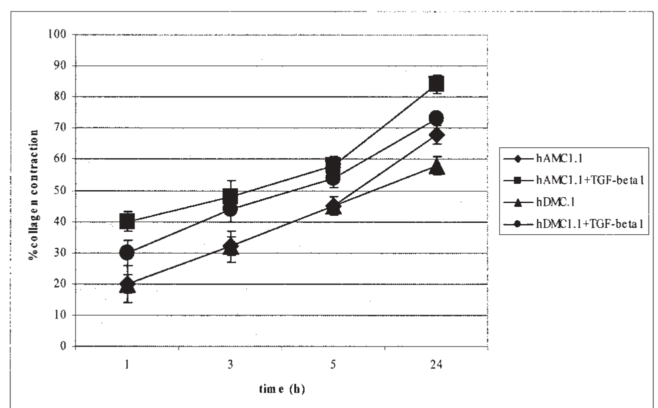


Figure 8: A, B) Collagen gel contraction. Both the rate and extent of collagen contraction was increased in TGF-β1-treated human (hAMC1.1 and hDMC1.1) and sheep (sAMC 1.1 and sDMC 1.1) mesenchymal cells.

myosin and cytokeratin, the intermediary filament found primarily in epithelial cells. These findings indicate that isolated cells were of mesenchymal cell type and actively synthesizing collagen. Neither cells isolated from arterial nor dermal tissue were homogeneously positive for α-SMA, but comprised at least two phenotypes depending on whether or not they expressed α-SMA; this was in keeping with findings reported by others (9).

Smooth muscle α-actin is expressed by, and is characteristic of, myofibroblasts. The percentage of cells expressing α-SMA in isolated cultures ranged from 10 to 43% - a factor that might be limiting when these isolates are seeded onto scaffolds in tissue valve engineering.

Interestingly, sAMC.1.1 and sDMC.1.1 express significantly more α-SMA compared to hAMC.1.1 and hDMC.1.1. Whilst such variation may be species-dependent, sAMC.1.1 were derived from carotid arteries subjected to systemic circulatory pressures, while hAMC.1.1 were derived from pulmonary arteries in the lower-pressure pulmonary circulation. The transdifferentiation of fibroblasts to myofibroblasts in response to tension has been documented. Arora and co-workers (18) demonstrated an association between local intracellular tension and the expression of α-SMA by fibroblasts. This observation could, at least partially, explain the difference in α-SMA expression in untreated hAMC.1.1 and sAMC.1.1.

TGF-β1-treated cells were 100% positive for α-SMA, as shown by immunocytochemistry and Western blotting, and this indicated a transition to myofibroblasts that exhibited pronounced longitudinal stress fibers. The use of such transdifferentiated cell lines from both sheep and human in tissue valve engineering would result in higher densities of myofibroblasts in seeded cells. The ability of fibroblasts to transdifferentiate to a myofibroblastic phenotype with successive passages has been reported (27). This transdifferentiation implies a relationship between the number of cell passages and α-SMA expression. Whilst this phenomenon was not investigated in the present studies, it should be noted that early-passage cells were used in these studies.

TGF-β1-treatment resulted in an up-regulation of the extracellular proteins collagen and TNC. Up-regulation of collagen synthesis was evidenced by an increase in total collagen and an accompanying increase in prolyl 4-hydroxylase activity. TNC is a disulfide-linked hexameric matricellular glycoprotein that is prominent in embryonic and adult tissues that are actively remodeling, such as in wound healing and cancer, where TNC is often expressed by myofibroblasts (28). When present in mixed substrata, the matricellular TNC protein may antagonize the pro-adhesive activities of other matrix proteins, such as collagen type 1 (29). TNC can modulate cell mobility, and might therefore be important for the invasion of myofibroblasts into a three-dimensional collagen matrix.

Cadherins are transmembrane glycoproteins linked to the actin cytoskeleton via the catenins, α-catenin and β-catenin or γ-catenin; N(eural)-cadherin is a pathfinding, promigratory molecule which is expressed in fibroblasts, myofibroblasts, neurons, smooth muscle cells and endothelial cells. The presence of N-cadherin at cell-cell borders of myofibroblasts may provide a site for the insertion of α-SMA filaments. An N-cadherin-actin association could support actin-based force generation in valve leaflets and efficient migration.

It has been reported that TGF-β-treated fibroblasts (myofibroblasts) contract a collagen gel more rapidly and to a greater extent than do fibroblasts (30). Consequently, in the present studies fibroblastic cultures were pretreated with 1 ng/ml TGF-β1. Mechanically loaded (stressed) collagen gel contractions were performed as described, and contraction was easily measurable after a few hours. These results indicated that TGF-β1-pretreated cells contract collagen gel faster and to a greater extent than do untreated cells - an observation which also suggests that the myofibroblastic phenotype is maintained in the absence of TGF-β1.

Arterial mesenchymal cells contracted collagen more than dermal cells under both control and TGF-β1-treated conditions, and are consistent with higher α-SMA expression, as shown by immunocytochemistry in arterial cells.

In conclusion, early-passage human and sheep arterial and dermal mesenchymal cells contained less than 50% myofibroblasts. Arterial mesenchymal cells isolates however, contained more myofibroblasts than dermal isolates in both sheep and human cells. TGF-β1 successfully transdifferentiates arterial and dermal fibroblasts from human and sheep to metabolically active and functional myofibroblasts. Nevertheless, the use of dermal mesenchymal cells is a less-invasive alternative to arterial mesenchymal cells as a source of myofibroblasts using this model. Moreover, the model is potentially useful in obtaining high densities of myofibroblasts for use in tissue valve engineering. The ability of these cells to repopulate biological matrices is currently being investigated by the present authors.

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Meeting discussion

DR. PATRICIA TAYLOR (United Kingdom): For how long do you expect this effect to last?
DR KISHAN NARINE (Ghent, Belgium): When the TGF-β treatment is removed, and the cells are transdifferentiated, the effect ceases - that seems to be the case.
DR. TAYLOR: Have you looked at the cells later on to see if they have maintained the phenotype?
DR. NARINE: Yes - when the cells were removed from TGF-β treatment, the contractility increased for a few hours. So the phenotype was met even in the absence of TGF-β for a short interval. There is a trend that once the cells are transdifferentiated they will stay that way.
DR. ROBERT GALLEGOS (USA): You speak of a desire to obtain larger sources of mesenchymal cells. Have you tried using bone marrow-derived mesenchymal stem cell lineages? Could this system be used to test whether it is the environment that may induce differentiational expression?
DR. NARINE: Yes, we have, and this forms part of our ongoing experiments. We are actually looking at circulating stem cells.
DR. GALLEGOS: Do you see any difference in terms of expression of, for example, the collagen content of these cells rather than cells derived from venous or arterial sites?
DR. NARINE: No.
DR. PETER ZILLA (South Africa): TGF-β is probably the most colorful growth factor, with various facets and contradictory effects regarding collagen storage. Might you be playing with fire by tissue engineering valve leaflets where you need to retain a very thin dimension but end up with a fibrotic leaflet?
DR. NARINE: Perhaps. But in many previous reports where isolated cells have been re-sewn onto valves and the valves then either implanted into animals or used in bioreactors, the cells have transdifferentiated to myofibrolastic phenotypes. This was a way of achieving a head's start by sewing myoblastic phenotypes onto the matrices. But this is not a cancerous situation, if that is what you are inferring.
SIR MAGDI YACOUB (United Kingdom): Have you looked at the specific receptor subtype through which that effect in this cell is being mediated? In other words, have you tried to block it with specific receptor blockers, because TGF-β is a molecule with many, many facets and produces opposite effects through specific receptors. It really is the receptor which is more important.
DR. NARINE: No, we have not. I agree that there is some fear about TGF-β, especially the association with cancerous cells. We know that myofibroblasts are at the forefront of cancer invasion, and that TGF-β does play a role here. However, it is not the effect of TGF-β on the cells that is an important factor in the invasion process and the production of an extracellular matrix for the invasion of cancer cells - it may be simply a recruiting factor rather than a transdifferentiating factor in a cancer setting. But we must wait for further results.