Development of cardiac support bioprostheses for ventricular restoration and myocardial regeneration

Abdel Shafy¹, Trine Fink¹, Vladimir Zachar⁴, Nermine Lila¹, Alain Carpentier⁵ and Juan C. Chachques⁶

¹ Department of Cardiovascular Surgery, Laboratory of Biosurgical Research, Pompidou Hospital, University Paris Descartes, Paris, France
² Laboratory for Stem Cell Research, Aalborg University, Aalborg, Denmark
⁴ Corresponding author. Department of Cardiovascular Surgery, Pompidou Hospital, 20 rue Leblanc, 75015 Paris, France. Tel: +33-613144398; e-mail: j.chachques@egp.aphp.fr (J.C. Chachques)

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Abstract

OBJECTIVES: Ventricular constraint devices made of polyester and nitinol have been used to treat heart failure patients. Long-term follow-up has not demonstrated significant benefits, probably due to the lack of effects on myocardial tissue and to the risk of diastolic dysfunction. The goal of this experimental study is to improve ventricular constraint therapy by associating stem cell intrainfarct implantation and a cell-seeded collagen scaffold as an interface between the constraint device and the epicardium.

METHODS: In a sheep ischaemic model, three study groups were created: Group 1: coronary occlusion without treatment (control group). Group 2: postinfarct ventricular constraint using a polyester device (Acorn CorCap). Group 3: postinfarct treatment with stem cells associated with collagen matrix and the polyester device. Autologous adipose mesenchymal stem cells cultured in hypoxic conditions were injected into the infarct and seeded into the collagen matrix.

RESULTS: At 3 months, echocardiography showed the limitation of left ventricular end-diastolic volume in animals both treated with constraint devices alone and associated with stem cells/collagen. In Group 3 (stem cell + collagen treatment), significant improvements were found in ejection fraction (EF) and diastolic function evaluated by Doppler-derived mitral deceleration time. In this group, histology showed a reduction of infarct size, with focuses of angiogenesis and minimal fibrosis interface between CorCap and the epicardium due to the interposition of the collagen matrix.

CONCLUSIONS: Myocardial infarction treated with stem cells associated with a collagen matrix and ventricular constraint device improves systolic and diastolic function, reducing adverse remodelling and fibrosis. The application of bioactive molecules and the recent development of nanobiotechnologies should open the door for the creation of a new semi-degradable ventricular support bioprosthesis, capable of controlled stability or degradation in response to physiological conditions of the left or right heart.

Keywords: Heart failure • Ischaemic heart disease • Ventricular constraint • Stem cell transplantation • Tissue engineering • Ventricular restoration

INTRODUCTION

Ischaemic and non-ischaemic cardiomyopathies induce adverse geometric remodelling of ventricular cavities, which change from a natural elliptical (conical) shape to a spherical shape. Ventricular chamber dilatation and spherical deformation are the important causes of morbidity and mortality of patients with congestive heart failure. A limitation of cardiac enlargement and reduction in mechanical ventricular wall stress was first observed in cases treated with biological restraint therapy, i.e. Lattisimus Dorsi dynamic cardiomyoplasty [1]. This autologous source of circulatory assistance in which an electrically stimulated grafted skeletal muscle works in concert with the myocardium requires a rather long and complex surgical procedure. For this reason, less-invasive alternative approaches have been proposed like ventricular restraint therapy using polyester or nitinol devices and more recently biological approaches for myocardial support and regeneration such as stem cell transplantation associated with tissue engineered scaffolds [2]. Since intrinsic myocardial regeneration takes place but is reduced during a normal life span, it can be assisted by extrinsic bioactive procedures, like stem cell transplantation [3].

Ventricular constraint therapy using polyester and nitinol devices for biventricular wrapping did not demonstrate clear haemodynamic benefits, probably due to the lack of myocardial tissue regeneration and the impairment of diastolic function, mainly for the right ventricle [4]. Echocardiographic studies using tissue velocity imaging following the implantation of polyester constraint devices showed that the positive effect on left ventricle (LV) dimensions was not accompanied by any improvement in cardiac output but rather right ventricular dysfunction [5]. In addition, it was shown that ventricular restraint nitinol devices applying epicardial transmural pressure can alter myocardial blood flow patterns in dilated cardiomyopathy [6].

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The objective of this study performed in a myocardial ischaemic model was to evaluate a combination procedure for myocardial repair and ventricular chamber remodelling, associating a ventricular constraint device with stem cells and three-dimensional (3D) collagen scaffolds.

MATERIALS AND METHODS

In female Rambouillet sheep weighing 32–37 kg (mean 35 ± 2.2 kg), subcutaneous tissue was removed for autologous adipose mesenchymal stem cell (MSC) isolation and expansion [7]. One month later, LV myocardial ischaemia was surgically created and then treated either with the CorCap ventricular constraint device or using a combined surgical procedure: associating stem cells, collagen scaffolds and the CorCap device.

All experiments were performed in accordance with the principles in the use of animals for scientific research of the French National Institute of Health and Medical Research (INSERM) and received care in compliance with the European Conventions. This study was approved by the ethical committee for animal research of the University of Paris.

Biopsy extraction

After preoperative medication with Vetranquil 1 mg/kg (Acepromazine, Ceva, Libourne, France) and induction of anaesthesia with Diprivan 6 mg/kg (Propofol, AstraZeneca, Rueil-Malmaison, France), animals were intubated and mechanically ventilated with an Aestiva 5 system (Datex-Ohmeda, Helsinki, Finland). Anaesthesia was maintained with oxygen inhalation of Forene 2–3% (Isoflurane, Abbot, Rungis, France). Adipose tissue biopsies were obtained by the removal of subcutaneous fat tissue (40–60 g) from the right thoracic wall and stored in phosphate-buffered saline (PBS) at room temperature until processing.

Isolation of adipose-derived MSCs (ASC)

The tissue samples were finely minced and digested by incubation in a 0.14 Wünsch units/ml Liberase Blendzyme 2 (Roche Applied Science, Hvidovre, Denmark) solution at 37°C for 2 h. The digests were centrifuged at 400 g for 10 min, and the top fluid and fat layers were discarded. Contaminating erythrocytes were lyzed by the resuspension of the pellet in sterile milli-Q water for 20 s, after which the salt concentration was adjusted through the addition of 10 × PBS. The cells were filtered through a 100-μm cell strainer, centrifuged at 400g for 10 min and resuspended in 25 ml of growth medium, consisting of minimum essential medium alpha (A-MEM; GIBCO/Invitrogen) supplemented with 10% foetal bovine serum and penicillin (10 U/ml), streptomycin (10 mg/ml) and gentamycin (10 mg/ml) (all from GIBCO/Invitrogen Laboratory). The cells were seeded in T75 flasks and transferred to a CO₂ incubator overnight, after which non-adherent cells were removed.

Adipo-, chondro- and osteogenic differentiation of adipose-derived stem cells

To determine if the isolated cells were stem cells, they were induced to differentiate into adipo-, chondro- and osteocytes. The adipogenic and osteogenic differentiation and the subsequent staining of intracellular lipid accumulations with Oil Red O and calcium deposits in the extracellular matrix with Alizarin Red were performed in monolayer cultures, as previously described [7]. The chondrogenic differentiation and the staining of sulphated glycosaminoglycans with Alcian Blue 8X were performed in pellet cultures.

Hypoxic culture and labelling with bromodeoxyuridine

Immediately following the isolation procedure, the flasks were transferred to a hypoxic workbench/incubator (Xvivo; Biospherix, Lacona, NY, USA), allowing for uninterrupted cell culture and passing in a controlled atmosphere of 5% O₂ and 5% CO₂ balanced with nitrogen. During expansion of the cells, the media was changed twice a week. When cells were 90% confluent, they were detached from the culture flasks using 0.125% trypsin/0.01% ethylene diamine tetracetic acid and transferred to new flasks.

For each sample, the cells were expanded until eight T175 culture flasks were 75% confluent, then the cells were labelled with bromodeoxyuridine (BrdU). Briefly, cells were incubated with growth media containing 10 μg BrdU (Sigma) for 48 h, and then the cells were washed several times with PBS and frozen in aliquots of approximately 10 × 10⁵ cells.

Experimental myocardial infarction

After preoperative medication and induction of anaesthesia (same protocol as fat tissue biopsies) animals were intubated and mechanically ventilated with an Aestiva 5 system. Electrocardiogram (ECG) was monitored during operation, a central venous line was placed through the external jugular vein and blood pressure was assessed using an arterial line from the animal ear.

Left thoracotomy was performed at the level of the fifth intercostal space, and the heart was exposed. To reduce the risk of ventricular fibrillation, a continuous i.v. infusion (2 mg/kg/h) of Xylocaine 1% (Lidocaine, AstraZeneca) was performed during the entire surgical procedure.

In all animals, an LV myocardial infarction was surgically created by transitory ligation (60 min) of the diagonal branch of the left coronary artery, followed by reperfusion. A 4-0 non-absorbable Prolene suture was passed underneath the coronary artery branch; the flow was interrupted using a Teflon pledget compressed by a polyurethane occluder. This occluder was released after 60 min, thus the myocardial ischaemic territory was reperfused. Significant EKG changes, including widening of the QRS complex and elevation of the ST segment, and colour and kinetics changes of the area at risk were considered indicative of coronary occlusion.

Treatment groups

After myocardial infarction, animals were randomized into three groups:

Group 1 (n = 5): Myocardial infarction without treatment (control group).
Group 2 (n = 5): Implantation of the CorCap ventricular constraint device.
Group 3 (n = 5): Intrainfarct injection of stem cells, implantation of the CorCap device and placement of the collagen matrix as interface.

Stem cells and collagen matrix implantation

In Group 3, sheep were treated with intramyocardial cell injection associated with a collagen matrix grafted with cells, placed onto the epicardium and covered by the CorCap device.

First step: stem cell treatment. 50 ± 4 million cells diluted in 2 ml of culture medium were injected into the myocardium with six needle points using a 27-gauge needle. Bulging over the infarcted area was confirmed in every case after injection. Criteria to guide the epicardial injections were the ischaemic ventricular surface discolouration and hypokinesia. To avoid regurgitation of the cell solution (channel leakage), we used finger compression for 1 min.

Second step: CorCap implantation. For CorCap device choice, the size of the heart was measured by special circumferential tape. The CorCap model Gen2 CSD Size B (Acorn Cardiovascular Inc., St. Paul, MN, USA) fabricated into a multifilament mesh knit of polyethylene terephthalate (PET-polyester) was chosen in all cases and placed around the ventricles.

Third step: collagen matrix. A collagen matrix was prepared from a commercially available CE Mark collagen kit (Pangen 2, Urgo Laboratory, Chenove, France). This 3D biodegradable scaffold (size: 5 × 7 × 0.6 cm) was manufactured using a lyophilized, non-denatured, native type I collagen. The matrix pores measured 50–100 μm. In the operating room and under high sterility conditions, the matrix was placed into a Petri dish; afterward, a cell suspension (50 ± 6 million cells diluted in 5 ml medium) was seeded onto the matrix. To promote a regular distribution of ASC into the matrix pores, Petri dishes containing the 3D collagen scaffolds were shaken continuously for 10 min at 160 g using an Orbital Shaker (Stuart Scientific, Stone, Staffordshire, UK). Then, the collagen slab was placed under the Acorn fabric (Fig. 1) that was fixed with epicardial sutures (Prolene 4-0) to the heart, at the level of the atrio-ventricular groove.

At the end of the operation, a chest drain was placed into the pleural cavity. Thoracotomy was then closed and the sheep ventilated until recovery. The drain was removed as soon as the sheep started spontaneous respiration. Postoperative sheep treatment consisted in cefazolin injected intramuscularly at 1 g per day over 5 days.

Evaluation

Evaluation includes postoperative (at 24 h) assessment of serum troponin levels and echocardiography. At 3 months, echocardiography and histopathological studies were performed to evaluate the evolution of the ischaemic lesions, the engraftment of the transplanted cell, new vessels formation and the CorCap device adaptation.

Echocardiography

By epicardial echocardiography, LV dimensions and function were assessed during surgery, before and after myocardial infarction, just before cell seeding and CorCap implantation. At 3 months, under general anaesthesia, the chest was reopened for epicardial echocardiography. Images were recorded using a 7-MHz phased-array transducer (Sonos 5500; Hewlett-Packard, Palo Alto, CA, USA) and subsequently analysed by an observer, blinded to the treatment groups (Ultrasound Image Workstation-300A, Toshiba).

Long and short axes and apical two- and four-chamber images were acquired. Endocardial borders were contoured for the assessment of end-diastolic and end-systolic images. Ventricular volumes and ejection fractions were calculated according to the biplane Simpson’s method.

Diastolic transmitral valvular flow was evaluated using conventional Doppler ultrasound. The following variables were measured: peak flow velocity of early filling (E), peak flow velocity at atrial contraction (A), their ratio (E/A) and deceleration time (DT) of early filling [8].

Histological studies

At postoperative month 3, all sheep were sacrificed after echocardiography assessment. The site of myocardial injury was identified and dissected. Four to 5 specimens were used, and each of these were fixed in 10% formalin, embedded in paraffin and sectioned to yield 10-μm-thick slices. The sections were stained with haematoxylin, eosin and saffron. For the identification of grafted ASC (labelled by BrdU), heart slices were tested with specific antibodies.

Statistical analysis

Data are summarized using median (inter-quartile range) or mean (standard deviation) values. Comparisons of LV function variables across groups were performed using analysis of variance or a non-parametric Kruskal–Wallis test with the exact P-value. Pairwise comparisons were analysed using analysis of
variance or analysis of variance on ranks, $P$-values were adjusted for multiple testing (Tukey adjustment). For all analyses, a two-tailed $P$-value <0.05 was considered statistically significant. Analyses were conducted using SAS 9.2 (Statistical Analysis System, Cary, NC, USA).

RESULTS

No animal mortality was observed during interventions and follow-up. The presence of the myocardial injury was confirmed by the raised serum level of cardiac troponin I (82.5 ± 15 μg/ml) after 24 h from myocardial infarction (MI). The quantitative determination was assessed with a cardiac troponin I fluorometric enzyme immunoassay (Stratus; Dade Behring, Deerfield, IL, USA).

Characterization of the cells

The isolated cells adhered to plastic and assumed a spindle shaped morphology after few days in culture (Fig. 2A). In addition, the cells could be induced to differentiate into either adipocytes (Fig. 2B), chondrocytes (Fig. 2C) or osteocytes (Fig. 2D), thus confirming their identity as multipotent MSCs.

Echocardiography

LV function and dimensions were quantified the day of myocardial injury and at 3 months. At baseline and after infarction, the mean left ventricular end-diastolic volume and EF were similar in all three groups (Table 1).

Over the course of 3 months, each group increased their LV end-diastolic dimension compared with their baseline value. A statistically significant attenuation of LV dilatation was demonstrated with CorCap without and with cells compared with the non-treated control group. Functional results at 3 months showed greater benefits in the hearts treated with CorpCap + cells/matrix compared with those receiving CorCap only or without treatment (Table 2).

Doppler-derived DT improved from 140 ± 6.3 to 195 ± 9.5 ms ($P = 0.03$) in the cell–matrix group but not in the other groups. In the CorCap-only group, the $E$ component was reduced, resulting in a lower $E/A$ ratio, this alteration can be related with impaired LV relaxation (Table 3).

Table 1: Initial echocardiography assessment (before treatment)

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>LVEDV (ml) Before infarction</th>
<th>LVEDV (ml) After infarction</th>
<th>LVEF (%) Before infarction</th>
<th>LVEF (%) After infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ($n=5$)</td>
<td>39 ± 2.5</td>
<td>48.6 ± 5.3</td>
<td>68.1 ± 2.4</td>
<td>41 ± 1.8*</td>
</tr>
<tr>
<td>CorCap ($n=5$)</td>
<td>37.4 ± 3.1</td>
<td>44.8 ± 4.5</td>
<td>67.5 ± 1.6</td>
<td>39.7 ± 2*</td>
</tr>
<tr>
<td>Cells + CorCap ($n=5$)</td>
<td>38 ± 3</td>
<td>47.4 ± 4.3</td>
<td>68 ± 2.1</td>
<td>38.8 ± 1.9*</td>
</tr>
</tbody>
</table>

Data are mean ± SD. LVEDV: left ventricular end-diastolic volume; LVEF: left ventricular ejection fraction.

*P = 0.001 vs baseline.
Histological results

Autopsy at 3 months showed the CorCap mesh covering both ventricles (Fig. 3A).

**Ventricular wall.** The ischaemia/reperfusion (I/R) model used for this study results in multifocal pathological areas, consisting in fibro-adipose lesions starting at the subendocardial layer. This mixed configuration of the I/R area (Fig. 3B and C) was different from the coronary artery occlusion model, with central fibrosis, intermediate borderzone, and all surrounded by healthy myocardium.

In the stem cell-treated sheep, the multifocal ischaemic areas were much less prominent. In this group, growing myocardial tissue containing BrdU-marked cells and focuses of angiogenesis were much less prominent. In this group, growing myocardial tissue containing BrdU-marked cells and focuses of angiogenesis were much less prominent.

**Interface.** At the level of the epicardium, an important difference was found between Group 2 (CorCap only) and Group 3 (CorCap with collagenized collagen). In the latter group, we found minimal fibrosis interface between the polyester mesh device and the epicardium probably due to the interposition of the cell-seeded collagen and/or anti-inflammatoric properties of the ASCs (Fig. 4). Contrary, in Group 2 (without seeded collagen), the CorCap was completely anchored by dense prominent fibrous tissue invading the nearby myocardium (Fig. 5, right).

**CorCap device.** Chronic inflammatory reaction was observed around the polyester fibres, consisting of giant’s cells, macrophages, fibroblasts and capillaries. An important fibrosis involved the multifilament mesh, spreading out the fibres penetrate into the ventricular epicardium (Fig. 5).

**DISCUSSION**

The objective of stem cell transplantation in myocardial disease is to repair the myocardial tissue by the implantation of myogenic and/or angiogenic cells. According to published clinical studies, the small percentage of cell survival in the scar and the absence of interaction between the implanted cells and the host myocardium (due to the absence of gap junctions) lead to minimal improvement in ventricular function [9]. Both large-animal and clinical studies have examined the potential role of endogenous and exogenous stem cells to alter the course of LV remodelling. Interestingly, there have been alterations in LV remodelling with stem cell treatment despite a lack of long-term cell engraftment. On the basis of these studies, there appears to be a relationship between stem cell treatment post-MI and the modification of proteolytic pathways, generating the hypothesis that stem cells leave an echo effect that moderates LV remodelling [10].

Increased level of stromal cell-derived factor-1 (SDF-1), a potent chemotaxin can be found in an ischaemic myocardium and might protect against ischaemia/reperfusion injury. In ischaemic models treated with stem cells overexpressing SDF-1, it was shown functional regeneration of ischaemic myocardium [11]. Myocardial tissue engineering is emerging as a new therapeutic tool, becoming a promising way for the creation of a ‘bioartificial myocardium’. The stem cell niche, a specialized environment surrounding native and grafted stem cells, provides crucial support needed for the maintenance of stem cells [12].

### Table 2: Echocardiography using the Simpson method: 3-month follow-up

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>CorCap (n = 5)</th>
<th>Cells + CorCap (n = 5)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic diameter (cm)</td>
<td>3.04 (0.08)</td>
<td>3.38 (0.48)</td>
<td>3.30 (0.29)</td>
<td>0.35</td>
</tr>
<tr>
<td>Systolic diameter (cm)</td>
<td>2.44 (0.10)</td>
<td>2.69 (0.38)</td>
<td>2.38 (0.17)</td>
<td>0.23</td>
</tr>
<tr>
<td>Shortening fraction</td>
<td>0.20 (0.02)</td>
<td>0.20 (0.01)</td>
<td>0.28 (0.05)</td>
<td>0.0051</td>
</tr>
<tr>
<td>Diastolic area 4C (cm²)</td>
<td>18.57 (1.15)</td>
<td>14.35 (1.86)</td>
<td>13.38 (1.10)</td>
<td>0.0013</td>
</tr>
<tr>
<td>Diastolic length 4C (cm)</td>
<td>6.89 (0.95)</td>
<td>5.24 (0.58)</td>
<td>6.56 (1.81)</td>
<td>0.19</td>
</tr>
<tr>
<td>Systolic area 4C (cm²)</td>
<td>14.75 (2.19)</td>
<td>9.30 (2.51)</td>
<td>7.45 (1.00)</td>
<td>0.0024</td>
</tr>
<tr>
<td>Systolic length 4C (cm)</td>
<td>6.24 (0.82)</td>
<td>4.89 (0.77)</td>
<td>4.40 (1.16)</td>
<td>0.052</td>
</tr>
<tr>
<td>Diastolic volume 4C (ml)</td>
<td>42.75 (1.25)</td>
<td>34.15 (9.03)</td>
<td>25.10 (9.36)</td>
<td>0.028</td>
</tr>
<tr>
<td>Systolic volume 4C (ml)</td>
<td>29.70 (1.38)</td>
<td>15.48 (5.82)</td>
<td>10.49 (4.86)</td>
<td>0.0005</td>
</tr>
<tr>
<td>EF area/LV length 4C</td>
<td>0.30 (0.05)</td>
<td>0.55 (0.08)</td>
<td>0.58 (0.07)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Diastolic area 2C (cm²)</td>
<td>15.47 (0.47)</td>
<td>15.05 (2.78)</td>
<td>13.93 (2.14)</td>
<td>0.56</td>
</tr>
<tr>
<td>Diastolic length 2C (cm)</td>
<td>6.21 (0.18)</td>
<td>5.81 (0.73)</td>
<td>5.68 (0.81)</td>
<td>0.50</td>
</tr>
<tr>
<td>Systolic area 2C (cm²)</td>
<td>11.14 (0.16)</td>
<td>9.75 (3.43)</td>
<td>7.35 (2.85)</td>
<td>0.28</td>
</tr>
<tr>
<td>Systolic length 2C (cm)</td>
<td>5.55 (0.03)</td>
<td>5.59 (0.36)</td>
<td>4.85 (0.95)</td>
<td>0.26</td>
</tr>
<tr>
<td>Diastolic volume 2C (ml)</td>
<td>31.55 (3.33)</td>
<td>34.63 (11.18)</td>
<td>30.33 (16.23)</td>
<td>0.91</td>
</tr>
<tr>
<td>Systolic volume 2C (ml)</td>
<td>19.01 (0.62)</td>
<td>15.47 (9.60)</td>
<td>9.72 (6.70)</td>
<td>0.40</td>
</tr>
<tr>
<td>EF area/LV length 2C</td>
<td>0.41 (0.03)</td>
<td>0.52 (0.16)</td>
<td>0.57 (0.13)</td>
<td>0.22</td>
</tr>
<tr>
<td>Diastolic volume biplane (ml)</td>
<td>37.40 (2.13)</td>
<td>37.68 (7.95)</td>
<td>25.37 (8.46)</td>
<td>0.54</td>
</tr>
<tr>
<td>Systolic volume biplane (ml)</td>
<td>24.53 (0.76)</td>
<td>16.31 (9.53)</td>
<td>9.23 (6.07)</td>
<td>0.0012</td>
</tr>
<tr>
<td>EF biplane</td>
<td>0.35 (0.04)</td>
<td>0.53 (0.09)</td>
<td>0.59 (0.09)</td>
<td>0.0053</td>
</tr>
</tbody>
</table>

Data are mean (standard deviation) or median (inter-quartile range).

2C and 4C: Two and four cavities echocardiographic assessments.

*P-value for analysis of variance or Kruskal–Wallis test across the three groups.
Ventricular constraint therapy has been used to prevent and reverse the progression of heart failure in ischaemic and non-ischaemic cardiomyopathies. Two devices have been used clinically: a polyester multifilament mesh (CorCap device, Acorn, St. Paul, MN, USA) and a nitinol mesh for ventricular wrapping (HeartNet device, Paracor, Sunnyvale, CA, USA). But these devices failed to demonstrate clear positive effects on cardiopulmonary exercise testing and survival [4–6]. We hypothesized that incorporating biological regenerative effects in ventricular restraint devices, should avoid adverse effects like restriction in diastolic function.

Worsening diastolic function significantly raises the risk of heart failure. Diastolic dysfunction is actually a fairly common, dynamic process that, when it further deteriorates, increases the risk of dying by almost 80%. Diastolic dysfunction, aside from being a marker of increased risk, seems also to be a direct contributor to the adverse progression of heart failure by limiting cardiac output reserve, accelerating neuroendocrine activation, increasing symptoms of breathlessness and promoting physical inactivity, deconditioning and frailty [13].

Adipose-derived MSCs cultured in hypoxic conditions

Transplantation of MSCs is increasingly being recognized as a treatment option for ischaemic heart disease. The beneficial effects of the stem cell treatment appear to be partly due to the secretion of proangiogenic and anti-inflammatory factors [3]. While most clinical trials so far have been performed using mesenchymal stem cells from bone marrow, recent years have seen a surge in the interest in the use of adipose tissue-derived stem cells (ASCs). The ASCs hold several advantages including a higher concentration per ml tissue, ease of isolation and better proangiogenic properties [14, 15]. Recent evidence shows that stem cells from adipose tissue protect cardiomyocytes against hypoxic injury; the anti-inflammatory properties of stem cells may reduce the magnitude of the immune response to the synthetic cardiac constraint material [16].

Our study combined adipose-derived mesenchymal stem cells with the CorCap technology. To further promote the proangiogenic effect of the ASCs, we propose to use a combination of hypoxic culture and trypsin treatment, prior to the injection of the cells. The protocols used in this study regarding ASC isolation and expansion were based on evidence from previous studies, where an array of different culture media and oxygen tensions have been assayed for effect on cell growth and induction of proangiogenic phenotype [7, 16]. Thus, the cells were cultured in A-MEM media in an atmosphere of 5% oxygen to ensure optimal cell growth, and best preservation of differentiation potential concurrent with the production of growth factors such as insulin growth factor 1 and vascular endothelial growth factor.

The presence of ASCs in areas of regeneration in the myocardium and in the walls of newly formed vessels lend support to the hypothesis that ASCs exert their beneficial effects mostly through the protection of cardiomyocytes from apoptosis and through the promotion of new blood vessel growth. A third potential benefit of the ASCs in combination with CorCap are the anti-inflammatory properties of stem cells, which may
have played a role in the prevention of fibrosis around the mesh [2, 3, 10]. This combined cell-based therapeutic approach combined with 3D collagen scaffolds and the rationale of cell dose was previously investigated by our group [17, 18]. Data derived from our previous animal experimentation suppose that the use of a cellularized collagen matrix favours intramyocardial cell retention and creates a microatmosphere that promotes cell survival. We also evaluated the impact of collagen matrix alone in myocardial infarct models survival [17]. These studies showed that the use of collagen matrix alone in myocardial infarct models did not improved EF, post-ischaemic remodelling and LV wall thickness, as was shown by echocardiography and histology.

It is important to remark that the resorbable collagen matrix used in our studies (Pangen) has high haemostatic capacity and is frequently indicated and used in our hospital and in other European centres as compresses for local haemostasis during cardiothoracic and abdominal surgery, when control of bleeding by ligaturing or other conventional means is ineffective or impractical. In cardiac use, long-term follow-up did neither demonstrate regenerative properties nor a restrictive process with impairment of diastolic function (i.e. constrictive pericarditis).

**Future directions in myocardial tissue engineering**

Preliminary experimental and clinical studies in ischaemic heart disease showed that combining cell transplantation with matrix scaffolds offer further benefits with respect to cell therapy alone [17, 18]. Unfortunately, patches of collagen, gelatin and hydrogels are compromised by the short-term biodegradation of the grafted material. Nanomaterials are emerging as the main candidates to ensure the achievement of a proper instructive cellular niche. The main purpose of these materials is to display

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Figure 3: (A) Autopsy at 3 months showing the CorCap mesh covering both ventricles (arrow). (B) Left ventricular infarct scar (arrow). (C) Histology at 3 months of the ischaemic/reperfused myocardium. Arrows show the mixed configuration: patchy fibrosis (1) and subnormal myocardium (2) (HE ×20).

Figure 4: Immunohistological study using anti-BrdU antibody on left ventricular wall. (Left) Growing myocardial tissue adjacent to the infarct scar including BrdU-labelled cells (arrows). (Right) Focuses of angiogenesis showing labelled cells in the vessels wall (arrows). BrdU antibody stains, ×10, ×20.
structural and functional properties similar to extracellular matrices, containing truly 3D nanonetworks [19, 20].

Ongoing research studies like the RECATABI European Project are developing ‘bioactive implants’ for myocardial regeneration and ventricular support (http://www.recatabi.com/). This approach includes an elastomeric microporous membrane (patch) having one synthetic non-degradable polymer and one partially degradable polymer (biological or synthetic) all associated with a peptide nanofibre hydrogel and stem cells. Therefore, a ‘bioactive implant’ for myocardial repair should provide a suitable environment for cell homing, growth and differentiation, as well as mechanical support to the heart.

The combination of degradable and non-degradable polymers should be advantageous because cells implanted in niches will organize, connect and contract more easily if they are surrounded by material that degrade with time. This partial degradation of the implant should reduce chronic fibrosis and the risk of diastolic function restriction. Some non-degradable prosthetic fibres that remain, seem necessary to avoid progressive heart dilatation (post-ischaemic remodelling).

Limitations of the study

This study is essentially an acute infarction study, whereas most heart failure based on an ischaemic pathology is chronic. Animal models of chronic heart failure generally need a second intervention to test surgical treatments. Myocardial infarction induced by interventional cardiology procedures is still related with high mortality. The design of our study is based in one-step surgical procedure to create the infarction followed by the implantation of the cells + collagen and the CorCap device. Likewise, we have avoided a two-step procedure, which would generate pericardial adhesions and fibrosis interfering with the fixation, the integration and the evaluation of our epicardial treatment (cells/collagen graft associated with the constraint device). We included an ischaemia/reperfusion model to evaluate treatment [21, 22]; further studies will be needed to assess the effect of this approach in chronic ischaemic diseases including complete occlusion of LV main arteries. The assessment of segmental wall motion abnormalities is recommended in future studies.

Myocardial infarct animals were not injected with cell vehicle or treated with collagen matrix alone. The main goal of our study was to evaluate how to improve ‘ventricular constraint therapy’, and for this reason, we compared the CorCap device with a new approach associating stem cells grafted in collagen scaffolds. Previous studies performed by our group and other groups showed poor results with cell therapy alone, and no effects on ventricular function and myocardial recovery using cell culture medium or collagen matrix alone [2, 9, 12, 14, 17]. In accordance with regulations of our University Ethical Committee, the design of research projects using large-animal models should strictly include the minimum necessary number of study groups and interventions to evaluate new surgical procedures and to satisfy statistical requirements.

CONCLUSION

In conclusion, our biosurgical procedure combines a regenerative biological approach (stem cells + collagen scaffold) reinforced by a mechanical ventricular constraint device (CorCap). Until now, these procedures separately failed to show clear benefits at long-term. It seems that collagen interface reduced the risk of CorCap foreign body reaction fibrosis impairing diastolic function. This study demonstrated improvements in systolic and diastolic functions in the cell–collagen-treated group, inducing cardiac angiogenesis. Reduced oxygen tension used to expand stem cells points to an important potential in the treatment of ischaemic myocardium [16, 23].
**Clinical implications.** The combination of ventricular constraint therapy together with a biological stem cell-based regenerative approach could be a promising way for the treatment of heart failure patients. The application of bioactive molecules and the recent development of nanobiotechnologies should open the door for the creation of a new bioprosthetic ventricular support system, in a form of a semi-degradable device designed according to the concept of 'helical ventricular myocardial band' [24]. It should be manufactured in different models for left and/or right ventricle diseases, capable of controlled stability or degradation in response to physiological conditions of the left or right heart [25].

**Conflict of interest:** none declared.

**REFERENCES**


