

## BASIC RESEARCH

## Interventional magnetic resonance imaging for guiding gene and cell transfer in the heart

I M Barbash, J Leor, M S Feinberg, A Tessone, S Aboulafia-Etzion, A Orenstein, J Ruiz-Cabello, J S Cohen, Y Mardor

Heart 2004;90:87–91

**Background:** Interventional magnetic resonance imaging (iMRI) has the potential for guiding interventional cardiac procedures in real time.

**Objectives:** To test the feasibility of iMRI guided gene and cell transfer to the heart and to monitor myocardial remodelling after myocardial infarction in a rat model.

**Methods:** The MRI contrast agent GdDTPA, together with either Evans blue dye, or a recombinant adenovirus encoding the *LacZ* gene, or primary fibroblasts tagged by BrdU, were injected into the myocardium of rats under iMRI guidance. Rats were killed seven days after the injection and the hearts sectioned to identify the blue dye, *LacZ* expression, or fibroblast presence, respectively. In a parallel study, left ventricular area was measured before and after myocardial infarction and in sham operated rats by T1 weighted MRI and by echocardiography.

**Results:** Location of GdDTPA enhancement observed with iMRI at the time of injection was correlated with Evans blue stain,  $\beta$ -gal expression, and the primary fibroblast location in histological studies. iMRI and echocardiography measured a comparable increase in left ventricular area at seven and 30 days after myocardial infarction. A good correlation was found between the iMRI and echocardiographic assessment of left ventricular area ( $r = 0.70$ ;  $p < 0.0001$ ) and change in left ventricular area with time ( $r = 0.75$ ;  $p < 0.0001$ ).

**Conclusions:** The results show the feasibility and efficiency of iMRI guided intramyocardial injections, and the ability to monitor heart remodelling using iMRI. Genes, proteins, or cells for tissue engineering could be injected accurately into the myocardial scar under iMRI guidance.

See end of article for authors' affiliations

Correspondence to:  
Dr Jonathan Leor, Neufeld  
Cardiac Research Institute,  
Sheba Medical Centre, Tel-  
Hashomer 52621, Israel;  
leorj@post.tau.ac.il

Accepted 22 July 2003

Recent advances in imaging technologies and software capabilities of magnetic resonance imaging (MRI) have allowed its emergence as a clinically available tool for the rapid three dimensional assessment of ventricular function and mass. Recently the use of interventional MRI (iMRI) has been expanded to providing real time images in interventional procedures<sup>1–4</sup> and to monitoring drug delivery.<sup>5</sup> iMRI is configured with an open magnet; it is therefore less noisy and less claustrophobic than conventional MRI machines, and allows the scanning of oversized patients as well. Thus in the era of economic medicine this tool can serve a dual purpose, both monitoring and interventional.

Progress in our understanding of the pathophysiology of heart failure and of molecular biological techniques has introduced new types of treatment.<sup>6</sup> Tissue engineering with or without gene therapy is a promising approach that makes the replacement of lost or failing tissues possible. Furthermore, percutaneous cell transplantation into the failing myocardium can reduce the risks of complications of open chest transplantation. Thus our aims in the present study were to assess the feasibility and efficiency of iMRI for interventional therapeutic procedures and for monitoring left ventricular remodelling in a rat model.

The study was conducted in two parallel arms: a treatment arm, in which iMRI guided percutaneous gene and cell transfer for tissue engineering were undertaken; and a monitoring arm, in which left ventricular remodelling was monitored by iMRI and compared with standard echocardiography.

## METHODS

The study was done in accordance with the guidelines of the animal care and use committee of Sheba Medical Centre,

Tel-Aviv University, which conform to the policies of the American Heart Association and the *Guide for the care and use of laboratory animals* (Department of Health and Human Services, NIH Publication No 85–23).

## Study arms

The study was conducted in two parallel arms: a treatment arm and a monitoring arm. In the treatment arm the feasibility of iMRI real time guided percutaneous injections into the myocardium of untreated rats was assessed. In the monitoring arm, the end diastolic left ventricular area of rats with myocardial infarction ( $n = 19$ ) or without infarction (control rats,  $n = 8$ ) was assessed on preset assessment intervals (days 0, 7, 14, 21, and 30). At each assessment interval, several rats from each group were assessed by measuring the end diastolic left ventricular area with both cross sectional echocardiography and iMRI.

The correlation between the two methods was assessed separately for each interval irrespective of the timing of its performance (that is, day 0, 7, 14, 21, and 30). Thus the data are presented and correlations calculated with regard to all assessment intervals irrespective of the timing of their performance. Rats without myocardial infarction (control rats) were included in the study because of the need to assess the correlation between the two methods in normal hearts not undergoing a remodelling process.

## Rat model of myocardial infarction

Our method for inducing myocardial infarction has been described previously.<sup>6</sup> Briefly, Sprague-Dawley rats were anaesthetised with a combination of ketamine (50 mg/kg) and xylazine (10 mg/kg). The chest was opened under sterile conditions by a left thoracotomy through the fourth

intercostal space. The pericardium was removed and the left main coronary artery was permanently occluded by an intramural stitch.

### Echocardiography

Transthoracic echocardiography was carried out before the infarcting operation (day 0 for control rats) and at preset assessment intervals up to 30 days after the operation.<sup>7</sup> Rats were anaesthetised as described above, the chest was shaved, and the animals were placed supine. Echocardiograms were done with a commercially available echocardiography system equipped with 12 MHz phased array transducer (Hewlett Packard Inc, Andover, Massachusetts, USA). The transducer was positioned on the left anterior side of the chest. The heart was imaged in cross sectional mode in parasternal long axis and short axis views of the left ventricle. Care was taken to avoid excessive pressure. Maximum left ventricular end diastolic dimension (at the time of maximum cavity dimension; cm<sup>2</sup>) was measured according to the leading edge method of the American Society of Echocardiography. All measurements were averaged for three consecutive cardiac cycles and were done by an experienced technician who was blinded to the treatment group.

### Magnetic resonance imaging

MRI was done before the infarcting operation (day 0 for control rats) and at preset assessment intervals up to 30 days after the operation. iMRI data were obtained using the 0.5 T GE iMRI machine at the Sheba Medical Centre with a specially constructed animal probe. In these experiments the T1 weighted images were averages of heart systole and diastole that provided excellent details and morphology without the need to trigger the acquisition by ECG.

For the treatment arm, axial images were acquired using an inversion recovery prepared gradient echo sequence with the following parameters: field of view 12×9 cm, 256×192 pixels; TE (time of echo) 13.9 ms; preparation time 500 ms; flip angle 60°; one slice of 5 mm. The images were displayed on a small screen in the magnet, so that the physician could see the updating magnetic resonance image while doing the procedure. The image updated every five seconds and the displayed image was averaged over the last seven acquired images for better signal to noise ratio. Averaging the images allowed high quality anatomical resolution, while the five second updates enabled real time guidance of the procedure.

For the monitoring arm, axial images were acquired using a spin echo sequence with the following parameters: field of view 12×9 cm, 256×128 pixels; TE/TR (time of repetition) 14.5/500 ms; 2 mm slices; four averages. Scan time was 6 minutes and 32 seconds. The area of the left ventricle was calculated from the three central slices. The data presented are the average and standard deviation (cm<sup>2</sup>) over these three slices.

### Primary fibroblast isolation and labelling

Sprague-Dawley rats were anaesthetised as described above. The chest was opened under sterile technique by left thoracotomy through the fourth intercostal space and the pericardium was removed. The pericardial tissue was dissected into 1–2 mm slices and plated onto 60 mm tissue culture dishes. Cells were cultured in Dulbecco modified eagle medium (DMEM) with 10% fetal calf serum, 1% L-glutamine, and 100 000 U/ml penicillin/streptomycin (Beit-Haemek) (that is, complete culture medium). Cells were first passed upon reaching 90% confluence. Immediately before transplantation, primary fibroblasts were detached from the culture plates using trypsin-EDTA (Beit-Haemek) and the cells were mixed with GdDTPA (0.005 mmol/ml).

One day before transplantation the cells were incubated overnight with the thymidine analogue 5-bromo-2' deoxyuridine (BrdU) (1:100, Zymed, address) for their identification one week after transplantation in histological preparations. One week after transplantation, formalin fixed slides were stained with biotinylated Ms anti-BrdU (Zymed Laboratories Inc, San Francisco, California, USA) in order to localise the transplanted cells.

### Adenoviral amplification and purification

We used recombinant E1a deleted adenovirus-5 carrying the *LacZ* reporter gene (Ad β-gal) under control of the CMV promoter. The virus was propagated with the 293 cell line. The adenovirus β-gal stock was prepared as described previously.<sup>7</sup> Purified virus was stored at –70°C until use.

### Intramyocardial injections

In the treatment arm, injections were aimed at the myocardium of untreated rats and were done by a percutaneous approach with a 22 g needle inserted in the left parasternum at the fourth intercostal space under the iMRI real time guidance (see “magnetic resonance imaging” section above).

As the needle does not produce a signal in MR images, it appears as a dark line on the brighter background of the tissue. In order to monitor the precise location of the injected solution, all injected solutions were mixed with GdDTPA (0.005 mmol/ml). In the first feasibility study methylene blue dye together with GdDTPA was injected in order to assess the correlation between the precise location of the needle in the myocardium, as seen in the real time imaging, and the actual location of the injection in histological preparations. In the following studies either a naked adenovirus carrying the *LacZ* gene, or primary fibroblasts ( $2 \times 10^6$  cells in 200 μl) tagged by BrdU were transplanted.

### Statistical analysis

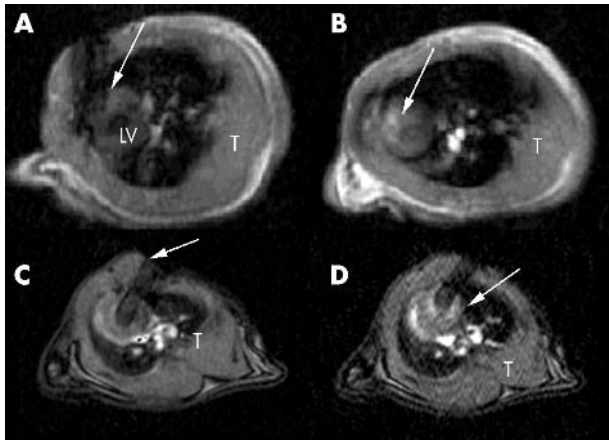
All values are shown as mean (SEM). Differences between MRI measurements before and after myocardial infarction were compared by unpaired *t* test (InStat, Version 3.01; GraphPad Software Inc). All tests were two tailed and significance was accepted at  $p < 0.05$ . Changes in left ventricular area following myocardial infarction as assessed by iMRI were calculated by one way analysis of variance; a probability value of  $p < 0.05$  was considered significant. The correlation between MRI and the echocardiographic assessment of the changes in left ventricular area was determined using the Pearson's correlation coefficient (denoted by *r*) where  $r = 1$  was regarded as a perfect linear relation with a positive slope between the two variables.

## RESULTS

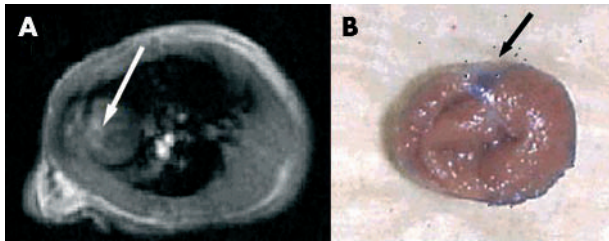
### Treatment arm

Real time iMRI provided a clear image of the needle and its relation to the chest wall and myocardium (fig 1). Additionally, following the injection a clear enhancement attributed to the injected GdDTPA indicated the exact location of the injection (fig 1). In the preliminary experiment in which methylene blue was injected, the location of the intramyocardial injection that was imaged by the iMRI was in the exact location (anterior wall) that was verified in macroscopic transverse sections of the heart (fig 2).

Similar results were found when a recombinant adenovirus encoding the *LacZ* gene was injected with GdDTPA percutaneously into the myocardium. The adenovirus efficiently transfected cardiomyocytes at the area of injection, as demonstrated in histological preparations done one week after the injection (fig 3). The inflammatory response resulting from the needle injury and directed against the transplanted cells and the adenovector was noticed.



**Figure 1** Intramyocardial injection guided by interventional magnetic resonance imaging (iMRI). Panels (A) and (C) show the location of the leading edge of the needle at the anterior wall of the left ventricle (LV). The left ventricle looks like a bagel. Panels (B) and (D) show GdDTPA related enhancement in the location of the injections. T, thorax.

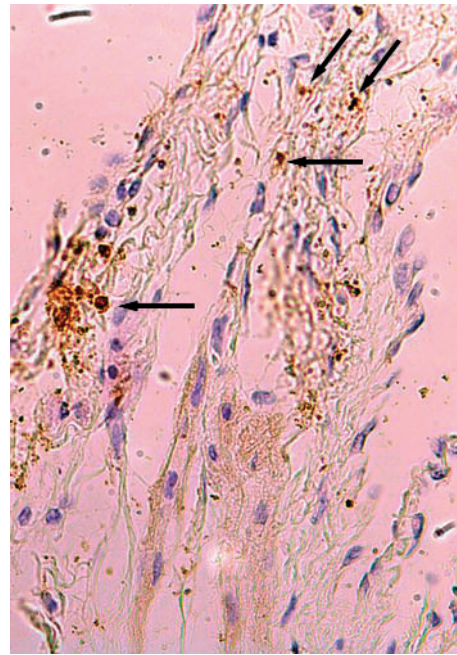


**Figure 2** Macroscopic view of heart injected by methylene blue dye with real time interventional magnetic resonance imaging (iMRI). (A) The site of GdDTPA related enhancement in anterior wall of the heart. (B) The actual location of the injection at the anterior wall of the heart, as indicated with the methylene blue dye in transverse histological sections of the heart.

As a method to transplant cells into the myocardium for tissue engineering, BrdU tagged fibroblasts were injected percutaneously together with GdDTPA under iMRI guidance. In formalin fixed slides of the hearts, the transplanted cells were found within the injection site up to one week after the transplantation (fig 4).

**Monitoring arm**

In this study, 19 rats with myocardial infarction and eight control rats were monitored. These 27 rats underwent a total of 65 measurements at the different assessment intervals and were included in the present analysis. Figure 5 shows the left



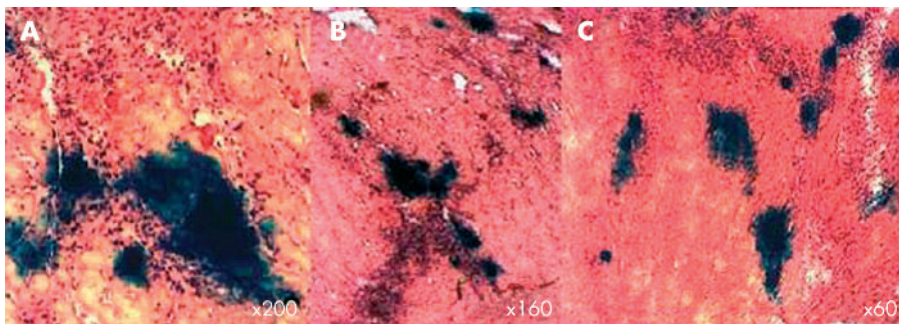
**Figure 4** Histological slides of hearts injected with primary fibroblasts tagged with BrdU. The implanted cells are positively brown stained (arrows).

ventricular dilatation and myocardial free wall thinning of the heart, as assessed by axial MRI slices before myocardial infarction (left ventricular area, 4.1 (0.4) cm<sup>2</sup> as calculated from the MR images), seven days post-infarction (9.7 (1.5) cm<sup>2</sup>), and at 30 days post-infarction (13.3 (1.1) cm<sup>2</sup>) (p = 0.002), as compared with echocardiographic imaging of the same rat, and with pathological examination of transverse sections of the heart 30 days after infarction. The increase in left ventricular area identified by iMRI was by a factor of 1.4 (0.3) at seven days and 1.9 (0.9) at 30 days after infarction (p = 0.0002). The echo identified a comparable increase in left ventricular area at seven days (1.5 (0.4)) and 30 days (1.9 (0.2)).

The correlation between MRI and echocardiography was good, both for assessing the left ventricular end diastolic area at a specific time after the infarction (r = 0.7) (fig 6A), and for assessing a change in the left ventricle over the defined period of time (r = 0.75) (fig 6B). Both correlations reached significance (p < 0.0001).

**DISCUSSION**

The major new findings of the present study are first, that iMRI can be used as an efficient method of guiding gene and



**Figure 3** Microscopic views of transgene expression in the myocardium of a rat heart treated by injection of recombinant adenovirus encoding the LacZ marker gene guided by magnetic resonance imaging. There is robust transgene expression in the myocardium.

cell transfer in the heart, and second, that iMRI can be used as a reliable tool with high anatomical resolution to assess left ventricular remodelling after myocardial infarction in a rat model.

In the setting of myocardial damage and heart failure, gene therapy can enhance neoangiogenesis by delivering genes encoding various growth factors directly to the ischaemic myocardium, thus improving myocardial perfusion and cardiac function.<sup>8,9</sup> A different approach for improving cardiac function after myocardial infarction is cell transplantation for tissue engineering, which has been shown to improve cardiac function.<sup>6</sup>

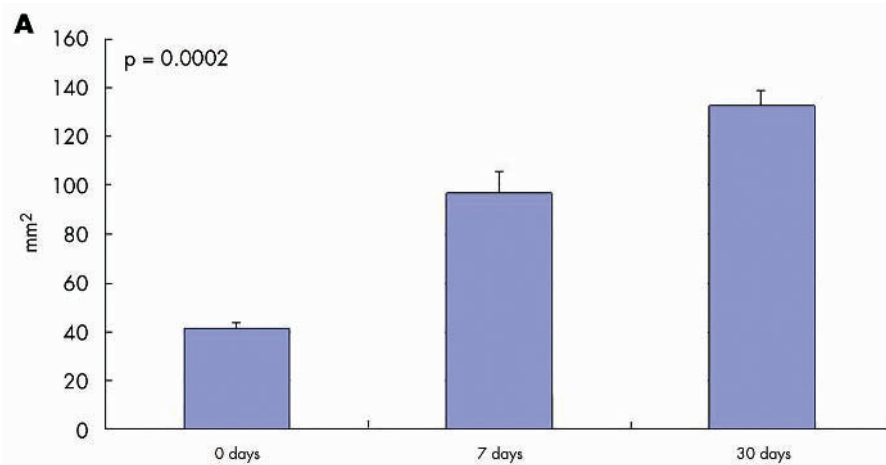
Recently, Sorger *et al* and Lederman *et al* presented preliminary data on testing the feasibility of using MRI real time imaging to guide invasive procedures in the heart.<sup>5,10</sup> Both groups injected GdDTPA mixed with coloured dye into the heart under real time guidance using a cardiac 1.5 T scanner and identified the dye location on histological examination. Our present study shows that good imaging quality can be achieved with a 0.5 T iMRI machine for real time image guided gene therapy and primary fibroblast transplantation. This resulted in efficient transfection of the myocardial tissue by recombinant adenovirus, and the survival of the transplanted cells for at least a week. Real time imaging with MRI has a theoretical advantage over other percutaneous methods for introducing cells into the myocardium as it allows a direct view of the needle, thus

improving both the accuracy and the safety of the procedure, and enabling direct injection to a specific location in the myocardium.

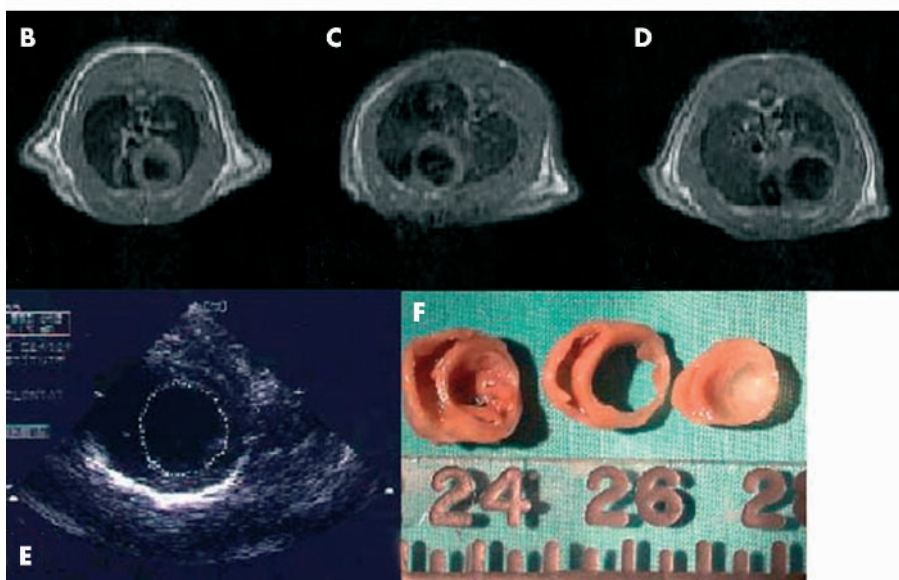
Furthermore, iMRI can also serve as a complementary tool to assess left ventricular remodelling after myocardial infarction, in view of its fundamental tomographic nature. Echocardiography is a widely accepted method of assessing left ventricular volume and function. However, it suffers from several limitations such as the need to extrapolate data from limited sampling of the left ventricle, both in M mode and in Simpson's biplane cross sectional method. iMRI can provide high spatial resolution imaging in any desired plane, without exposure to ionising radiation. Furthermore, cardiovascular magnetic resonance imaging has been shown in several studies to be accurate in assessing normal and dilated hearts, both in humans and in animal models,<sup>11-13</sup> and also to be well correlated with echocardiography.<sup>14,15</sup> The present study verifies and further extends these findings, indicating that iMRI is an efficient tool for assessing left ventricular remodelling.

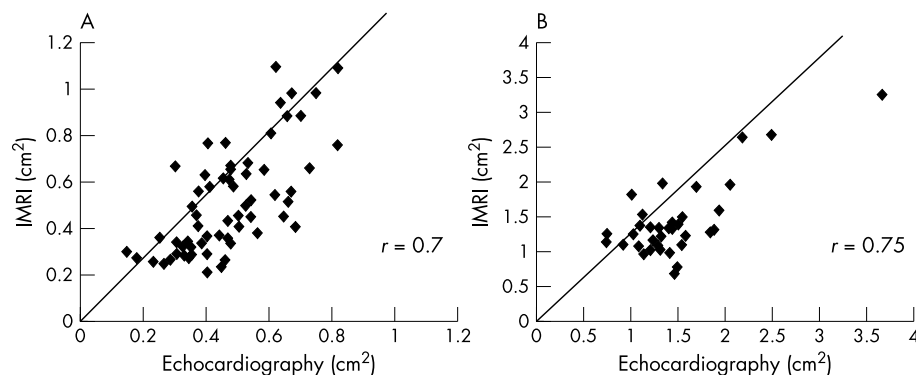
### Limitations

Our study lacks comparison with other strategies for gene and cell delivery to the heart. The ability to inject genes and cells during open chest surgery,<sup>6,16</sup> x ray fluoroscopy, and NOGA electroanatomical mapping<sup>17</sup> have been tested. These alternative approaches have certain limitations. Additional



**Figure 5** Left ventricular area assessment following myocardial infarction. (A) Left ventricular area assessed by magnetic resonance (MR) imaging. The increase in left ventricular area was by a factor of 1.4 (0.3) at seven days and 1.9 (0.9) at 30 days after the infarction ( $p = 0.0002$ ). (B) MR image of left ventricular area before myocardial infarction. (C) Left ventricular area seven days after the infarction. (D) Left ventricular area 30 days after the infarction. (E) Echocardiographic image of the left ventricular area 30 days after the infarction. Note the resolution as compared with the MR image in (D). (F) Pathological transverse sections of the heart 30 days after infarction.





**Figure 6** Correlation between interventional magnetic resonance imaging (iMRI) and echocardiography. (A) Correlation in the assessment of the left ventricular area (cm<sup>2</sup>) ( $y = 1.044x + 0.0248$ ). (B) Correlation in the assessment of the change in the left ventricular area up to 30 day follow up ( $y = 0.7485x + 0.3326$ ).

studies are needed to compare the advantages of iMRI over alternative imaging tools for introducing genes or cells into the heart. Another limitation is that the present study assessed the correlation between iMRI and echocardiography for only one index of left ventricular remodelling after myocardial infarction, and does not present correlation data for a complete assessment of left ventricular function in the post-infarction period.

### Conclusions

Our study shows for the first time that iMRI can serve as an effective tool for providing real time guidance for gene and cell delivery into the heart, as well as for assessing heart remodelling. These findings are preliminary, and improved imaging may be obtained by using interventional MRI systems with higher magnetic fields and with ECG triggered acquisition. Further investigations to confirm the beneficial effects of iMRI guided gene and cell delivery on left ventricular remodelling and function are needed. If successful, iMRI may evolve into an alternative tool for interventional cardiac procedures.

### ACKNOWLEDGEMENTS

This work was supported in part by grant No 95-00294/1 from the United States–Israel Binational Science Foundation (BSF), the Izmel programme of the Israel Ministry of Industry and Commerce and Tel-Aviv University, and the Itzhak Tiber and Slezak Funds, Tel-Aviv University.

### Authors' affiliations

I M Barbash, J Leor, M S Feinberg, A Tessone, S Aboulafia-Etzion, Neufeld Cardiac Research Institute, Sheba Medical Centre, Tel-Hashomer, Israel

A Orenstein, J S Cohen, Y Mardor, Advanced Technology Centre, Sheba Medical Centre

J Ruiz-Cabello, Universidad Complutense, Madrid, Spain

### REFERENCES

- Daniel BL. Intraprocedural magnetic resonance imaging-guided interventions in the breast. *Top Magn Reson Imaging* 2000;**11**:184–90.
- Gehl HB, Frahm C. MRI-controlled biopsies. *Radiologe* 1998;**38**:194–9.
- Seifert V, Zimmermann M, Trantakis C, et al. Open MRI-guided neurosurgery. *Acta Neurochir (Wien)* 1999;**141**:455–64.
- Buecker A, Spuentrup E, Grabitz R, et al. Magnetic resonance-guided placement of atrial septal closure device in animal model of patent foramen ovale. *Circulation* 2002;**106**:511–15.
- Sorger JM, Guttman MA, Wassmuth R, et al. Real-time MRI guided drug delivery [abstract]. *Proc Int Soc Magn Reson Med* 2001:abstract 2187.
- Leor J, Patterson M, Quinones MJ, et al. Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation* 1996;**94**(9 suppl):II332–6.
- Etzion S, Battler A, Barbash IM, et al. Influence of embryonic cardiomyocyte transplantation on the progression of heart failure in a rat model of extensive myocardial infarction. *J Mol Cell Cardiol* 2001;**33**:1321–30.
- Nabel EG. Gene therapy for cardiovascular disease. *Circulation* 1995;**91**:541–8.
- Lafont A, Guerot C, Lemarchand P. Prospects for gene therapy in cardiovascular disease. *Eur Heart J* 1996;**17**:1312–17.
- Lederman RJ, Guttman MA, Peters DC, et al. Catheter-based endomyocardial injection with real-time magnetic resonance imaging. *Circulation* 2002;**105**:1282–4.
- Semelka RC, Tomei E, Wagner S, et al. Interstudy reproducibility of dimensional and functional measurements between cine magnetic resonance studies in the morphologically abnormal left ventricle. *Am Heart J* 1990;**119**:1367–73.
- Siri FM, Jelicks LA, Leinwand LA, et al. Gated magnetic resonance imaging of normal and hypertrophied murine hearts. *Am J Physiol* 1997;**272**:H2394–402.
- Rudin M, Pedersen B, Umemura K, et al. Determination of rat heart morphology and function in vivo in two models of cardiac hypertrophy by means of magnetic resonance imaging. *Basic Res Cardiol* 1991;**86**:165–74.
- Herregods MC, De Paep G, Bijnens B, et al. Determination of left ventricular volume by two-dimensional echocardiography: comparison with magnetic resonance imaging. *Eur Heart J* 1994;**15**:1070–3.
- Mogelvang J, Stokholm KH, Saunamaki K, et al. Assessment of left ventricular volumes by magnetic resonance in comparison with radionuclide angiography, contrast angiography and echocardiography. *Eur Heart J* 1992;**13**:1677–83.
- Leor J, Quinones MJ, Patterson M, et al. Adenovirus-mediated gene transfer into infarcted myocardium: feasibility, timing, and location of expression. *J Mol Cell Cardiol* 1996;**28**:2057–67.
- Vale PR, Losordo DW, Milliken CE, et al. Left ventricular electromechanical mapping to assess efficacy of phVEGF(165) gene transfer for therapeutic angiogenesis in chronic myocardial ischemia. *Circulation* 2000;**102**:965–74.