Microencapsulation to reduce mechanical loss of microspheres: implications in myocardial cell therapy

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Abstract

Objective: Previous regenerative studies have demonstrated massive cell losses after intramyocardial cellular delivery. Therefore, efforts at reducing mechanical losses may prove more successful in optimising cellular therapy. In this study, we hypothesized that escalating mesenchymal stem cells (MSCs) dose will not produce corresponding improvement in cardiac function due to washout of the small cells in microcirculation. Using microspheres similar in size to MSCs, that are encapsulated in alginate-poly-L-lysine-alginate (APA), we tested the hypothesis that size is an important factor in early losses. Methods: In experiment I, five groups of rats (n = 9 each) underwent coronary ligation; group I had no treatment; the other groups received escalating 0.5 \times 10^6, 1.5 \times 10^6, 3 \times 10^6 and 5 \times 10^6 of MSCs each. Echocardiogram was performed at baseline, 2 days and 7 weeks after surgery. In experiment II, cell-sized microspheres (10 \mu m) were encapsulated in APA microcapsules. In group I (n = 16), rats received bare microspheres, group II (n = 16) microspheres within 200 \mu m microcapsules and in group III (n = 16), microspheres within 400 \mu m microcapsules. After 20 min, hearts were quantified for the amount retained. Results: Myocardial function did not improve further with escalating cell doses beyond an initial response at 1.5 \times 10^6 cells. Encapsulated microspheres in 200 \mu m and 400 \mu m microcapsules demonstrated a fourfold increase in retention rate compared with 10 \mu m microspheres. Conclusion: We concluded that suboptimal functional improvement in this animal model starts at 1.5 \times 10^6 cells and does not respond to escalating cell doses. Improving mechanical retention is possible by increasing the size of the injectate. Microencapsulation could be used to encapsulate donor cells and facilitate functional improvement in cellular heart failure therapy.

1. Introduction

With advances in medical and surgical therapies, patients with cardiovascular diseases currently have their best survival rates ever. However, this, in return, has led to an epidemic of congestive heart failure (CHF) in the developed world. In developed countries, CHF is the most common cause of hospitalisation in patients more than 65 years of age with an incidence that has increased by 155% in the past 20 years. The current estimate is that over 550 000 new cases are diagnosed annually in the USA alone. These patients have frequent hospitalisation with high morbidity and mortality reaching as high as 40% annually [1].

Although the mechanism is still controversial, animal studies and clinical trials have shown that mesenchymal stem cells (MSCs) could potentially improve ventricular function following ischaemic injury [2]. However, for such therapy to become clinically relevant, it is crucial to ensure that the transplanted cells remain within the myocardium after implantation. In this light, many studies have attempted to limit the extent of cell loss by reducing biological losses of implanted cells through anti-apoptotic, angiogenic or anti-free radical therapies. However, recent studies have shown that a large proportion of the injected cells are lost from the myocardium within the first few minutes post-injection [3–6]. Biological factors are thus unlikely to account for this early cell loss.

This has led us to suspect, based on a previous study from our lab, that massive mechanical, rather than biological losses, account for the substantial initial decrease in retained...
cells. This study, which used a fluorescent microsphere model not susceptible to biological factors, showed that washout of the implanted microspheres into the punctured microvascular network played a major role in the mechanical loss [6]. Other studies with cells suggest that larger cells have better retention rate [3—5]. One hypothesis is that they are prevented from being 'washed out' into the blood vessels by virtue of their size.

To overcome massive losses, one could suggest increasing the number of cells injected. However, higher cell loads into the heart are not without setbacks. Ishida et al. demonstrated that larger volumes of injectate compromised heart function, resulting in diastolic failure [7]. In addition, initial ischaemic conditions in the heart would not be able to sustain the viability of more injected cells. Thus, there is a limitation on how much volume and cell load one can deliver to the heart.

We hypothesized that increasing cell load through conventional direct epicardial injection will not significantly improve the cell engraftment or further optimise functional improvement of the treated ischaemic heart. Increasing the size of the injectate through microencapsulation, however, may decrease mechanical losses.

Developed in the early 1960s, microcapsules are semi-permeable polymeric membranes surrounding a solid matrix core. They have been used to trap and target large quantities of cells in different organs [8]. The membrane allows the bidirectional diffusion for the entry of nutrients and oxygen as well as the exit of therapeutic protein products and cellular waste materials. The capsule could be made non-biodegradable, protecting the content against larger molecules such as antibodies, white blood cells and tryptic enzymes or biodegradable where the content is released into the surrounding environment in a controlled fashion. Alginate-poly-L-lysine-alginate (APA) microcapsules are the most extensively studied material for cell and tissue microencapsulation. They have demonstrated the unique features of biocompatibility, durability and reproducibility [9].

To our knowledge, this is the first attempt to use microcapsules in cellular cardiomyoplasty.

2. Methods

All experiments were performed on female Lewis rats (200—250 g, Charles River, Quebec, Canada) in accordance with the guidelines set forth by the Canadian Council on Animal Care and were approved by the institutional ethics committee.

2.1. Cellular implantation studies

2.1.1. Cell isolation and labelling

Isolation and primary culture of MSCs from rats were performed as described previously by Caplan [10]. Marrow plugs from the tibia bones of male rats were disaggregated and the dispersed cells were centrifuged and resuspended twice in complete medium consisting of Dulbecco’s Modified Eagle’s Medium (DMEM) containing selected lots of 10% calf serum and antibiotics (100 U ml⁻¹ penicillin G, 100 μg mg⁻¹ streptomycin, 0.25 μg amphotericin B) (Gibco Laboratories, North Andover, MA, USA). A total of 10 ml of complete medium containing these cells were then introduced into tissue culture dishes. The medium was replaced every 3 days and the non-adherent cells including haematopoietic cells and fibroblasts were discarded. Each primary culture was twice divided into three new plates and the cell density of the colonies grown approximately to 90% confluence. After these passages, cells were almost confluent, and attached cells in culture flaks were stained with Lac Z as described by Bittira et al. [11] for unique identification among native cardiomyocytes.

2.1.2. Ligation of the left coronary artery and intramyocardial injections of MSCs

Rats were anaesthetised using 5% isoflurane, intubated and mechanically ventilated at 80 breaths min⁻¹. Via a left thoracotomy, the left coronary artery was ligated 2 mm from its origin with a 7/0 polypropylene suture (Ethicon, Inc, Somerville, NJ, USA). The ischaemic myocardial segment rapidly became identifiable through its pallor and akinesia. Fifteen minutes after ligation of the artery, three equal peri-infarct intramyocardial injections using a 27-G needle were performed. In the first group of rats (n = 9) 150 μl of culture medium only was delivered. In the subsequent groups II—V (n = 9 each), the same procedure was repeated using 150 μl of incremental doses of 0.5 × 10⁶, 1.5 × 10⁶, 3 × 10⁶ and 5 × 10⁶ MSCs, respectively.

2.1.3. Echocardiography

Transthoracic echocardiography was performed on all animals at baseline, 2 days post-infarct and at 7 weeks. An experienced echocardiographer (JFA), who was blinded to the experimental groups, performed the procedure. Echocardiograms were obtained with a commercially available system (Sonosite, Titan-Washington, Seattle, WA, USA), equipped with a Linear Probe 7—13 MHz 25-mm footprint Turbo transducer. Briefly, left ventricle end-diastolic (LVEDD) and end-systolic (LVESD) diameters were measured with M-mode tracings between the anterior and posterior walls from the short-axis view just below the level of the papillary muscles of the mitral valve. Following the American Society of Echocardiology leading-edge method, two images on average were obtained in each view and averaged over three consecutive cycles. Left ventricular fractional shortening (LVFS) was determined as = [(LVEDD — LVESD)/LVEDD] × 100% [12].

2.1.4. Histological and scar area analysis

After echocardiography at 7 weeks, hearts were stained for evidence of Lac Z labelled cell engraftment using X-gal (bromo-chloro-indolyl-galactopyranoside) staining and haematoxylin and eosin (H&E), as described by Bittira et al. [11,13].

To measure the scar area, picro-sirius red stain was used. Sectional slices of the myocardium were obtained at the mid-papillary level. The sections were stained in picro-sirius red for 1.5 h. Then they were washed in alcohol: 70%, 90% and 100%, respectively. Whereas normal heart tissue had a bright yellow—orange appearance, scar areas stained red—orange. Slides were digitised using an HP scanner (8300 Professional)
2.1.5. Polymerase chain reaction analysis

Random samples were selected from each group for polymerase chain reaction (PCR) analysis to confirm the survival of the implanted gender-mismatched cells in the hearts at 7 weeks. Genomic DNA was purified using DNeasy (Quiagen, Valencia, CA, USA). A total of 25 mg of cut myocardium was placed in a 1.5-ml microcentrifuge tube and 180 µl of buffer ATL was added. As much as 20 µl of proteinase K was added and mixed. The mixture was incubated at 55 °C in a shaking water bath until the tissue was completely lysed in 4 h. A total of 200 µl of buffer ATL was added to the sample, vortexed and incubated at 70 °C for 10 min. The sample then underwent multiple steps of washing with buffer solutions and vortexing. The final step was to form the gel and perform the electrophoresis of the purified DNA. The presence of living male cells in female hearts was confirmed by targeting a specific microsatellite sequence within the Y chromosome. The primer pairs used were 5’ AGA GGC ACA AGT TGG CTC AAC 3’ and 5’ TTC CAC TGA TAT CCC AGC TGC T 3’.

2.2. Microencapsulation of microsphere studies

2.2.1. Microencapsulation

Each vial of fluorescent microspheres from Molecular Probes (Invitrogen, Carlsbad, CA, USA) contained 36 million microspheres in 10 ml. To determine the number of microspheres per fluorescence unit, a standard regression line was first obtained. Seven samples of 50 µl of the microspheres (180 000 units) were taken by pipette and dissolved each in 3 ml of 2-ethoxyethyl acetate 98% (Sigma Laboratories, St. Louis, MO, USA) [14]. The samples were allowed to stand for 24 h and then were diluted as follows: 1:50, 1:100, 1:200 and 1:400. From each sample, 200 µl was read twice using a microtitre spectrophotometer (PerkinElmer, Waltham, MA, USA). The averaged values provided a standard curve of fluorescence versus microspheres. A total of 7.5 ml of microsphere suspension was centrifuged for 15 min at 2000 × g (Fig. 1(a)). The pelleted microspheres was then mixed with 0.9% saline and alginic acid (200 cps, Sigma Laboratories), such that the final alginate concentration was 1%. It was then run through the Encapsulator (Inotech Biotechnologies Ltd, Basel Switzerland) with the 100 µm nozzle and the following settings: frequency at 2000 Hz; voltage at 0.917 V; and speed 4.0 m min⁻¹. The microcapsules produced (200—250 µm in diameter) were collected and solidified for 10 min in a stirred solution of 0.1 M calcium chloride. To produce APA microcapsules (Fig. 1(b)), the solidified beads were filtered and washed in 0.1% poly-l-lysine and 0.1% alginate for 10 min [9]. The microcapsules were then suspended inside saline solution (Fig. 1(c)).

To determine the microsphere content per millilitre of encapsulated solution, seven samples of 50 µl of the microcapsules were taken by pipette and each dissolved in 10 ml of saturated sodium citrate solution for 1 h. Following centrifugation, the pellet was re-dissolved in saturated ethylenediamine tetraacetic acid (EDTA) (Fisher, ACS grade), re-centrifuged and washed with deionised water. These steps are taken to break the APA microcapsules and release their microspheres. Finally, the pellets were dissolved each in 3 ml of 2-ethoxyethyl acetate. The fluorescence obtained indicated a content of 2.11 million microspheres (±8%) per millilitre of encapsulated solution. Non-encapsulated microspheres were then diluted to this new concentration.

To produce the 400 µm microcapsules, modifications to the previously described methodology were the 200-µm nozzle; 2066 Hz; voltage at 1.332 V and speed at 14.2 m min⁻¹. The microcapsules produced were collected, filtered and washed in the same manner described earlier. The final microcapsules produced were 400 ± 50 µm (Fig. 1(d)).

2.2.2. Intramyocardial injections of microspheres into rat myocardium

A total of 150 µl of the non-encapsulated solution (317 000 microspheres) were divided into three equal left ventricular intramyocardial injections using a 27-G needle as described earlier (n = 16). In the second group (n = 16), rats received the same volume of 200-µm microencapsulated microsphere solution. The third group (n = 16) received 150 µl of 400 µm microcapsules but with a 25-G needle. Following the injections, rats were sacrificed after 20 min. Hearts were washed on the surface, and flushed with phosphate-buffered saline (PBS) through the aorta to remove microspheres that may have lodged inside the ventricles. The syringes were also flushed. Since 317 000 microspheres were injected in each group, subtracting the amount of microspheres remaining in the syringes would yield the amount of injected microspheres. After we quantified the amount of microspheres retained in the heart at the end of the experiment, it was then possible to calculate the intramyocardial retention rate.

2.2.3. Quantitative analysis of microspheres

Individual heart samples were digested in 4 ml of 4 M ethanolic KOH (ethanol: Commercial Alcohol Inc., Ontario,
Canada; KOH: Sigma, ACS grade). The samples were left for 48 h inside a warm water bath at 40 °C. To recover the microspheres, the samples were centrifuged and the pellet was washed with 10 ml deionised water with 0.25% Tween 80 and re-centrifuged. The process, including centrifugation, supernatant removal, and resuspension, was repeated with PBS to neutralise the basicity of the solution. Deionised water was used to rinse the pellet. Washings were repeated with saturated sodium citrate for 1 h, saturated EDTA for 2 h and deionised water to break the microcapsules. Only the last three steps (sodium citrate, EDTA and deionised water) were performed for the syringe washes. The resulting pellets were dissolved in 3 ml of 2-ethoxyethyl acetate and left overnight. Fluorescence was quantified the following day in the same manner described earlier.

2.2.4. Statistical analysis

All statistical analyses were carried out using the SAS software, version 9.2 (SAS Institute, Cary, NC, USA). All statistical tests were two tailed. p Values of 0.05 or less were considered to indicate statistical significance.

2.2.4.1. Experiment I (dose/response LVFS). We used analysis of covariance (ANCOVA), to compare mean LVFS at 7 weeks post-infarct between the five groups, after adjusting for possible differences in LVFS levels at 2 days post-infarct. Preliminary checks were conducted to ensure that there was no violation of the assumptions of normality, linearity, homogeneity of variances and homogeneity of regression slopes. When warranted by a significant analysis of variance (ANOVA) F-test for the overall difference between the groups, post hoc pairwise comparisons of means, adjusted at the mean LVFS values 2 days post-infarct, were performed. p-Values from these comparisons were adjusted for multiple testing.

2.2.4.2. Experiment I (percentage scar area). ANOVA was used to compare mean percentage scar area between the groups. Post hoc comparisons of means are performed with a Tukey method for the adjustment of p-values and 95% confidence intervals (CIs) for multiple testing, which is recommended for balanced ANOVA.

2.2.4.3. Experiment II (microspheres retention rate). ANOVA was used to compare mean retention rate between the groups. Post hoc comparisons of means are performed with a Tukey method for adjustment of p-values and 95% CIs for multiple testing. An analysis of residuals showed that a log-transformation of the retention rate was needed to achieve homogeneity of variance between the groups. Results are reported as geometric means and ratios after transforming them back to the original scale.

3. Results

3.1. Cellular implantation studies

3.1.1. Marrow stromal cell culture

MSCs from male rats proliferated in culture medium and developed spindle-shaped morphology. β-Galactosidase staining in vitro demonstrated a transfection efficiency of 90—100% (Fig. 2).

3.1.2. Sample size and mortality

A total of 56 female Lewis rats were included in this part of the study. A mortality rate of 20% (11 rats) was observed with a total of 45 rats surviving to the experimental end point at 7 weeks. All of the mortalities occurred during the first 48 h after coronary ligation. No late deaths were observed in surviving rats.

3.1.3. Echocardiographic assessment of cardiac function

Table 1 shows means and standard deviations of LVFS at baseline, 2 days post-infarct and 7-week post-infarct. ANCOVA showed that there was a highly significant difference between the groups on the mean LVFS at 7-week post-infarct, F (4, 39) = 210.38, p < 0.0001. Post hoc comparisons show that mean LVFS at 7-week post-infarct for group II is not statistically significant compared with group I (95% CI (—2.0, 1.3), p-value 0.97). However, mean LVFS at 7-week post-infarct for group III, group IV and group V were higher than mean LVFS for group I and the differences were highly statistically significant, with differences of 10.1% (95% CI (8.6, 11.6), p < 0.0001), 10.3% (95% CI (8.7, 11.8), p < 0.0001) and 10.0% (95% CI (7.9, 12.1), p < 0.0001), respectively. Similarly, mean LVFS for group III, group IV and group V are also highly significantly different from mean LVFS for group II (data not shown).

The mean values LVFS between group III, group V and group V are not significantly different. The difference between group IV and group III is 0.2% (95% CI (—1.6, 1.3), p = 0.99), between group III and group V, 0.1% (95% CI (—1.7, 1.9), p = 0.99) and between group IV and group V, 0.3% (95% CI (—1.4, 1.9), p = 0.99).

3.1.4. Histological assessment and scar area measurement

After X-gal staining to detect β-galactosidase activity, all hearts that contain engrafted cells show distinct areas of blue discolouration. We were able to visually detect transplanted
MSCs in groups III, IV and V (1.5 x 10⁶, 3 x 10⁶, 5 x 10⁶ cells) at 7 weeks. However, no engrafted cells were seen in group II, neither was there any false-positive staining in group I (control). From the groups that had detectable cells, the amount of cells detected was very low. Few sections from the heart contained any detectable cells. The density of engrafted cells between the different groups was grossly the same and no significant difference was observed, despite the escalating doses of implanted cells (Fig. 3(a) and (b)).

3.1.5. Measurement of scar area
Mean percent scar and standard deviation (SD) per group are presented in Table 2. ANOVA showed that there was a statistical significant difference in mean percent scar between the groups, F (4, 40) = 39.3, p < 0.0001. Post hoc comparisons showed no statistical significant difference in mean percent scar between group I and group II, with a difference of 0.4% (95% CI (−5.1, 5.8), p = 0.99). However, mean percent scar for group III, group IV and group V were smaller than mean percent scar for group I, and the differences were highly significant, with mean differences 16.5 (95% CI (11.0, 22.0), p < 0.0001), 16.8 (95% CI (11.3, 22.3), p < 0.0001) and 13.2 (95% CI (7.8, 18.7), p < 0.0001), respectively. Means for group III, group IV and group V showed no statistically significant differences.

Table 2
Scar area (percentage of total area).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean (%)</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>28.9</td>
<td>3.58</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>28.6</td>
<td>3.88</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>12.4</td>
<td>3.69</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>12.1</td>
<td>3.69</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>15.7</td>
<td>5.29</td>
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</tbody>
</table>

3.1.6. PCR results
Genomic DNA of donor cells was used to confirm the presence of engrafted cells. PCR detected the presence of male rat Y chromosome in all treated groups, II—V. This is in contrast to the X-gal staining where we failed to demonstrate any detectable transplanted cells in group II. There was no false-negative donor DNA that could be amplified in group I (control) (Fig. 4).

3.2. Microencapsulated microsphere retention rate
Mean retention rate and SD per group are presented in Table 3. The ANOVA showed that there was a statistical significant difference in mean log-transformed retention rate between the groups, F (2, 45) = 76.1, p < 0.0001. The geometric means for group I, group II and group III were 3.9 (95% CI (3.3, 4.7)), 16.0 (95% CI (13.5, 19.0)) and 12.3 (95% CI (10.4, 14.7)), respectively. Post hoc comparisons showed higher geometric mean for group II and group III compared with group I, with ratios of 4.1% (95% CI (3.0, 5.4), p < 0.0001) and 3.1% (95% CI (2.3, 4.2), p < 0.0001) between group I and each of group II and group III, respectively. Although group II had a slightly higher mean than group III,

Table 3
Microspheres retention rate.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>4.3</td>
<td>1.72</td>
</tr>
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<td>2</td>
<td>16</td>
<td>16.5</td>
<td>3.58</td>
</tr>
<tr>
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<td>16</td>
<td>12.9</td>
<td>3.96</td>
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there was no statistically significant difference (ratio 1.3%, 95% CI (0.9, 1, 7), p = 0.1).

4. Discussion

4.1. The cellular dose—response issue

Although recent studies have indicated the potential cardiac improvement resulting from implanted progenitor stem cells [15] or skeletal myoblasts [16], the mechanism behind such observations remains highly controversial. Different studies have, in turn, implicated the role of transdifferentiation [17], cellular fusion [18], and paracrine effects [19] as the main mechanisms behind such improvements. However, an issue that has not been adequately addressed remains the optimal dose required for this cellular therapy to work and whether higher cell doses would ameliorate the current suboptimal therapeutic effect of the transplanted cells. Various clinical trials have already been undertaken throughout the world, with cell dosage varying from 1 million to 100 million without any clear rationale [20]. To improve on the therapeutic potentials of cellular cardiomyoplasty, the first major step would be to understand the fate of the stem cells post-injection. Indeed, the traditional view of a relationship between injected cells versus functional improvement may not be appropriate, as it is the number of cells retained and not injected that is relevant to the functional improvement.

In many reported studies, the number of engrafted cells appears very small in the weeks or even days following injection [3—5]. Because the injections take place in a peri-infarct area, the ischaemic environment has been pointed as the main reason for observed low survival rates. Therefore, increasing the cell load could expose the cells to a lower perfusion and augment the likelihood of cell death due to ischaemia. A large bolus of cells could create a necrotic core that secretes pro-inflammatory cytokines, killing healthy cells and thus counteracting the main aim of increasing cell load [4]. Thus, it is desirable that a sustainable cell dose is delivered that will not perpetuate the destructive process that is described.

So far, the main efforts have been directed at limiting cellular loss following implantation through angiogenic [21], anti-apoptotic [22], and anti-free radical strategies [23]. Such approaches to minimise biological losses are built upon the presumption that the majority of injected cells are retained within the myocardium. Although reported results varied, a clear trend emerges: there is a significant cellular loss early on. In most studies and clinical trials, cell implantation, whether by direct intramyocardial or transvascular catheter approach, involves injection within the beating myocardium. Observations that supported this idea came from studies indicating the high retention rates of labelled implanted cells in the non-contracting organs such as the lung, the liver or the kidneys [5,7]. Until very recently, little effort has been focussed on the potential mechanical ‘washout’ immediately following injection. Our previous study with microspheres has isolated the contribution of mechanical losses. Although microspheres differ from cells in the aspects of shape, adhesion, etc., initial losses (<10 min) account for a significant portion of the injected material. The retention rate obtained was extremely low: 8.83 ± 3.29% for multiple injections in rodents and 11.1% in larger pig models [6]. There was also very little retention difference between a simple injection group (5.44 ± 5.66%) and another group where suturing of the puncture hole was carried out immediately after injection (6.19 ± 4.05%). This led us to believe that suspected backward leakage in fact accounted for a very small proportion of the mechanical losses.

Our current rat study was carried out to improve upon the mechanical cell losses, as well as to test our hypothesis of ‘mechanical washout’. In the first part of the study, we have investigated the roles of different cell dosages and the potential of increasing the cell load on engraftment in an acutely infarcted myocardium. We observed that although an increased dosage of MSCs is assumed to result in overall better ventricular function compared with low-dose therapy, as assessed by echocardiography and scar measurement, this effect could not be documented in the current study. Beyond a ‘minimal dose’, increasing the number of injected cells failed to induce further functional improvement. The groups that received the 3 × 10⁶ and 5 × 10⁶ cells were similar in effect to the groups which received 1.5 × 10⁶, at least in this rodent model. The ‘plateau phenomena’, along with histological slides, show that there is little difference in gross number of cells retained in these groups. Although this result can be explained by either a late biological or an early mechanical loss and thus does not explain the mechanism of functional improvement, it nevertheless suggests that increasing the amount of injectate alone is insufficient to guarantee a higher yield, which leads to the rationale for our second experiment.

4.2. The role of microencapsulation

By microencapsulating microspheres inside APA microcapsules (200 μm or 400 μm), we are effectively limiting their mobility. Despite any pressure generated, larger beads would be very hard to squeeze into the disrupted microcirculation at the moment of injection. Our present findings—a fourfold increase in retention rate—support our initial hypothesis, as the significant increase in the amount of microspheres retained indicates that the microcapsule model limited the initial dispersion of the injected material. Our model of cell loss may explain the disparity between the findings of Suzuki et al. (44.8%) [3] and Muller-Ehmsen et al. (57%) [4]. Transplantation of larger, partially differentiated cells may result in a higher retention, as they are by virtue of their size, less likely to be washed out into the circulation. The finding that an increased microcapsule size, from 200 to 400 μm, does not further enhance the retention rate is consistent with our model of mechanical washout, in which any size of microcapsule that is not able to squeeze into the microvasculature would have the same effect.

With these results, another issue is raised: whereas the retention rate did increase, how were the rest of the microspheres lost? Our previous study has already established that post-injection backflow of microspheres accounted for an insignificant amount of mechanical loss. A possibility is the limitation of the rat model itself. Because of the extremely thin and contracting myocardium walls, it is technically difficult to reproduce consistent injections, as the needle can...
easily pierce into the ventricle and inject into systemic circulation. This can explain the high standard deviation obtained in our results. A limitation to our current study is that although we have shown that the myocardial retention rate has increased, we did not analyse organs other than the heart for microspheres content.

Although the encapsulated microspheres demonstrated better retention than bare microspheres, we cannot be sure how this will translate when MSCs are used. A promising point is the fact that APA microcapsules, as well as other types of microcapsules, have been shown to provide an adequate medium for implanted cell survival. Indeed, a recent in vitro study indicated that the survival of adult human MSCs inside alginate microcapsules exceeds 80% after 15 days [24]. Despite the evidence of this viability, the behaviour of these cells while being delivered and in vivo needs to be further investigated. Evidence demonstrates decreased viability of bare stem cells depending on the way and speed they are delivered [25]. Factors such as manipulation in syringe, increased periods of storage, needle diameter used to deliver cells and temperature are all factors that effect cell viability. Our own preliminary work (data not shown) has demonstrated that damage to the capsule structure occurs when an inappropriate size needle is used.

5. Conclusion

Our study suggests that in cellular cardiomyoplasty, microencapsulation may provide the means to increase the initial retention of the injected cells. Microcapsules can be designed with different mechanical and physical properties, such that they could be biodegradable or non-biodegradable. It is feasible also to create time-dependent biodegradable microcapsules that disintegrate gradually, releasing the cells. By that time, the disrupted microvasculature incurred at the time of injections could have time to heal and close off, and thus minimising mechanical cell loss. This feature would be important if cell-to-cell contact or angiogenesis rather than a paracrine effect is primarily important in the positive effect of cell therapy. Such studies could further advance cellular myocardial regeneration.

Acknowledgement

We would like to thank Minh Duong for her technical assistance in cell culturing and her expertise in countless days of staining. Her help is greatly appreciated.

References