Encapsulated Glucagon-Like Peptide-1-Producing Mesenchymal Stem Cells Have a Beneficial Effect on Failing Pig Hearts

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Abstract

Stem cell therapy is an exciting and emerging treatment option to promote post-myocardial infarction (post-MI) healing; however, cell retention and efficacy in the heart remain problematic. Glucagon-like peptide-1 (GLP-1) is an incretin hormone with cardioprotective properties but a short half-life in vivo. The effects of prolonged GLP-1 delivery from stromal cells post-MI were evaluated in a porcine model. Human mesenchymal stem cells immortalized and engineered to produce a GLP-1 fusion protein were encapsulated in alginate (bead-GLP-1 MSC) and delivered to coronary artery branches. Control groups were cell-free beads and beads containing unmodified MSCs (bead-MSC), n = 4–5 per group. Echocardiography confirmed left ventricular (LV) dysfunction at time of delivery in all groups. Four weeks after intervention, only the bead-GLP-1 MSC group demonstrated LV function improvement toward baseline and showed decreased infarction area compared with controls. Histological analysis showed reduced inflammation and a trend toward reduced apoptosis in the infarct zone. Increased collagen but fewer myofibroblasts were observed in infarcts of the bead-GLP-1 MSC and bead-MSC groups, and significantly more vessels per mm² were noted in the infarct of the bead-GLP-1 MSC group. No differences were observed in myocyte cross-sectional area between groups. Post-MI delivery of GLP-1 encapsulated genetically modified MSCs provided a prolonged supply of GLP-1 and paracrine stem cell factors, which improved LV function and reduced epicardial infarct size. This was associated with increased angiogenesis and an altered remodeling response. Combined benefits of paracrine stem cell factors and GLP-1 were superior to those of stem cells alone. These results suggest that encapsulated genetically modified MSCs would be beneficial for recovery following MI. Stem Cells Translational Medicine 2012;1:759–769

Introduction

Glucagon-like peptide-1 (GLP-1) is a major incretin hormone that functions to regulate blood glucose homeostasis [1]. In addition to its benefits in the treatment of type 2 diabetes, GLP-1 also exhibits cardioprotective properties, as shown in various models of myocardial infarction (MI) and heart failure [2–6]. GLP-1 has an extremely short half-life in vivo, which limits its clinical usefulness. More recently, therefore, studies have been investigating the effects of longer lasting GLP-1 analogs, such as exenatide for the treatment of MI, with results showing decreased infarct size and reduced apoptosis levels [7, 8].

Another promising avenue for treating heart disease is stem cell therapy. Preclinical trials [9, 10] and clinical trials [11–13] using various types of stem cells have shown promising improvements in left ventricular ejection fraction (LVEF) and reduction in infarct size; however, the retention, differentiation, and survival rates of such cells remain a concern [14, 15]. Increasingly, stem cell therapy is not thought to achieve its benefits by direct replacement of damaged cardiomyocytes but rather because of a paracrine effect from factors such as vascular endothelial growth factor, monocyte chemotactic protein-1, and interleukins [16]. Pigs treated with mesenchymal stem cell (MSC) preconditioned medium exhibited reduced infarct size and increased number of vessels following MI [17], suggesting that the factors released from the cells are more important than direct implantation of cells.

GLP-1 CellBeads (CellMed AG, Alzenau, Germany, http://www.cellmed.com) are spherical alginate matrices that encase genetically modified human MSCs and can be manufactured in sizes from 150 to 600 μm in diameter [18]. The MSCs secrete a GLP-1 fusion protein, known as CM1, which comprises two GLP-1 (7–37) molecules bound by an intervening peptide (IP-2), giving it an extended half-life in vivo. The alginate matrix of the GLP-1 CellBeads allows passage of
oxygen and nutrients to enable MSC survival while also protecting MSCs from host immune rejection. The alginate beads, once resident in an area, therefore provide the opportunity for long-term, localized delivery of GLP-1, along with the additional paracrine benefit of factors released by the MSCs. The MSCs are predicted to survive for a maximum of 6 months in vivo, whereas the alginate beads are predicted to be stable for up to 1 year, following which they would be biodegradable and the breakdown products cleared from the body. Previous in vivo use of these beads has been tested in a range of animal models, demonstrating a good safety profile [19–23]. Additionally, the GLP-1 CellBeads are currently undergoing clinical testing in the treatment of intracerebral hemorrhage. The aim of the current study was to determine the effects of localized delivery of GLP-1 CellBeads in a porcine model of multiple coronary microinfarctions leading to early LV dysfunction.

**MATERIALS AND METHODS**

**Engineering and Encapsulation of GLP-1-Producing MSCs**

Primary cells were obtained from a healthy, male, 33-year old donor and immortalized following stable transduction by a retroviral vector containing human telomerase reverse transcriptase. Transduced cells showed a karyotype, growth rate, and osteogenic potential similar to those of nontransduced MSCs and demonstrated no tumorigenic potential [24]. Approximately 3,300 ± 800 MSCs were embedded into each spherical barium cross-linked alginate bead with a diameter of 400 ± 30 μm. To achieve immunoprotection, the core beads were surrounded by a selectively permeable shell of pure alginate, which resulted in a CellBead diameter of 600 ± 75 μm (Fig. 1A). The mean production rate of GLP-1 per bead is approximately 7 fmol/hour, which decreases with time after administration [21]. GLP-1 CellBeads were cryopreserved in 10% dimethyl sulfoxide and stored in liquid nitrogen prior to use. Following washing, CellBeads were suspended in Ringer’s solution and further diluted immediately prior to intracoronary infusion. Control experiments consisted of cell-free beads and beads containing MSCs that did not secrete GLP-1 (bead-MSC), which would distinguish whether effects were due to GLP-1 or paracrine factors released from the MSCs. Beads containing cells expressing green fluorescent protein (GFP) were also used to evaluate longevity in vivo.

**Pig Myocardial Injury Model**

All animal work was performed using Yorkshire white pigs (n = 32, 6–8 weeks old, weighing 20–37 kg) and was covered by the necessary U.K. Home Office project and personal licenses, under approval of the University of Manchester local ethics committee. An electrocardiogram (ECG) was used to monitor each animal during the procedure, and a transthoracic echocardiogram (TTE) was performed before and after each embolization procedure and immediately prior to sacrifice at 1 or 4 weeks.
Selective catheterization of the left coronary system was performed via the right carotid artery. Embolization using CellBeads and relevant controls was then achieved in the left anterior descending (LAD) coronary artery branches (two diagonal branches of 1–1.5 mm diameter per experiment or pig) in order to create areas of microinfarction. Approximately 200 μl (1,900 CellBeads) was diluted in a total volume of 2 ml of Ringer’s solution and infused via a single intracoronary injection (<1 minute), as guided by the ECGs and transthoracic echocardiogram (n = 4–5 per group; Fig. 1C). Heparinized saline (3 ml) was used to flush through the LAD immediately following embolization. MI was confirmed by ECG. The appropriate dose of analgesia was administered (0.3 mg buprenorphine, i.m.) at the end of the procedure, and animals were allowed to recover for the duration of the experiment. Identification of beads was concealed so that all analysis could be performed blind. Data were unblinded when the experiments were completed.

Echocardiographic Assessment of LV Function
A TTE machine (Accuson; Siemens, Munich, Germany, http://www.medical.siemens.com) and the appropriate probe (3.5 MHz) were used to quantify the degree of acute mild LV dysfunction (i.e., at the time of microembolism/infarctions), compared with the baseline (i.e., normal) function. The GE Echopack software (GE Healthcare, Little Chalfont, U.K., http://www.gehealthcare.com) was then used to analyze the LV areas in apical long axis views to calculate LVEF and therefore LV function. Echocardiographic moderate LV dysfunction was defined as an ejection fraction (EF = LVDA – LVSA/LVDA × 100) of 30%–40%, with an EF of 40%–50% defined as mild LV dysfunction. (Where EF indicates ejection fraction, LVDA is left ventricular diastolic area, and LVSA is left ventricular systolic area.) The animals were euthanized at 1 and 4 weeks postprocedure by lethal injection of pentobarbital (i.v.).

Pathological and Histological Analysis
The animals were euthanized at either 1 or 4 weeks post-MI. The gross pathology of the heart was photographed to highlight the epicardial areas of infarction for subsequent semiquantitative morphometry. For comparative analysis, infarct area was represented as a percentage of the LV-free wall area. LV myocardial tissue was processed for histology.

Determination of Cellular Changes and Remodeling
Apopotic cells were detected using an ApopTag Plus terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) kit (Millipore, Billerica, MA, http://www.millipore.com) as per the manufacturer’s instructions. Cardiomyocyte apoptosis was examined by containing for active caspase-3 (ab13847; Abcam, Cambridge, MA, http://www.abcam.com) and cardiac troponin T (LVMS 295P0; Fisher Scientific International, Hampton, NH, http://www.fisherscientific.com). Fluorescent sections were mounted with Vectashield containing 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, http://www.vectorlabs.com). Immunohistochemical staining of endothelial cells was performed using a primary antibody against von Willebrand factor (vWF), with only single-cell-thick angiogenic vessels being counted (ab6994; Abcam). Counting was verified independently by a second person. Staining of granulocytes and monocytes was performed using a MAC387 antibody (M0747; Dako, Glostrup, Denmark, http://www.dako.com). Myocyte cross-sectional area was measured by staining for wheat germ agglutinin (RL-1022; Vector Laboratories), as described previously [25]. To visualize collagen, sections were soaked in picrosirius red for 1 hour before examination under polarized light. Myofibroblasts were identified by fluorescent costaining for α-smooth muscle actin (ab5694; Abcam) and vimentin (sc-6260; Santa Cruz Biotechnology Inc., Santa Cruz, CA, http://www.scbt.com). Cell viability was monitored by examining unstained sections under fluorescent light for GFP-tagged MSCs.

For analysis, photomicrographs were taken of five random fields of view within each area of the myocardium (infarct zone, border zone, and remote region). The numbers of TUNEL+ cells, caspase-3/cardiomyotrope T (cTnT)+ cells, MAC387+ cells, and vWF+ vessels were counted as a percentage of the total cell number (TUNEL and caspase-3/cTnT) or area of myocardium (MAC387 and neovessels) using ImageJ analysis software (NIH), with the observer blinded to the experimental conditions. Collagen levels were measured as a percentage of collagen per tissue area. Myocyte cross-sectional area was calculated using ImageJ.

Statistical Analyses
All data are expressed as mean (± SEM). One-way analysis of variance with a Bonferroni post hoc test was used to test for differences among treatment groups.

RESULTS
Unless otherwise stated, all results are presented in the following order: bead versus bead-MSC versus bead-GLP-1 MSC.

LV Function
Baseline echocardiographic LV function (Fig. 2A) was >50% (i.e., normal) and similar in all three groups (57.20 ± 1.66% vs. 53.20 ± 1.53% vs. 54.20 ± 1.29%; p = not significant [ns]). In the surviving animals (26 of 32; 6 animals died during the procedure), TTE confirmed onset of mild LV dysfunction in all groups (EF: 43.40 ± 1.17% vs. 42.40 ± 0.93% vs. 44.00 ± 1.29%, p = ns among groups, p < .001 in each group compared with prior to MI; Fig. 2A). Four weeks after intervention, repeat echocardiography demonstrated a significant return toward baseline LV function in the bead-GLP-1 MSC group (49.75 ± 1.03%, p < .001), but not in the bead group or the bead-MSC group (EF: 41.20 ± 2.29% vs. 44.20 ± 0.86%, p = ns compared with post-MI; Fig. 2A).

Myocyte Cross-Sectional Area
At 1 week post-MI, border zone myocytes were significantly larger in the bead group compared with normal LV tissue (control) (361.3 ± 79.22 vs. 132.7 ± 13.01, p < .01). Border zone (BZ) myocytes in the groups treated with beads-MSCs (265.5 ± 11.87) and beads-GLP-1 MSCs (248.1 ± 33.93) were not significantly larger than myocytes from control tissue (132.7 ± 13.01, p = ns; Fig. 2C).

Four weeks post-MI, BZ myocytes from all three groups were significantly larger than those from control tissue (378.4 ± 31.32, 364.6 ± 15.71, and 344.7 ± 38.91 μm² vs. 132.7 ± 13.01 μm², p < .005, p < .005, and p < .01, respectively; Fig. 2E). No differences were observed in myocyte area in the remote zones at either time point (week 1: 198.3 ± 14.68 vs. 234.9 ± 22.30 vs. 181.8 ± 16.05 μm² vs. 163.3 ± 16.56 μm², Fig. 2D; week 4:...
Infarct Size
LV surface area morphometry showed significantly reduced surface infarct area 4 weeks post-MI in the bead-GLP-1 MSC group compared with the bead-MSC group (17.7 ± 4.69% vs. 3.21 ± 0.90%, p < .05) and the bead group (16.71 ± 1.60% vs. 3.21 ± 0.90%, p < .05; Fig. 3Ai). No difference was observed among the three groups at 1 week post-MI (9.78 ± 1.81% vs. 7.46 ± 2.24% vs. 6.21 ± 0.64%, p = ns). Both the bead group (p = ns) and the bead-MSC-treated group (p < .05) demonstrated surface area infarct expansion between 1 and 4 weeks, with the bead-GLP-1 MSC (p < .05) group showing a significant reduction in epicardial infarct area (Fig. 3Aii).

Descriptive Histological Analysis
Three distinct zones of the myocardium were present within the samples: infarct zone (IZ), BZ, and remote zone (RZ). Eosin staining was reduced in the IZ (Fig. 3Bi), with a sharp demarcation between BZ and IZ (Fig. 3Bii). Tissue staining in the BZ and RZ was similar (Fig. 3Biii).

Total Apoptosis
More TUNEL + cells were observed in the infarct zone compared with the border and remote zones in all groups at 1 week post-MI (Fig. 4G). However, there were no differences among the three experimental groups in any of the specific areas analyzed (infarct: 1.47 ± 0.17% vs. 1.47 ± 0.34% vs. 1.46 ± 0.57%, p = ns; border: 0.44 ± 0.12% vs. 0.31 ± 0.10% vs. 0.77 ± 0.33%, p = ns; remote: 0.28 ± 0.10% vs. 0.12 ± 0.10% vs. 0.34 ± 0.05%, p = ns).

At 4 weeks post-MI, there was a trend toward less apoptosis in the IZ of the bead-GLP-1 MSC group compared with the two other experimental groups (1.84 ± 0.625 vs. 1.36 ± 0.24% vs. 0.51 ± 0.18%, p = ns), with no difference in border and remote zones (border: 0.26 ± 0.06% vs. 0.23 ± 0.06% vs. 0.21 ± 0.04%, p = ns; remote: 0.12 ± 0.04% vs. 0.09 ± 0.04% vs. 0.13 ± 0.04%, p = ns; Fig. 4F).

Cardiomyocyte Apoptosis
One week post-MI, more cardiomyocyte apoptosis was observed in the IZ, with no differences observed among groups (infarct: 0.90 ± 0.14% vs. 0.71 ± 0.33% vs. 0.52 ± 0.22%, p = ns). No differences were observed among groups in other zones analyzed (border: 0.21 ± 0.06% vs. 0.15 ± 0.02% vs. 0.28 ± 0.11%,
At 4 weeks, increased cardiomyocyte apoptosis was still observed in the IZ compared with the other two zones, with no differences observed among groups in any region (infarct: 1.62 ± 0.60% vs. 1.10 ± 0.57% vs. 1.50 ± 0.23%, p = ns; border: 0.076 ± 0.08% vs. 0.31 ± 0.07% vs. 0.05 ± 0.05%, p = ns; remote: 0.07 ± 0.04% vs. 0.02 ± 0.02% vs. 0.07 ± 0.07%, p = ns; Fig. 4J).

Angiogenesis

One week post-MI, there were significantly fewer vessels per mm² in the IZ of the bead-GLP-1 MSC group compared with the bead group (398.5 ± 41.05 vs. 237.8 ± 29.47, p < .05), but not the bead-MSC group (277.0 ± 43.29 vs. 237.8 ± 29.47, p = ns). No differences were observed in the BZ or RZ (border: 294.1 ± 34.63 vs. 199.0 ± 22.36 vs. 286.1 ± 34.99, p = ns; remote: 175.0 ± 26.73 vs. 229.0 ± 36.17 vs. 226.7 ± 24.78, p = ns; Fig. 5D).

Four weeks post-MI, significantly more vessels were observed in the infarct zone of the bead-GLP-1 MSC group compared with controls (160.0 ± 32.51 vs. 232.0 ± 37.72 vs. 722.2 ± 68.49, p < .001), with again no differences in other zones (border: 304.4 ± 109.1 vs. 358.4 ± 54.39 vs. 633.3 ± 38.76, p = ns; remote: 347.2 ± 141.7 vs. 312.8 ± 45.39 vs. 472.2 ± 73.91, p = ns; Fig. 5E).

Inflammation

One week post-MI (Fig. 6D), there were significantly more MAC387^+ cells per mm² in the IZ compared with BZ and RZ in all three experimental groups. Within the infarct, there were significantly more positive cells in the bead-GLP-1 MSC group, compared with both the bead group (15.86 ± 5.83 vs. 111.8 ± 25.09, p < .01) and the bead-MSC group (111.8 ± 25.09 vs. 47.03 ± 11.22, p < .05). No differences among groups were observed in the BZ (4.87 ± 1.06 vs. 13.38 ± 5.55 vs. 17.84 ± 6.21, p = ns); however, significantly more MAC387^+ cells were identified in the RZ treated with bead-MSCs compared with both the bead group (6.76 ± 0.93 vs. 13.66 ± 1.77, p < .01) and the bead-GLP-1 MSC group (13.66 ± 1.77 vs. 5.83 ± 1.04, p < .001).

Four weeks post-MI (Fig. 6E), overall inflammation was reduced; however, significantly more MAC387^+ cells were again observed in the IZ of the bead-GLP-1 MSC group compared with controls (160.0 ± 32.51 vs. 232.0 ± 37.72 vs. 722.2 ± 68.49, p < .001), with again no differences in other zones (border: 304.4 ± 109.1 vs. 358.4 ± 54.39 vs. 633.3 ± 38.76, p = ns; remote: 347.2 ± 141.7 vs. 312.8 ± 45.39 vs. 472.2 ± 73.91, p = ns; Fig. 5E).
observed in the bead-MSC group compared with the other groups in both IZ and RZ (infarct: 3.27 ± 0.76 vs. 36.97 ± 6.56 vs. 5.95 ± 1.50, p < .001; remote: 2.38 ± 0.53 vs. 12.43 ± 1.60 vs. 4.05 ± 0.70, p < .001), whereas in the border zone, there was significantly more inflammation in the bead-MSC group compared with the bead group (3.46 ± 0.77 vs. 14.70 ± 3.16, p < .001) but not the bead-GLP-1 MSC group (14.70 ± 3.16 vs. 8.78 ± 0.87, p = ns).

**Collagen Content**

One week post-MI (Fig. 7A), there were no differences in collagen content among groups in any zone (infarct: 9.95 ± 1.42% vs.
5.60 ± 1.38% vs. 5.14 ± 1.19%, p = ns; border: 1.63 ± 0.21% vs. 2.98 ± 0.71% vs. 1.90 ± 0.37%, p = ns; remote: 1.46 ± 0.15% vs. 1.34 ± 0.38% vs. 1.74 ± 0.22%, p = ns). Four weeks post-MI, there was significantly more collagen in the IZ of all three experimental groups, compared with the respective border and remote zones (Fig. 7B). Within the infarct zone, there was more collagen in the bead-MSC group compared with the bead group (6.87 ± 2.92 vs. 19.63 ± 1.37, p < .05), with no significant difference compared with the bead-GLP-1 MSC group (6.87 ± 2.92% vs. 16.88 ± 6.61%, p = ns). No differences were observed in border or remote zones among the three experimental groups (border: 0.56 ± 0.34% vs. 1.85 ± 0.42% vs. 1.52 ± 0.84%, p = ns; remote: 0.34 ± 0.15% vs. 0.77 ± 0.27% vs. 0.58 ± 0.17%, p = ns).

Myofibroblast Levels

Significantly more myofibroblasts were detected per mm² in infarct and border zones of the bead group compared with the bead-MSC and bead-GLP-1 MSC groups (Fig. 7C) (infarct: 405.6 ± 107.4 vs. 123.8 ± 44.34 vs. 200.3 ± 11.22, p < .05; border: 50.64 ± 12.28 vs. 8.65 ± 2.89 vs. 34.62 ± 11.75, p < .05; remote: 2.56 ± 2.56 vs. 0.0 ± 0.0 vs. 0.0 ± 0.0). Negligible numbers of myofibroblasts were observed in remote regions of all groups. Four weeks post-MI (Fig. 7D), there was a trend toward fewer myofibroblasts in the infarct of the bead-GLP-1 MSC-treated group (185.0 ± 96.54 vs. 181.7 ± 49.14 vs. 34.62 ± 3.85). No differences were observed in the border zone of the bead-treated group (29.62 ± 11.27 vs. 4.09 ± 1.59 vs. 24.36 ± 8.41), with again very few cells in the border regions (0.0 ± 0.0 vs. 0.0 ± 0.0 vs. 5.13 ± 5.13). No differences were observed in the remote zone of the three experimental groups.

Viability and Localization of GLP-1 CellBeads In Vivo

Examination of unstained sections using fluorescent microscopy identified beads containing multiple GFP-expressing cells in the infarcted myocardium at day 0 (Fig. 3Ci). Beads expressing GFP cells were also found at 7 and 28 days post-MI (Fig. 3Cii, 3Ciii); however, there were notably fewer cells at these time points. No beads were found in border or remote zones in any of the animals.

DISCUSSION

The main finding of this study is that delivery of GLP-1-expressing genetically modified human MSCs (hMSCs) encapsulated within a biocompatible alginate shell (GLP-1 CellBeads) restores LV function in a porcine model of early ischemic LV dysfunction. This is associated with reduced epicardial infarcted area, increased angiogenesis, and an increased initial inflammatory response. Collagen scar content was increased but myofibroblast number was decreased within infarct and border regions, and no effect was observed on remote collagen remodeling.

Viability of the cells within the beads up to 1 month post-delivery in vivo was confirmed by identification of GFP cell-containing beads in the myocardium at this time point. The number of cells observed after 4 weeks was dramatically reduced from 1 week; it is therefore predicted that no cells will remain by 6 months, but longer term studies are required to confirm this.

Although early studies showed promising results with the use of MSCs for the repair of damaged myocardium, more recent randomized controlled trials have demonstrated less convincing results [14, 15]. In addition, evidence for the transdifferentiation and integration of functional cardiomyocytes from transplanted...
MSCs remains an area of intense investigation [26]. It is well established that MSCs also secrete various cardioprotective factors, and this “paracrine” hypothesis is increasing in credibility as an explanation for some of the benefits on myocardial function following MSC transplantation [27]. It is therefore plausible that the therapeutic benefits of MSCs would be enhanced in vivo by ensuring a greater life span, as well as establishing a prolonged presence within the myocardium.

In the current study, we demonstrated the enhanced benefits of using encapsulated hMSCs that have the advantage of a prolonged presence within the myocardium while being immunoprotected. The number of CellBeads delivered translated to more than 6 million MSCs being delivered and retained in the myocardium, which is a much larger number than that retained in cell infusion studies, enabling a longer period of therapeutic potential. Furthermore, the MSCs, although encapsulated within a highly biocompatible alginate matrix, allow diffusion of molecules across the alginate, which may explain their significant yet local therapeutic effects on post-MI repair process in our model. The alginate encapsulation might also offer similar protection.

**Figure 6.** MAC387 immunohistochemistry for detection of granulocytes and monocytes. (A–C): MAC387-positive cells were detected in the infarct of animals treated with beads (A), beads-MSCs (B), and beads-GLP-1 MSCs (C). (D, E): Numbers of MAC387⁺ cells per mm² observed in different regions at 1 (D) and 4 (E) weeks post-myocardial infarction. Arrowheads indicate positively stained cells. Significance: *, p < .05; **, p < .01; ***, p < .001. Scale bars = 100 μm. Abbreviations: GLP-1, glucagon-like peptide-1; MSC, mesenchymal stem cell.

**Figure 7.** Effect of CellBeads on collagen remodeling following myocardial infarction (post-MI). (A, B): Collagen levels (picrosirius red staining) in infarct, border, and remote regions in hearts at 1 (A) and 4 (B) weeks post-MI. (C, D): Myofibroblast numbers in different regions of the heart at 1 (C) and 4 (D) weeks post-MI. Significance: *, p < .05. Abbreviations: GLP-1, glucagon-like peptide-1; MSC, mesenchymal stem cell.
from other known adverse reactions to transplanted MSCs, such as myocardial tumor formation [28] and areas of calcification/ossification [29], although we did not examine these in our study. The alginate may also offer the additional advantage of a scaffold for the damaged area of myocardium, although this would require further investigation.

Patients showing infarct expansion have a greater chance of developing heart failure [30]. This study found that pigs that were administered GLP-1 CellBeads showed no infarct expansion between 1 and 4 weeks, which is in contrast to both control groups, showing that the effect is due to the GLP-1 and not factors released from the MSCs. Although some of the reversal of this dysfunction after 4 weeks could be attributed to a degree of remodeling, in the therapy group this reversal of dysfunction was greater compared with the empty bead group. The associated reduced areas of histopathological infarctions noted on explantation of these pig hearts support our hypothesis that most of the improvements of ejection fractions at 4 weeks are attributable to regeneration of the myocardial tissue (a desired function of the stem cell therapy) rather than remodeling alone. This result is in keeping with several studies that have shown that prolonged infusion of GLP-1 reduces infarct size following MI [3,31]. Likewise, the restoration of LVEF at 4 weeks with bead-MSC GLP-1, but not with the bead-MSCs, indicates that restoration of LV function is due mainly to prolonged and local delivery of GLP-1.

GLP-1 exhibits antiapoptotic properties [32], which are thought to contribute to the reduced infarct size observed in numerous GLP-1 studies [33]. Our results showed a trend toward decreased apoptosis with GLP-1 treatment, which is in agreement with such a mechanism of action for locally delivered GLP-1. This is in accordance with previous data, where we showed that bead-GLP-1 MSCs reduced apoptosis at both 2 and 7 days post-MI in comparison with control beads [23]. No difference was observed in apoptosis rates between the bead group and the bead-MSC group, despite antiapoptotic factors also being released from MSCs [34].

Previous studies have identified that both GLP-1 and GLP-1 receptor agonists inhibit apoptosis via several mechanisms, including cAMP/GMP activation, mitogen-activated protein kinase-extracellular signal-regulated kinase-1/2 phosphorylation, and activation of prosurvival pathways (e.g., phosphatidylinositol 3-kinase and Akt) [33,35,36]. It is assumed that the bead-GLP-1 MSCs also exert their effect via this mechanism, and work is ongoing to investigate this.

The bead-GLP-1 MSC-treated group demonstrated a significant reduction in infarct size between 1 and 4 weeks. This could be accounted for by both decreased apoptosis and enhanced reperfusion of the infarcted tissue. Another possible explanation could be that the GLP-1 is recruiting progenitor cells to regenerate the infarct; however, further studies are required to investigate this.

Significantly more vessels were observed in the infarct of animals treated with bead-GLP-1 MSCs compared with the other two groups at 4 weeks (Fig. 5E), suggesting that GLP-1 has an angiogenic effect, aiding reperfusion of infarcted tissue. No difference was observed between groups treated with empty beads or beads-MSCs, which contrasts with other studies that have shown similar proangiogenic effects of MSCs following post-MI implantation [37]. Likewise, MSCs are known to influence the cardiac response to injury, releasing both pro- and antiinflammatory cytokines. In this study, significantly more MAC387 cells were observed within the infarct of the bead-MSC group 1 week post-MI compared with the two other groups (Fig. 6D). This large reaction could partly be explained by human factors being detected by the host animal, creating a xenogenic response. Four weeks post-MI, an increased response in the bead-MSC group compared with bead-GLP-1 MSCs and bead groups was observed in all areas of the heart (Fig. 6E). The sustained inflammatory reaction could be explained again by a xenogenic response. Further experiments to test a human-human or porcine-porine reaction are therefore warranted. The bead-GLP-1 MSC group had a significantly reduced inflammatory reaction compared with beads-MSCs, which suggests that secreted GLP-1 is reducing the inflammatory response to the MSCs. This finding is in accordance with a study that showed GLP-1 can decrease neutrophil activation post-MI [38].

Increased collagen content observed in the groups treated with beads-GLP-1 MSCs and beads-MSCs at 4 weeks post-MI, which is likely due to the increased inflammation seen in this group. No difference was observed between any groups in border or remote zones, showing that the bead-GLP-1 MSCs have no effect on general fibrosis up to this time point. Similarly, no differences in myocyte cross-sectional area were seen among groups at 4 weeks, suggesting that longer term studies are required to investigate any effect on hypertrophy.

Significantly more myofibroblasts were observed in both the infarct and border zones of the bead group 1 week post-MI; however, this did not correlate with collagen levels. One explanation for this could be increased levels of matrix metalloproteinases breaking down the formed collagen in the tissue. Although myofibroblasts initially have a beneficial role in healing post-MI, they are known to remain in the heart for long time periods, and prolonged collagen deposition can lead to ventricular dysfunction and heart failure. Therefore, the observation of a large amount of collagen in the scar of the treatment group but a low number of observed myofibroblasts can be seen as a good prognostic indicator.

**Limitations**

In this study, several microinfarcts were created by embolization of LAD branches by delivery of the beads. The main purpose was to investigate the mechanism of action of GLP-1 CellBead therapy post-MI. The use of larger (600 μm) beads helped to test the potential effects of localized GLP-1 therapy delivered at the exact sites of microinfarctions. This method was decided upon over that of LAD occlusion because of the much lower mortality rate and the ability to create microinfarctions of a reproducible size. In any clinical application, smaller beads (150 μm) would be used. We have previously shown that infusing these smaller beads following left circumflex coronary artery occlusion in pigs resulted in local retention of the beads in the infarcted area, caused no microvascular obstruction, and decreased apoptosis levels [23], all of which is in agreement with the results from the current study.

This study used beads that contained human MSCs that were then implanted into pigs. Although the cells themselves were encapsulated in alginate and therefore not directly exposed to the host tissue, human factors released from these cells were able to diffuse out of the bead and could therefore possibly cause a xenogenic response. Additional investigations into the inflammatory response are therefore warranted.
CONCLUSION

We have demonstrated that delivery of alginate-encapsulated human mesenchymal stromal cells expressing GLP-1 (GLP-1 CellBeads) reduces infarct size and improves LV function in a porcine model of early LV dysfunction. These findings are associated with increased angiogenesis and increased collagen deposition within the infarct region. The MSCs have a proinflammatory effect, which is partially negated by the GLP-1. Reduced infarct size could be explained by the increased perfusion, the GLP-1 benefits, and the GLP-1 receptor effect. Further investigation into precise mechanisms is warranted. Beneficial effects produced by the CellBeads are due to a synergistic combination of localized GLP-1 and paracrine factor secretion. The findings of the current study suggest that this treatment may be useful in the treatment of damaged myocardium and would be more effective than either GLP-1 or MSC treatment alone, warranting further investigation prior to clinical application of smaller GLP-1 CellBeads.

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AUTHOR CONTRIBUTIONS

E.J.W.: collection and assembly of data, data analysis and interpretation, manuscript writing; K.A.F.: collection and assembly of data, data analysis and interpretation; N.M.: conception and design, collection and assembly of data, data analysis and interpretation; M.K.: provision of study material; A.L.L.: conception and design, financial support; C.W.: conception and design, financial support, provision of study material; C.M.H.: conception and design, administrative support, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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