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Intracoronary Delivery of Self-Assembling Heart-Derived Microtissues ("Cardiospheres") for Prevention of Adverse Remodeling in a Pig Model of Convalescent Myocardial Infarction

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Abstract

Background—Preclinical studies in rodents and pigs indicate that the self-assembling microtissues known as cardiospheres (CSp) may be more effective than dispersed CSp-derived cells (CDCs). However, the more desirable intracoronary (IC) route has been assumed to be unsafe for CSp delivery: CSp are large (30-150 μm), raising concerns about likely micro-embolization. We questioned these negative assumptions by evaluating the safety and efficacy of optimized IC delivery of CSp in a porcine model of convalescent MI.

Methods and Results—First, we standardized the size of CSp by modifying culture conditions. Then, dosage was determined by infusing escalating doses of CSp in the LAD of naïve pigs, looking for acute adverse effects. Finally in a randomized efficacy study, 14 mini-pigs received allogeneic CSp (1.3×10^6) or vehicle one month following MI. Animals underwent MRI before infusion and 1 month later to assess left ventricular (LV) ejection fraction (EF), scar mass and viable mass. In the dosing study, we did not observe any evidence of micro-embolization after CSp infusion. In the post-MI study, CSp preserved LV function, reduced scar mass and increased viable mass whereas placebo did not. Moreover, CSp decreased collagen content, and increased vessel densities and myocardial perfusion. Importantly, IC CSp decreased LV end diastolic pressure and increased cardiac output.

Conclusions—Intracoronary delivery of CSp is safe. Intracoronary CSp are also remarkably effective in decreasing scar, halting adverse remodeling, increasing myocardial perfusion and improving hemodynamic status post-MI in pigs. Thus, CSp may be viable therapeutic candidates for IC infusion in selected myocardial disorders.

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Keywords

stem cell; cardiospheres; myocardial infarction; adverse remodeling

Ischemic heart disease is the leading cause of death in the world, accounting for at least 7 million deaths per year[1,2]. New treatments to improve outcome following myocardial infarction (MI) are desirable. Heart-derived cell products have particular promise in this regard[3]. The first described human cardiac stem cell products were self-assembling multicellular three-dimensional microtissues which Messina et al dubbed "cardiospheres" (CSp)[4]. These microtissues had diameters of ~30->200 μm; when injected intramyocardially (IM), CSp improved function and structure in a mouse model of acute MI. Given that capillaries have diameters of only ~8 μm, intracoronary (IC) delivery of CSp was assumed to be implausible given the likelihood of coronary microembolization[5, 6]. In contemplating translation to the clinic, we preferred to use the IC route whose safety had been well-validated in prior clinical trials of cell therapy. Thus, we developed cardiosphere-derived cells (CDCs) as a dispersed single-cell product grown from replated CSp. CDCs delivered IC turned out to work quite well, both in animal models and in humans[7-11], but we were left wondering whether CSp might have worked even better. When compared head-to-head in small- or large-animal MI models using IM delivery, CSp were at least as effective as CDCs, and often more so. Therefore, in the present study, we questioned our negative assumptions regarding the viability of IC CSp infusion. We reasoned that perhaps, if CSp sizes were standardized and dose/delivery were carefully optimized, we might be able to infuse CSp safely down a coronary artery. We initially optimized CSp size using different culture conditions, and performed a dose-ranging study in minipigs to assess the feasibility and safety of IC CSp infusion. Finally, we performed a blinded, prospective placebo-controlled study in a porcine model of convalescent MI to assess the safety and efficacy of IC CSp infusion.

Methods

For detailed methods please see the supplement material. Animal studies were performed in an American Association for Accreditation of Laboratory Animal Care accredited facility with approval from the Cedars-Sinai Institutional Animal Care and Use Committee.

Growth of CSp and optimization of culture conditions

CDCs were grown from a freshly-explanted heart obtained from a male Sinclair mini-pig, and secondary CSp were made from CDCs at the 5th passage using CSp media as described[4, 12]. Culture time and cell seeding density were optimized to obtain a minimal percentage of CSp >150 μm (which may cause severe microvascular obstruction) and a high percentage of CSp >50 μm (to avoid the infusion of single cells). For this purpose, we compared 5 different seeding densities and 4 different harvest time points (Figure 1A). Size, number and distribution of the CSp obtained were determined using a particle counter. The CSp generated (from 12M plated CDCs) were counted before and after *ex vivo* passage through a micro-catheter (Finecross®, Terumo, Tokyo, Japan) to quantify retention of CSp in the catheter.

For *in vivo* studies, secondary CSp from 12.5M CDCs were frozen and thawed the day of infusion. Viability of the thawed CSp was assessed using homo-ethidium bromide (which stains dead nuclei red).

Study design

Two separate experimental protocols were performed as depicted in Figure 2. Briefly, a study was first performed to determine the maximal feasible dose and, in a second step, a blinded placebo-controlled study was performed to assess efficacy of infused CSp. A total of 26 Yucatan mini-pigs were used: 7 completed the dose study and 14 completed the efficacy study. Three pigs died, 2 following MI creation and 1 following placebo infusion. Two pigs were excluded because of technical issues during MI creation (one balloon deflation leading to inadequate MI, and one left anterior descending artery [LAD] dissection).

MI creation and CSp infusion

For the feasibility study, increasing doses of CSp were administered in naïve Yucatan minipigs. The doses were defined by the number of single cells used to manufacture the CSp (single-cell equivalent [SCE]). Pigs were infused with 12.5×10^6, 25×10^6 and 50×10^6 SCE, corresponding to $\sim3.25\times10^5$, 6.5×10^5 and 1.3×10^6 multicellular particles respectively (Supplemental Figure 1). All IC infusions were performed using continuous flow (no flow interruption during infusion, no balloon inflation), with a microcatheter (Finecross®, Terumo, Tokyo, Japan) placed in the mid-LAD. Safety was assessed by adverse events during infusion (e.g., arrhythmias, dissection, hypotension), TIMI flow after infusion, left ventricular ejection fraction (LVEF) measured by LVgram before and after infusion (to detect potential myocardial stunning related to micro-embolization), and troponin I increase 24 hours after infusion.

For the efficacy study, MIs were created in female adult Yucatan mini-pigs by inflating an angioplasty balloon in the mid-LAD just after the 1st diagonal branch for 2.5 hours. Three weeks later, animals were randomized to receive CSp (50×10^6 SCE, 1.3×10^6 particles) or vehicle using continuous flow infusion. Safety was assessed as in the dose study. LV end-diastolic pressure was recorded using a pigtail catheter placed into the LV cavity. Left ventriculography was then performed to assess LV function. Minipigs were euthanized one month after infusion. All procedures and analysis were performed blinded to treatment allocation.

MRI

MRI was performed on a 3.0 Tesla MRI scanner (Siemens Magnetom Verio, Erlangen, Germany) at baseline (3 weeks following MI, before infusion) and one month post-infusion.

Coronary flow reserve (CFR) measurement

Coronary flow reserve was quantified with a Doppler wire as described in the supplemental material.

Histology

Histological analyses were performed on 8 μm sections from myocardial samples (fixed in 10% formalin and embedded in paraffin) obtained from infarct and border zone (3-6 slides per heart) and remote zone (2 slides per heart).

Statistical analysis

Continuous variables are presented as mean ± standard deviation in the text and mean ± standard error in the figures. Categorical variables are expressed as absolute number and percentage. Independent groups (CSp and placebo) were compared using Mann-Whitney U test. Wilcoxon test was used to compare paired groups (changes from baseline). All *P* values are two sided, and a *P* value <0.05 was considered statistically significant.

Results

CSp culture optimization

Figure 1B shows a histogram of CSp size distribution at various times and seeding densities. Increased seeding density is associated with a higher proportion of spheres >50 μm (Figure 1C) whereas longer culture time mostly increased the percentage of very big particles (>150 μm; Figure 1D). Therefore a high seeding density (1.5×10^6 CDCs in 75 cm^2 flasks, i.e. 20×10^3 CDCs/cm^2) and short culture duration (48 hours) were deemed optimal for CSp manufacturing. The number and size of secondary CSp manufactured from 12.5M CDCs using these conditions are shown in Supplemental Figure 1.

Using optimally-manufactured CSp, we observed 5±9% total retention of CSp in the catheter and ∼10% retention for CSp>50 μm (Supplemental Figure 1). Therefore, for the *in vivo* study we decided to increase the doses by 10% to offset in-catheter retention. Fortuitously, the extent of loss dramatically increased with particle size, reaching ∼70% for CSp>150 μm, providing an additional safeguard against microvascular occlusion.

Safety of CSp infusion

A dose escalation study was performed in 7 naïve animals to assess feasibility and safety. Pigs were infused with 12.5×10^6 SCE (n=2), 25×10^6 SCE (n=3) and 50×10^6 SCE (n=2). No arrhythmia occurred during CSp infusion and no impairments of TIMI flow or LVEF following infusion were observed (Figure 3 A, B). Troponin increase 24 hours after infusion was low without any difference between the 3 doses (Figure 3C). To further assess safety, the heart of a pig infused with 50M SCE and euthanized 24 hours after infusion was stained with TTC. No myocardial necrosis was evident, either in the infused region or in remote areas (Supplemental Figure 2). Therefore the highest dose (50M SCE) was used for further studies.

Safety was then confirmed in infarcted animals. Following infusion of CSp (dose of 50M SCE) we observed no impairment of LVEF when compared to vehicle infusion; also, troponin I and CK at 24 hours were similar in CSp and placebo groups (Figure 3 D-G). No impairment in TIMI flow was observed after vehicle infusion, whereas it decreased from TIMI 3 to TIMI 2 in 1 of 7 pigs infused with CSp. This decrease in TIMI flow was most

likely related to coronary spasm, for three reasons: 1) coronary flow normalized immediately after nitroglycerin infusion, along with visible dilatation of the epicardial vessel; 2) there was no decrease in LV function; 3) troponin did not increase at 24 hours. These considerations exclude micro-embolization as the cause of the decrease in TIMI flow grade. No other adverse events occurred during CSp infusion, whereas one pig died of severe hypotension followed by cardiac arrest immediately after placebo infusion. Analysis of the MRI performed in that pig before infusion revealed a major myocardial scar involving both ventricles with severe bi-ventricular dysfunction. Therefore, only 14 pigs were analyzed at the 1 month post-infusion endpoint.

Preclinical study: Benefits associated with CSp infusion

Having established a safe dose, we performed a preclinical study in which animals were blindly allocated to CSp (50×10^6 SCE, 1.3 CSp) (n=7) or vehicle infusion (n=8). The average diameter of the CSp infused was 45±23 μm; 30.2±6.8%, 3.4±1.8% and 0.24±0.17% of the CSp were >50, 100 and 150 μm respectively. Viability of the infused CSp was 92.1±3.9%. MRI characteristics of the animals before treatment are shown in Supplemental Table 1.

LV function preservation and scar reduction—Figure 4A shows representative non-enhanced MR images at end-diastole and end-systole in placebo (left) and CSp groups (right). Pooled data (Figure 4B) reveal that, at baseline, LVEF was similar in the two groups. At 1 month follow up, however, LVEF was preserved in CSp-treated animals (P=0.35 within group) whereas it significantly decreased in placebo (P=0.02 within group, P=0.004 between groups). In parallel, both indexed LV end systolic and end-diastolic volumes significantly increased in the placebo group but not in the CSp group (Figure 4C), indicative of attenuated adverse remodeling with CSp treatment. In accordance with preservation of LV volumes, CSp-treated pigs showed better 1 month regional function (assessed by end systolic thickening) compared to placebo both in infarcted and remote zones (Figure 4D,E).

Analysis of late Gd-enhanced images (Figure 5A) revealed that scar mass decreased significantly in CSp-treated animals (P=0.02 within group) but not in placebo (P=0.18 within group, P=0.002 between group) leading to a five-fold higher relative decrease in the CSp group (-11.6±2.7% vs. -2.3±4.6%, P=0.003) despite similar scar mass at baseline (Figure 5B, C). Consistently, the decrease in scar size (scar mass divided by total LV mass; Figure 5D) was significantly greater in CSp group than in control. In addition to the reduction of scar mass, we observed a strong trend toward a higher increase in viable mass in CSp-treated animals (Figure 5B, D). We have previously noted that viable mass recovery tends to lag behind the reduction of scar, such that changes in viable mass that are not quite significant at one month (as is the case here) tend to increase with longer follow-up [11, 13, 14].

Myocardial perfusion increase—Myocardial perfusion was assessed using 2 different methods: 1) CFR of the infarct-associated artery before CSp infusion and 1 month later using a Doppler wire, and 2) First-pass MRI to quantify perfusion in the infarcted area. CFR values at endpoint were higher in CSp-treated pigs than in control despite similar baseline values (Figure 6A). Consistent with these findings, MRI first-pass sequences revealed a

significant increase in myocardial perfusion at endpoint in the CSp group but not in placebo controls (Figure 6B; *P*=0.02 in CSp, vs. *P*=0.18 in control, P=0.13 for intergroup comparison).

Histology: fibrosis, vascular density and architecture—Functional measurements reviewed above indicate an increase in myocardial perfusion with CSp treatment. Consistent with these findings, histological analysis revealed twofold higher arteriolar density (i.e., isolectin and α-smooth muscle actin-positive vascular structures) in the infarcted and border zones of CSp-treated pigs compared to controls, but no differences in the remote zone (Figure 6C,D). Capillary density was also higher in the peri-infarct zone, but not in the remote zone (Figure 6E).

MRI also revealed a decrease in myocardial scar after CSp infusion. To confirm this histologically, we first evaluated the transmurality of the scar using Masson trichrome staining. Then, collagen content in the infarcted, border and remote areas was quantified using Picrosirius red (which offers superior contrast between fibrotic and non-fibrotic areas in the remote and border zones relative to Masson trichrome, as evident from the representative images in Figure 7A1-4). Scar transmurality was less in CSp-treated pigs vs. placebo (Figure 7B). In parallel, collagen content was reduced in the CSp-treated pigs in the infarct zone and in border zone, but also in the remote zone (Figure 7C-E).

These differences in fibrosis and vascularity were not accompanied by cardiomyocyte hypertrophy, as demonstrated by the similar cross-sectional areas of cardiomyocytes from CSp and placebo pigs (Supplemental Figure 3).

Hemodynamic improvement—To investigate whether the observed functional and architectural improvements were associated with hemodynamic improvement, we measured LV end diastolic pressure (LVEDP) and cardiac output (CO, from stroke volume and heart rate). At baseline, LVEDP and CO were similar in CSp- and placebo-treated pigs. After treatment, LVEDP significantly decreased in CSp but not in placebo, while CO increased only in the CSp group (Figure 8A; Table). We did not observe any differences in systolic or diastolic blood pressure or heart rate that might have confounded the LVEDP and CO measurements (Table).

Immunological safety: cellular infiltration and allo-antibodies—Histological analysis revealed more lymphocyte infiltration in the infarcted and border zone of the treated animals compared to placebo (5/7 animals vs. 0/7 animals, P=0.002). Importantly, no area of cardiomyocyte damage related to immune response was observed, indicating that no animal had a grade of immune reaction >1 (Supplemental Figure 4). Slightly higher levels of allo-antibodies were observed in CSp-treated animals as compared to placebo (Supplemental Figure 4), but the significance of this observation is uncertain.

Discussion

Adverse remodeling is a major contributor to MI-related mortality and morbidity[15, 16]. Despite the improvement of care and faster reperfusion, mortality remains >10% at 1 year [2].

Here, we demonstrated the ability of 3-dimensional multicellular cardiospheres to halt the ongoing process of adverse remodeling in a clinically-relevant porcine model of convalescent MI. Furthermore, we established the safety of the desirable IC route for the administration of this cell product.

Safety of intracoronary infusion

Intracoronary infusion is an easy, convenient and widely-available technique for delivery of biological products. Prior to this study, IC infusion of CSp had been avoided, because of the prevailing assumption that these multicellular microtissues may cause microvascular obstruction resulting in myocardial damage. Indeed, some authors have raised the concern that IC infusion of particles >10 μm may be unsafe because of microvascular obstruction [17]. However, the safety of IC infusion of large particles (CellBeads of 160-400μm) has previously been reported, although the number of particles infused was low (up to 60000), and those studies aimed to cause a certain degree of micro-vascular occlusion [18-20]. Here, we have been able to deliver IC $>1\times10^6$ CSp with an average diameter of 45 μm without any adverse events. The first study evaluating IC infusion of dispersed CDCs used minipigs with convalescent MI and traditional stop-flow delivery; IC infusion of $\geq 25\times10^6$ CDCs, which have a diameter ∼20 μm, led to elevation of serum troponin I at 24 hrs, establishing the maximal safe dose as 12.5×10^6 (or ∼300,000 CDCs/kg)[8]. The combination of careful CSp formulation and the use of continuous-flow infusion[21] allowed us to deliver up to an equivalent of 50×10^6 cells IC in the same model. This ability to deliver a higher number of cells may augment efficacy, although cell therapy does not necessarily follow conventional dose-response relationships[22-24].

Anti-fibrotic and pro-angiogenic properties of CSp

CDCs have been extensively studied pre-clinically, leading to several Phase I/II clinical trials involving first autologous and, more recently, allogeneic products[9, 25]. Thus, the ability of CDCs to decrease scar mass and to increase viable tissue has been thoroughly validated. In the porcine post-MI model, we confirmed the impressive anti-remodeling properties of CSp that had been observed by IM injection in small-animal models[13, 14, 26-28]. Indeed, CSp infusion completely halted further LVEF deterioration. Moreover, we observed an improvement in regional function and a decrease in fibrosis both in infarcted and in non-infarcted zones, indicating a positive effect on adverse global remodeling, and not just a decrease in scar content of the infarcted area. These “global” effects on LV remodeling and fibrosis may be related to the higher engraftment and survival of CSp as compared to CDCs and to the secretion of growth factors, endoglin and MMP as described [12, 13, 27, 29, 30]. Taken together, these properties may enable a more sustained and diffuse paracrine effect. Paracrine signaling is a major contributor to cardiac regeneration following cell therapy[29, 31-35], as benefits far outlast measurable cell engraftment[7, 10, 28]. It seems likely that the benefit observed in this study might increase over time[8,10] and potentially surpass the benefit observed with CDCs[11]. The benefits seen here are indeed generally superior to those seen 1 month after IC infusion of CDCs or after intramyocardial injection of a comparable dose of CSp (comparison with historical results from Yee et al.[6], and unpublished 1-month follow-up results from the study by Malliaras et al.[11] are summarized in Figure 8B,C). However, a direct comparison was beyond the scope of the present study.

In addition to this anti-fibrotic effect, we describe a strong angiogenic effect following CSp infusion. Indeed, we observed a twofold greater number of arterioles in the infarcted and border zones of CSp-treated animals compared to placebo. This increased arteriolar number was associated with improved perfusion in the infarcted area as assessed by two different methods (invasive and non-invasive). Given the chronicity of the model, the increase in myocardial perfusion is likely attributable to the growth of new vessels, not to preservation of existing vasculature. This impressive angiogenic effect might be useful to recruit in selected clinical situations, e.g. in patients with intractable angina and/or micro-circulatory deficits.

Hemodynamic improvement

Consistent with the structural changes, we observed hemodynamic improvements following CSp infusion, including increased CO and, importantly, decreased LVEDP. High LVEDP is a strong predictor of adverse events (i.e. acute heart failure or death)[36-39]; if reproduced in patients, the twofold decrease observed in this study could be highly meaningful clinically.

Immunological safety

The structural and functional improvements associated with allogeneic CSp infusion were associated with minimal immune reaction, confirming the safety of allogeneic heart-derived cell therapy[10, 11, 28]. While no immunosuppressive treatment was given, we did not observe any reaction >grade 1 ISHLT in the tissue, and the level of allo-antibodies that were detected was not very different than in the negative control.

Limitations

This study has several limitations. First, the MI model, although mimicking human adverse remodeling, is made on a background of young healthy animals. Therefore their ability to recover and generate new cardiac tissues might be superior to what can be observed in patients with cardiovascular disease risk factors and other comorbidities. Second, engraftment was not quantified in this study; engraftment of CSp has been shown to be greater than that of CDCs, but only in intra-myocardial injection models [6, 12, 27]. However, it is not clear whether the benefit of CSp is related to the higher engraftment, to properties of the secretome, or both. While engraftment correlates roughly with efficacy, the relationship is vague, particularly over the longer term[8, 10, 11]. Nevertheless, increased short-term engraftment would be expected to boost the availability of secreted factors, so the two possibilities are undoubtedly intertwined.

Conclusions

We have demonstrated the safety of IC infusion of multicellular 3-dimensional cardiospheres. Moreover, CSp treatment decreases scar, attenuates adverse remodeling, increases myocardial perfusion and improves hemodynamics in a pig model of convalescent MI. Cardiosphere infusion may deserve testing as a therapeutic approach to attenuate adverse remodeling and/or to achieve angiogenesis in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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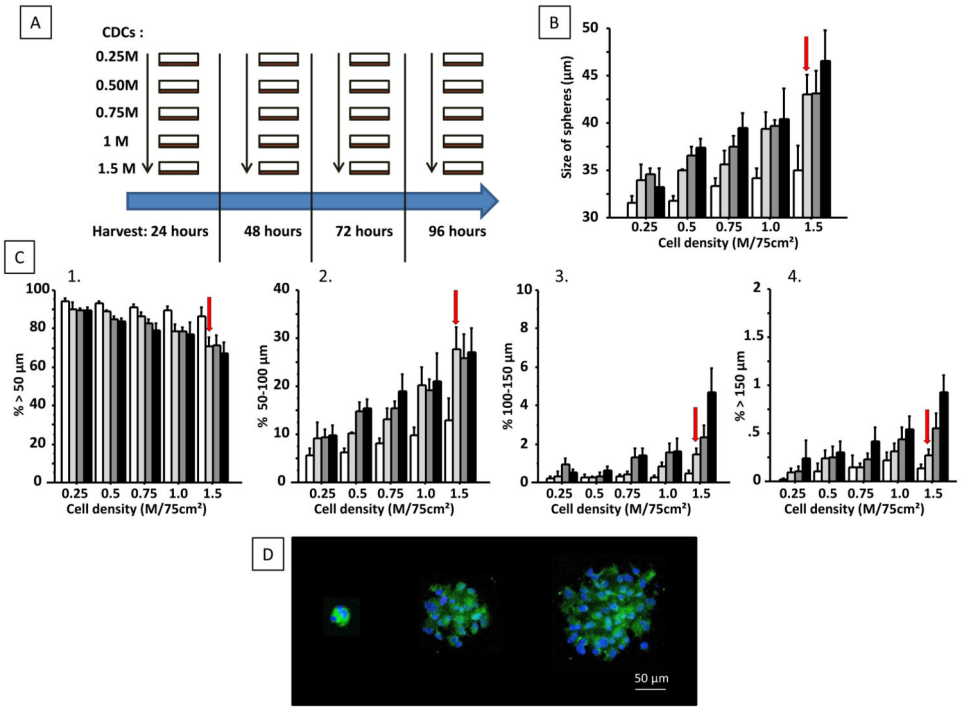

Figure 1.

Schematic representation of the in vivo study (A); 5 different seeding densities and 4 different times for harvest were used to optimize cardiosphere (CSp) size. Increasing cell density increases the average size of CSp (B), decreases the proportion of small CSp (<50 µm) (C1) and increases the proportion of 50-100 µm CSp (C2). In contrast, increasing culture duration increases the number of CSp in the 100-150 µm range (C3) and CSp>150 µm (C4). The conditions chosen for the in vivo studies are those giving a high numbers of CSp > 50 µm with few CSp > 150 µm (labeled with red arrow in panel B and C1-4). Images of a small, mid-size and large CSp are shown in (D); nuclei are stained with DAPI (blue) and cell membranes are stained with calcein acetomethoxy (green)".

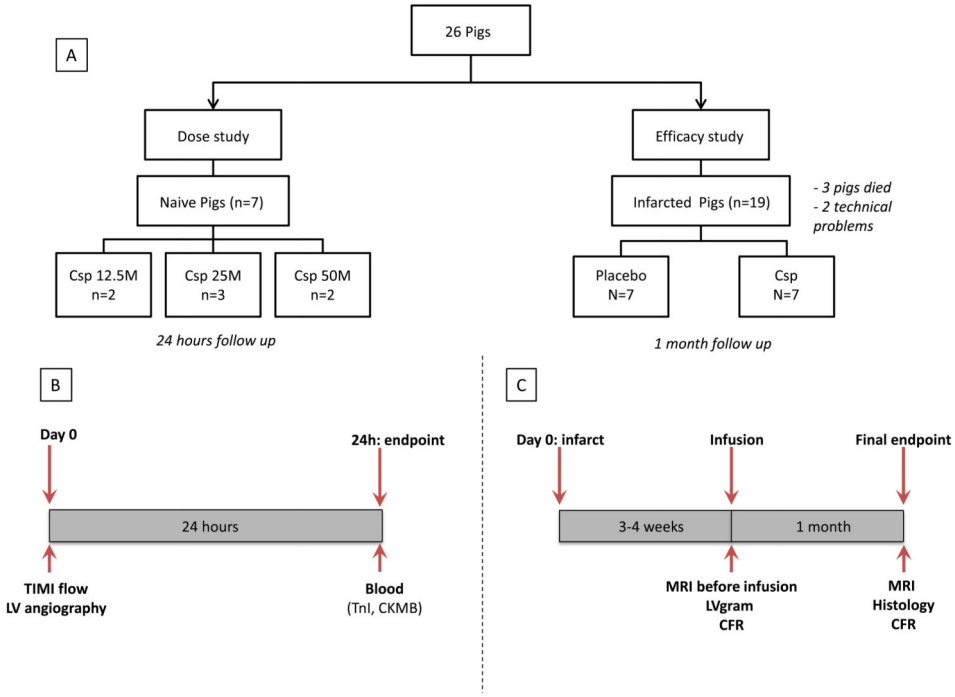

Figure 2. Design of the whole study (A) and of the feasibility (B) and efficacy (C) studies

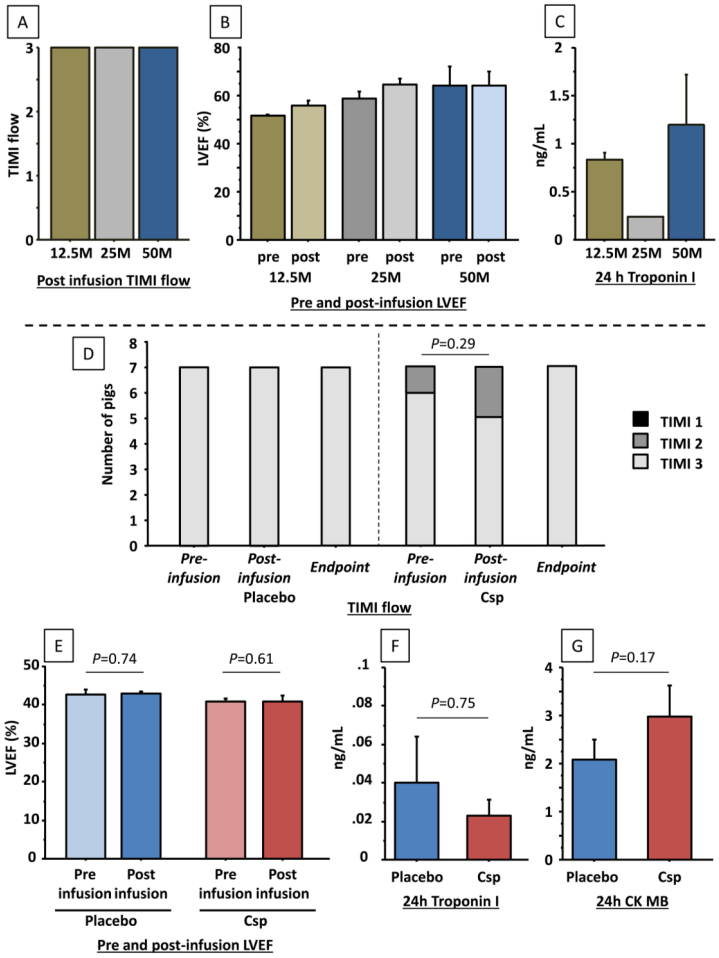

Figure 3.

Intracoronary infusion of CSp is safe in both naïve pigs and in pigs with convalescent myocardial infarction. TIMI flow (A), LVEF (B) and troponin I (C) following infusion of increasing doses of CSp (dose labeled in single-cell equivalents, SCE) in naïve pigs (feasibility study). In infarcted pigs (efficacy study), no differences were observed in TIMI flow (D), Troponin I (E), CK MB (F) or LVEF (G) following infusion of CSp or placebo (N=7 per groups).

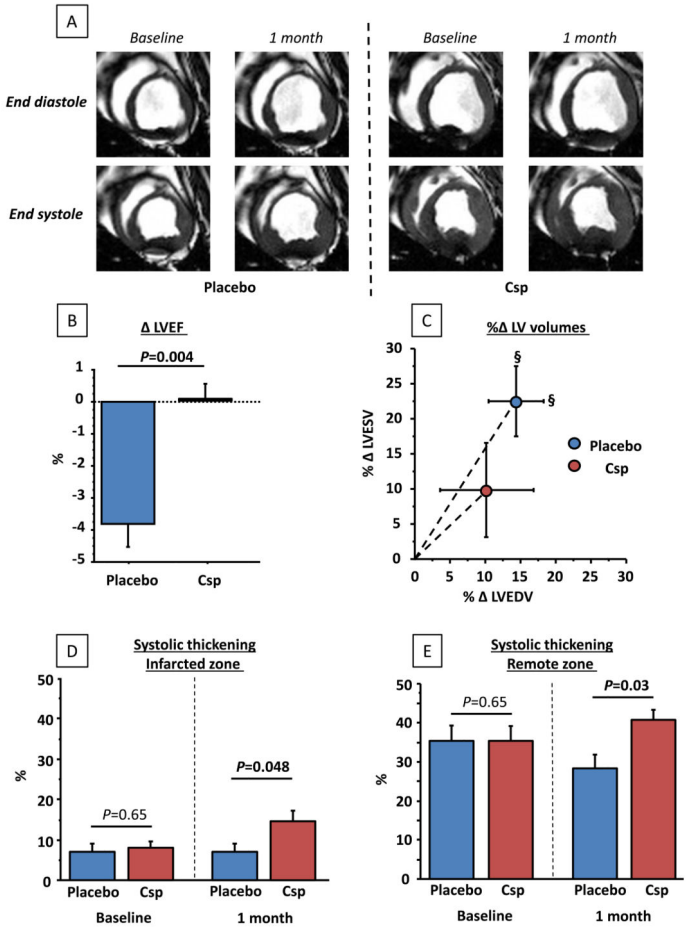

Figure 4.

Cardiopheres preserve LV function and stop adverse remodeling compared to control. (A) Matched representative end-diastolic and end-systolic MR images of placebo and CSp-treated minipigs before infusion and at 1 month follow-up. Changes in LVEF (B), relative changes in LV end-diastolic and end-systolic volumes (indexed/BSA) (C) and changes in regional function from the infarcted (D) and remote (E) zones in CSp- and placebo-treated pigs. (N=7 per groups)

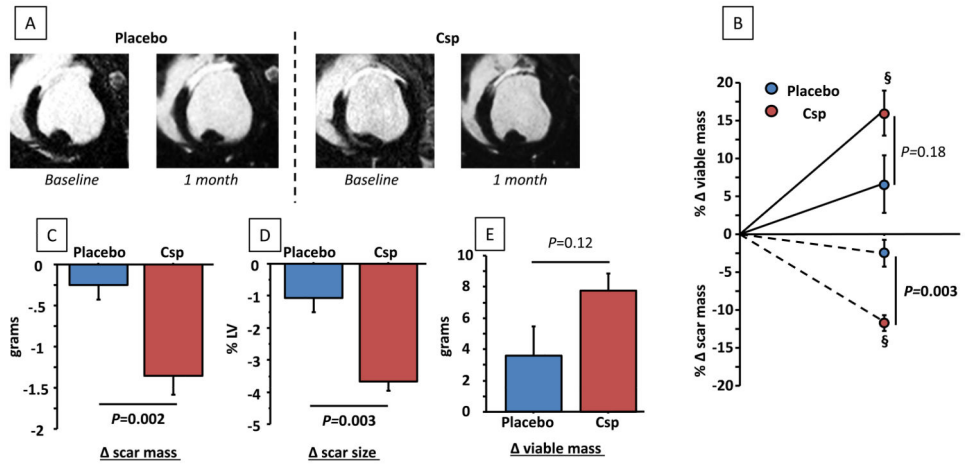

Figure 5.

Cardiospheres but not placebo decreased scar size and tended to increase viable mass at 1 month. (A) Representative MR images showing late gadolinium enhancement for placebo and CSp-treated pigs. Changes in scar mass (B), scar size (scar mass/total LV mass) (C) and viable mass (D). Relative changes in scar mass and viable mass (E). § P<0.05 vs. baseline (paired analysis). (N=7 per groups)

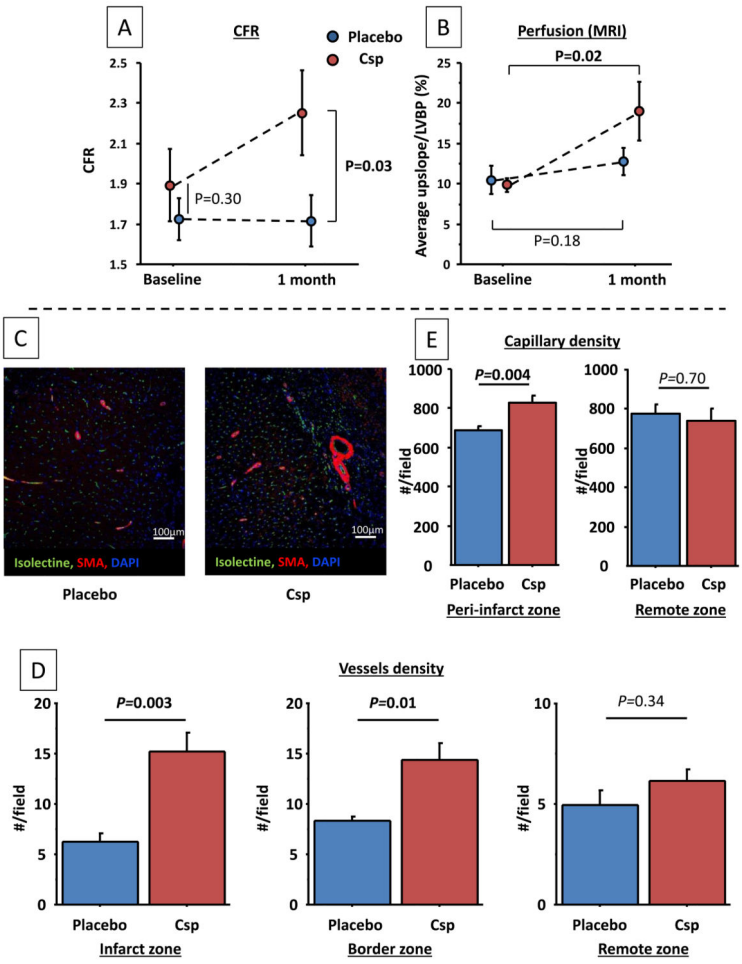

Figure 6.

Cardiospheres increase myocardial perfusion of the infarcted zone assessed by CFR (A) and MRI (1st pass sequences) (B). Representative images of arterioles and capillaries in the peri-infarcted area in CSp- and placebo-treated pigs (C). Arteriolar density is increased in the infarcted (D) and border zones but not in the remote zone. Capillary density (E) is increased in the peri-infarct zone but not in the remote zone. (N=7 per groups)

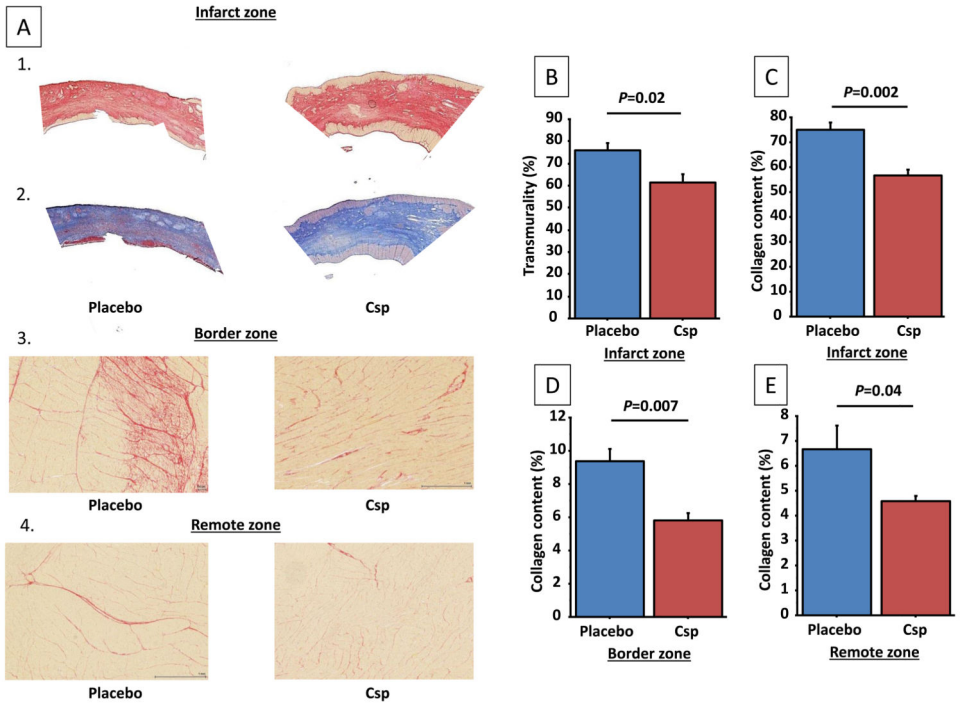

Figure 7.

Cardiosphere treatment decreases fibrosis in the heart. (A) Collagen-stained sections of infarct zone by Picrosirius red (A1) and Masson trichrome staining (A2), and of border (A3) and remote (A4) zones by Picrosirius red. Transmurality of the scar (B) and collagen content of the infarcted (C), border (D) and remote (E) zones. (N=7 per groups)

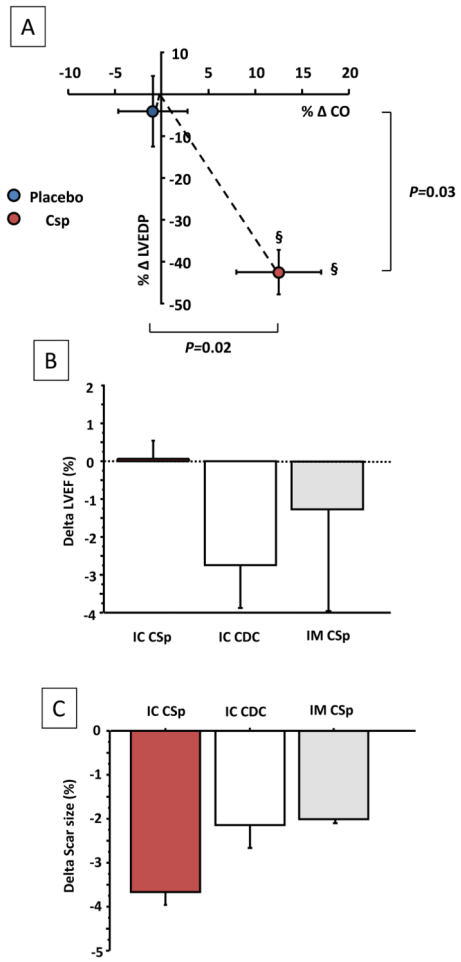

Figure 8.

(A) Cardiospheres increase cardiac output and decrease LV end diastolic pressure compared to placebo. Relative changes are shown. § P<0.05 vs. baseline (paired analysis) (N=7 per groups). (B) and (C) Effects on LVEF preservation (B) and scar size decrease (C) of IC CSp compared to IC CDC and to intra-myocardial injection (NOGA) of a comparable dose of Csp (45M SCE). The results for IC CDC and intra-myocardial CSp are derived from previous studies (no statistical comparisons were therefore performed) [6, 11]

Table

Baseline and endpoint values of Left Ventricle End-Diastolic Pressure (LVEDP), Cardiac Output (CO), Systolic blood pressure (SBP), Diastolic Blood Pressure (DBP) and Heart Rate (HR) in CSp- and Placebo-treated pigs (N=7 per group).

	Baseline			**Endpoint**			**Delta**		
	Placebo	**CSp**	**P**	**Placebo**	**CSp**	**P**	**Placebo**	**CSp**	**P**
LVEDP (mmHg)	**16.4±2.5**	**18.2±1.8**	**0.31**	**15.0±3.3**	**11.8±4.5**	**0.06**	**-1.4±3.7**	**-6.3±3.8**	**0.02**
CO (L/Min)	**3.5±0.6**	**3.6±0.3**	**0.65**	**3.5±0.4**	**4.1±0.5**	**0.05**	**-0.1±0.3**	**0.5±0.5**	**0.01**
SBP (mmHg)	71±11	71±8	0.61	86±4	93±14	0.25	18±16	21±8	0.65
DBP (mmHg)	51±5	53±7	0.89	63±5	67±16	0.36	13±3	14±16	0.58
HR (bpm)	108±11	111±27	0.84	104±16	98±7	0.40	-6±18	-10±11	0.65