A new *in vivo/in vitro* model for assessing the capacity of human derived oral mucosa stem cells to colonize the infarcted myocardium

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Abstract

Ischemic heart failure (IHF) is a leading cause for mortality and morbidity worldwide. Stem cell (SC) therapy is a promising strategy for IHF treatment. A major prerequisite for successful SC therapy is homogenous colonization of the infarcted myocardium. This process implies SC migration from the transplantation site. The purposes of the present study were to develop a new model system capable of quantifying SC migration within the infarcted myocardium and to assess the effect of post myocardial infarction (MI) remodeling on the migration of a newly identified neural crest SC population derived from the human oral mucosa (hOMSC). MI was induced in rats. Myocardial scar tissue was collected at 0,1,3,7,14, and 28 days post-MI. Dil-labeled-hOMSC were injected in the center of cylindrical scar tissue specimens harvested from each post-MI time point and transferred to organ culture. Changes in host and hOMSC cell densities were quantified in the center and periphery of specimens after 0, 3, 7, 14, and 28 days of culture by morphometric fluorescence microscopy. The results indicate a decrease in hOMSC density in the central zone and an increase in density in the peripheral zones indicating hOMSC migration from the central transplantation site to the rest of the infarcted myocardium. The average hOMSC density increased overtime due to cell proliferation. The level of hOMSC migration and proliferation was significantly affected by post-MI remodeling phase being higher in post-MI tissue that still comprised cardiomyofibers than in granulation and fibrotic tissues. Preliminary hOMSC transplantation in nude rat supports these findings. The present study shows that the new *in vivo/in vitro* model system is sufficiently sensitive to detect and quantify changes in the migratory capacity of stem cells within the infarcted myocardium and suggests that cell therapy applied in the early stages (up to 3 days) of post-MI remodeling facilitates the process of tissue colonization.

Introduction

Chronic ischemic heart failure (IHF) is one of the leading diseases in the industrialized world, and its main cause is ischemic heart disease.[1] In the United States alone, there is an incidence of 4,000,000-6,000,000 new patients every year. The increasing incidence of IHF is the result of better treatment of coronary artery disease and acute ischemic syndromes that prevents early mortality following myocardial infarction (MI).[1] With current treatment, heart failure remains a lethal condition, with 5 year survival rates of almost 40% of the patients from the time of diagnosis.[2] Thus, a new solution is required for IHF treatment. Stem cells therapy for IHF treatment is an alternative emerging solution that is being tested in preclinical and clinical setups.[2] Endogenous populations of cardiac stem cells capable of responding to MI have been identified in the mammalian and human hearts[3] and bone marrow derived progenitors have been shown to be recruited to the infarcted site.[4] Recently, a neural crest-derived stem cell (NCSC) population has been shown to contribute to the developing cardiomyocites population of the mammalian heart and to remain as a dormant stem cell population capable of responding to MI stress by migrating to ischemic area and differentiating into cardiomyocytes.[5,6]

However, these endogenous mechanisms are not sufficient to regenerate the destroyed myocardium and the post-MI functional demand induces a remodeling process that results in the thinning of the left ventricle and its dilation that further worsens the myocardial function and aggravates the disease. Thus, during the past years exogenous stem cell therapy has been tested in preclinical and clinical setups.[1,2]

The outcome of exogenous stem cell therapy depends largely on the root of cell transplantation.[7] When endocardiac root delivery is undertaken,[8,9] major prerequisites for successful stem cell therapy are substantial cell engraftment and retention into the myocardial scar and homogenous colonization of the scar tissue by the engrafted cells, implying stem cell migration and proliferation. These processes might be affected by the nature of the myocardial scar tissue as determined by the post-MI remodeling process and by the migratory capacity of the transplanted stem cell population.

We have recently identified a novel primitive neural crest stem cell population in the lamina propria of the human oral mucosa (hOMSC).[10] hOMSC are highly expandable (trillions of cells are generated from a tiny biopsy); are positive for the NCSC markers p75 (95%) and nestin (80%) and for the pluripotency associated markers Oct4, Sox2 (70%) and Nanog (40%); differentiate into cell lineages of the 3 germ layers *in vitro*; and upon dexamethasone stimulation and implantation in scid mice form teratoma-like tumors that consists of ectodermal and mesenchymal lineages. To date, the experimental models that allow the quantification of stem cell migration within the infarcted myocardium following their endocardial transplantation are scarce. In the present study, we developed a new *in vivo/in vitro* model in the rat aimed at quantifying the effect of post-MI remodeling phase on the migration of hOMSC within the infarcted myocardium. Using this model, we found that the colonization of the infarcted myocardium is affected by the post-MI transplantation timing, mainly due to the different capacity of hOMSC to migrate within the infarcted rat myocardium at various stages of remodeling.

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Materials and Methods

In vivo experimentation

The laboratory study was approved by the Animal Ethical Committee of Tel Aviv University and by the Animal Ethical

Committee of the Israeli Ministry of Health. Female Sprague Dowley rats weighing 250 gm served as experimental animals (Harlan, Jerusalem, Israel, www.harlan.com). Following a lateral open thoracotomy, myocardial infarction was induced by ligation of the left anterior descending artery as previously described.[11] Rats were sacrificed at 6 time points: 0, 1, 3, 7, 14 and 28 days post MI induction. The infarcted myocardium was identified according to its whitish color, dissected out and immersed immediately in DMEM + 10% FCS and a solution of antibiotics comprising Penicillin (100 units/mL), Streptomycin (0.1 mg/mL) and Nystatin (12.5 units/mL) (organ culture medium) (Biological Industries Ltd., Kibbutz Beit Haemek, Israel). Cylindrical punch biopsies, 3 mm in diameter were obtained under aseptic conditions from the infarcted myocardium and maintained in organ culture as described below. The specimens obtained at time 0 days served as controls of the normal myocardium.

In vitro experimentation

Cell Culture

Oral mucosa derived stem cells (OMSC), were generated as described elsewhere.[10] Briefly, gingival and alveolar mucosa biopsies were obtained from donors following the study approval by the Institutional Helsinki Committee of Sheba Medical Center, Ramat-Gan, Israel. Biopsies were explanted in DMEM-LG + 10% FCS and a solution of antibiotics consisting of Penicillin (100 units/mL), Streptomycin (0.1 mg/mL) and Nystatin (12.5 units/mL). Cells that emerged from the explanted tissues were harvested with 0.25% Trypsin ((Biological Industries Ltd.), and propagated in the same medium. This, procedure has been shown to generate hOMSC with a reproducible phenotype.[10] Cultures of passages 4^{th} – 10^{th} were used for the experiments described below.

Human oral mucosa derived stem cell labeling, transplantation and organ culture

hOMSC were harvested as described above, centrifuged and resuspended in a solution of DiI (1,1-dioctadecyl-3,3,3,3-testramethylindicarbocyanine perchlorate) for 30 min, washed 2 times in phosphate buffer saline (PBS) counted and resuspended at a concentration of 10×10^6 cells/mL in PBS and transferred to a Hamilton syringe.

Each of the specimens that were obtained as described above was injected in its center with 5 μL of cell suspension containing 1×10^5 hOMSC. Then, the specimens were cultured on a stainless steel wire mesh (grid method) as described elsewhere.[12] Specimens were harvested in groups of 3 at 0, 3, 7, 14 and 28 days post-transplantation. The cylindrical biopsies were fixed in cold 4% paraformaldehyde, washed in PBS + 4M sucrose and then incubated in this solution overnight, embedded in OCT and cut transversally into serial sections that exhibited a spherical profile. Four sections from each specimen located 100 μm from the epicardial and endocardial surfaces and 2 central sections located 100 μm apart (Figure 1A) were covered with mounting material containing DAPI (Vectashield Mounting Medium with DAPI; Vector Laboratories, Inc., Burlingame, CA, USA) and examined by fluorescent microscopy. Adjacent sections were stained with hematoxylin and eosin (H&E).

Human oral mucosa derived stem cell engraftment and migration

hOMSC engraftment and migration were assessed by determining the density (number of cells/mm square) in 5 fields of each section: a central one and in 4 peripheral fields located at the periphery of the sections located on 2 perpendicular diameters as shown in Figure 1B. To do this, each field was captured at a magnification of X400 with a Zeiss Axioplan light and fluorescent microscope. Each field was photographed twice: once using a filter to visualize DAPI and once using a filter to visualize DiI. The images were superposed using Adobe Photoshop C54 software (Adobe Systems Incorporated, San Jose, CA, USA). The total number of nuclei and the total number of DiI labeled hOMSC were counted in each field in each of the 4 sections obtained from each specimen. Using a microscopic grid the photographed area was calculated and the cell density was computed and expressed as cells/mm^2.

The data was used to compute the following 4 parameters: i) the average nuclei density/specimen in the central zone. To do this, the mean of the 4 central fields, one from each section, was computed; ii) the average nuclei density/specimen in the peripheral zone. To do this the mean nuclei density/field/section was calculated and the results obtained for each of the 4 section were used to calculate the average nuclei density/specimen; iii) the average hOMSC density/specimen in the central zone was calculated as described above for the nuclei density; iv) the average hOMSC density/specimen in the peripheral zone was calculated as described above for the nuclei density.

The results were used to compute the average of each parameter per specimen for each of the *in vivo/in vitro* time points.

Student-t-test was used to assess the statistical significance of the effect of post-MI timing on cell engraftment and migration of hOMSC over time in culture.

Results

The new experimental model developed during this study consists of an *in vivo* experimental period followed by an *in vitro* one. Thus, the effects of two time courses (both of them 28 days long) on the colonization of the myocardial tissue were analyzed in this *in vivo/in vitro* model system. The *in vivo* time course refers to the effect of the time that elapsed from the MI induction to the animal sacrifice and hOMSC implantation and reflect the of post-MI remodeling phases. The time points of this time course will be referred as PMI(x) – where PMI stand for *post-MI* and x represents the number of days that elapsed between MI induction and hOMSC transplantation into the infarcted myocardial tissue. For example, PMI(14) refers to specimens obtained 14 days following MI induction that were implanted with hOMSC and transferred to organ culture.

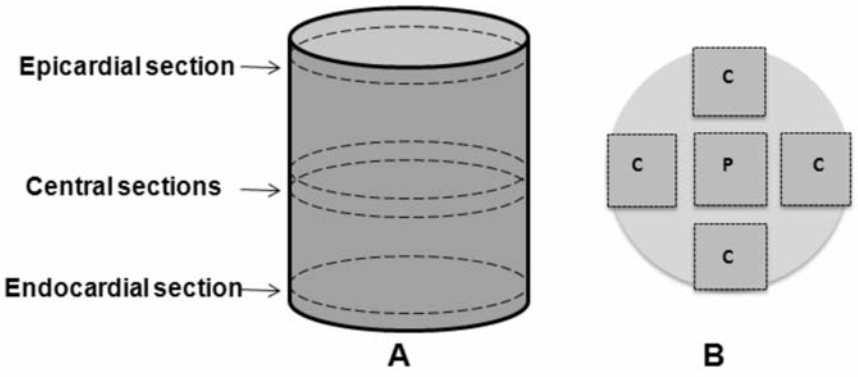

Figure 1. Schematic illustration of the processing of a cylindrical myocardial specimen. (A) The location of the sections obtained from each specimen relative to its endocardial and epicardial aspects. (B) The location on each section of the five fields used for determining the host and human oral mucosa derived stem cell densities.

The *in vitro* time course refers to the effect of culture time on hOMSC migration and proliferation in the organ culture system. The times points of the *in vitro* period will be referred as C(X) where C stands for *culture* and x represents the number of days during which the specimens were maintained in culture before being harvested for the analyses described in *Materials and Methods*. Thus, a specimen termed C(7) refers to specimen that was cultured for 7 days. Consequently a specimen termed PMI(14)C(7) refers to a specimen that was harvested 14 days post-MI, implanted with hOMSC and maintained in organ culture for 7 days.

Microscopic examination of the H&E sections obtained at the 6 post-MI time points revealed the characteristic histopathological changes described elsewhere.[13] Briefly, at PMI(3) coagulation necrosis and infiltration of acute inflammatory cells were observed; at PMI(7) early formation of granulation tissue was found; beginning of scar formation was observed at PMI(14) that continued to develop into mature scar tissue by PMI(28).

The effect of post-myocardial infarction time on the cell density of the infarcted myocardium

The cellularity of the host tissue at the various post-MI time points was assessed by deducting the total number of hOMSC from the total number of DAPI positive stained nuclei, which included those of hOMSC. Analysis of cell density at C(0) in the specimens obtained at the various post-MI time indicates a decrease of 4 folds ($P < 0.05$) between PMI(0)C(0) and PMI(28)C(0) (Figure 2A). The major drop [2.57 folds; ($P<0.05$)] occurred between PMI(0)C(0) and PMI(7)C(0).

Analysis of the effect of the *in vitro* time course on cell density of the infarcted myocardium revealed a progressive reduction in cell density overtime in specimens obtained at all post-MI time point (Figure 2). The highest and lowest reductions in cell density over the 28 days of culture were observed in specimens obtained at PMI(7) and PMI(28), respectively [$P<0.5$; (Figure 2B)].

Human oral mucosa stem cells

hOMSC engraftment was assessed by determining the cell density of DiI labeled cells in the central and peripheral fields at C(0) time points. The total average cell density [(central + peripheral)/2] at various post-MI time points is shown in Figure 3A and the differential engraftment in the central and peripheral parts of the specimens in Figures 3B, 3D. The level of engraftment as assessed by hOMSC density at C(0) at all PMI time points was similar indicating that the nature of the infarcted

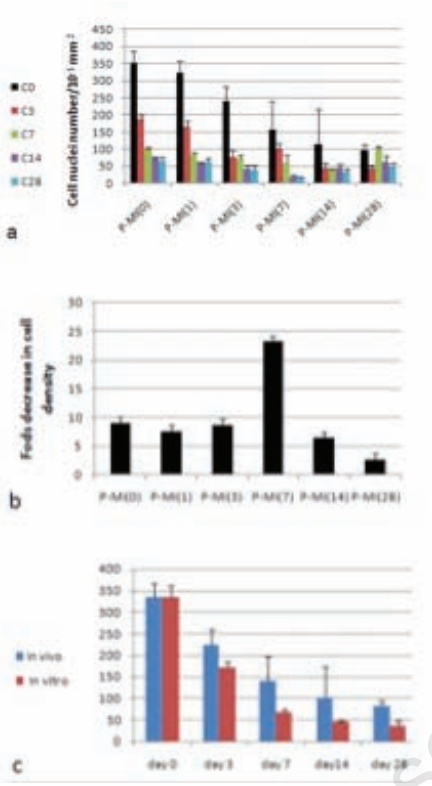

Figure 2. Changes in cell densities within the host infarcted myocardium as a function of post-myocardial infarction remodeling phase and culture time course. (A) The bars represent the total average cell density of the peripheral and central zones of both human oral mucosa derived stem cell and host cardiomyocytes. On the horizontal axis, the results are presented in groups according to the post-myocardial infarction harvesting time points [PMI(x)]. The different colors of the bars represent the time points in culture [C(x)] at which the specimens were harvested. (B) The folds decrease in host cell density during the 28 days of culture period in specimens harvested from animals at the various post-MI time points are depicted. The values were calculated by dividing the cell density at C(0) by the cell density at C(28) at each post-myocardial infarction time point. The highest decrease in cell density is observed in PMI(7) specimens and it differs statistically ($P < 0.05$) from the other time points. (C) Comparison between cell density decay in vivo and in vitro. The blue bars represent mean host cell densities at C(0) in specimens obtained immediately after sacrifying the animals at PMI(0), PMI(3), PMI(7), PMI(14) and PMI(28); the red bars represent mean host cell densities of PMI(0) specimens harvested from the culture after 0, 3, 7, 14 and 28 days of culture. Statistical analysis indicate significant differences between the means of the groups of PMI(14) and PMI(28) but not between those of PMI(0), PMI(3) and PMI (7).

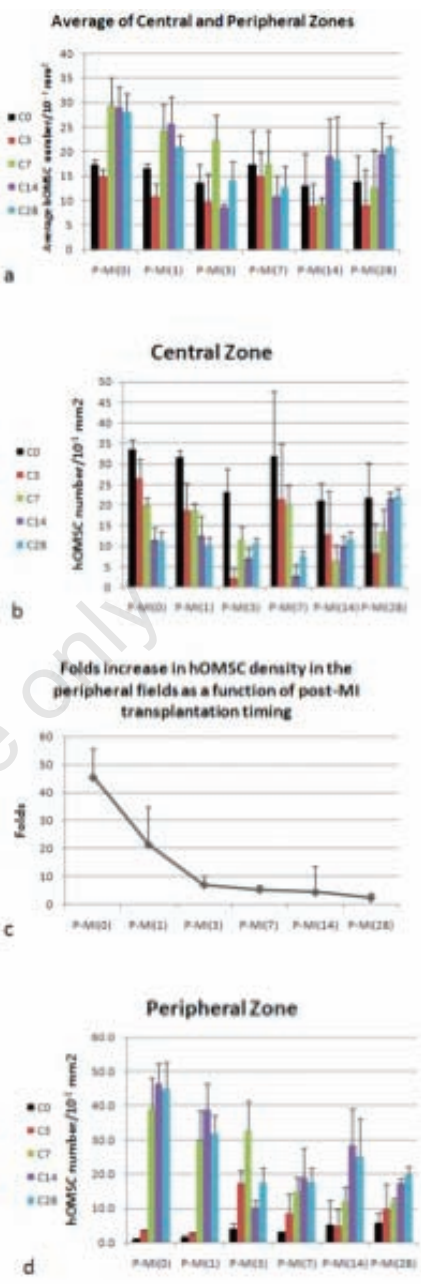

Figure 3. Changes in human oral mucosa derived stem cell densities in the central and peripheral zones as variables of post-myocardial infarction remodeling phase and culture time course. The results in (A), (B) and (C) are presented as explained in Figure 2A. (A) The bars represent the total average of human oral mucosa derived stem cell density of both the peripheral and central zones. (B, D) The bars represent the average human oral mucosa derived stem cell density in each of the central and peripheral, respectively. (C) The effect of post-myocardial infarction remodeling on the increase in human oral mucosa derived stem cell density is presented as the ratio between the human oral mucosa derived stem cell density at C(0) and that at C(7) for PMI(3) and PMI(7) specimens and at C(14) for the rest of the specimens

tissue did not affect the initial *in vitro* engraftment of hOMSC. Differential analysis between the central and peripheral fields indicate that in the PMI(0) and PMI(1) specimens, the vast majority of hOMSC were located in the central field and only negligible cell numbers were identified at the peripheral zone (Figures 3B, 3D). Starting from PMI(3) there was an increase in the cell density in the peripheral zones at C(0), pointing to the leakiness of the infarcted tissue that underwent more advanced remodeling (Figure 3D).

Human oral mucosa derived stem cell migration and proliferation

The effect of the nature of the scarred myocardium on hOMSC migration was determined by assessing the change in the density of the DiI labeled hOMSC in the central and peripheral fields over time in culture. Generally, in the central fields there was a gradual and statistically significant decrease ($P<0.05$) in the hOMSC cell density over the 28 days period of culture in PMI(0), PMI(1), PMI(3), PMI(7) and PMI(14) specimens (Figure 3B). The hOMSC density in the central zone did not changed in PMI(28) specimens (Figures 3B; Figure 4). Notable, in the peripheral zone there was a progressive increase in hOMSC density in all specimens obtained at all post-MI time points. This increase peaked at 7 days of cultures in the PMI(3) and PMI(7) specimens (t-test C(0) *vs* C(7); $p < 0.05$) and at 14 days of culture in the rest of the specimens (t-test-C(0) *vs* C(14); $p < 0.05$) (Figures 3C,D; Figure 4). The highest increase in hOMSC density in the peripheral zone was observed in the PMI(0)C14 (45 folds) and PMI(1)C14 (21 folds) (Figure 3C). In the remaining specimens, this increase in hOMSC density ranged between 7 [PMI(7)] to 2 [PMI(28)] folds. Taken together these data indicate that hOMSC migrated from the central zone to the periphery in specimens obtained from all post-MI time points, but the amount of migration was substantially affected by the remodeling phase of the myocardium.

Comparison between the total average of hOMSC density/specimen at C(0) and that at C(14) or C(28) reveals an increase in the hOMSC density overtime in specimens of each post-MI time points. However, this increase in hOMSC density, which on average was 45%, was statistically significant only in specimens obtained at PMI(0), PMI(1) and PMI(3) strongly suggesting proliferation of hOMSC during the culture period. These findings are furthered by analyzing the change in the proportion of hOMSC within the total cell population over time. As shown in Figure 5A there was a general increase in the proportion of hOMSC over time in culture at all post-MI time points increasing from an average of 5.4% at C(0) to 37.5% at C(14,28). The increase in the proportion of hOMSC was statistically significant at all post-MI time points [t-test for C(0) vs. C(14); $p < 0.05$] except for PMI(14).

In parallel to developing the *in vivo/in vitro* model system, we performed a preliminary experiment aimed at obtaining qualitative preliminary results on the capacity of hOMSC to colonize the post-MI myocardium *in vivo*. In this pilot experiment, a suspension 2×10^6 hOMSC in 100 µL of DMEM was transplanted

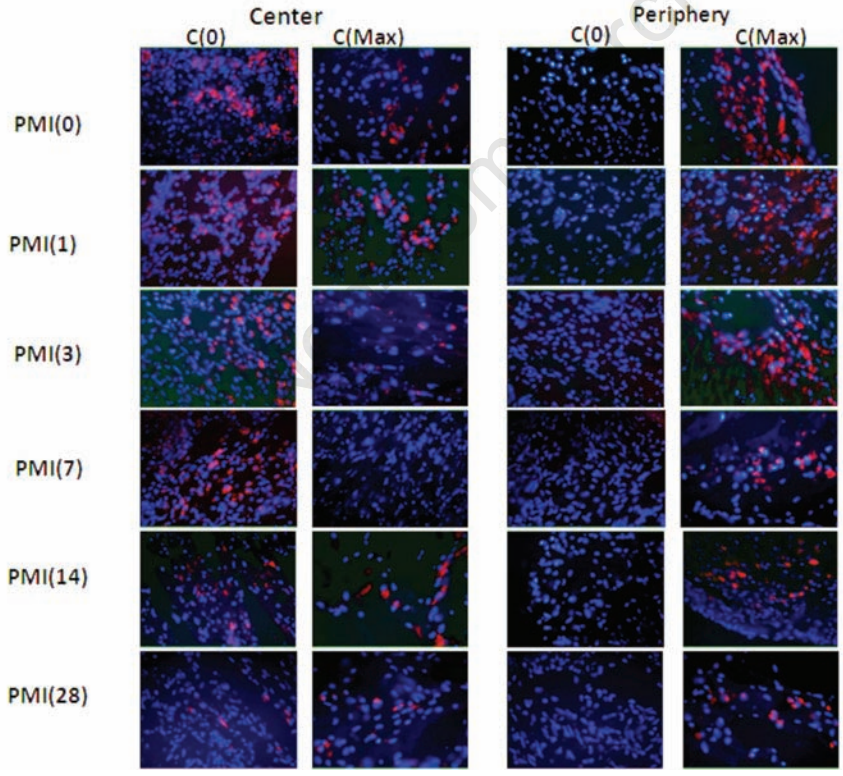

Figure 4. Representative qualitative fluorescent microscopic illustration of Dil-labeled human oral mucosa derived stem cells (stained red) in the central and peripheral fields in specimens harvested from cultures at C(0) and at C(max) when max stands for the highest human oral mucosa derived stem cell density determined in the peripheral fields. (See Legend of Figure 3).

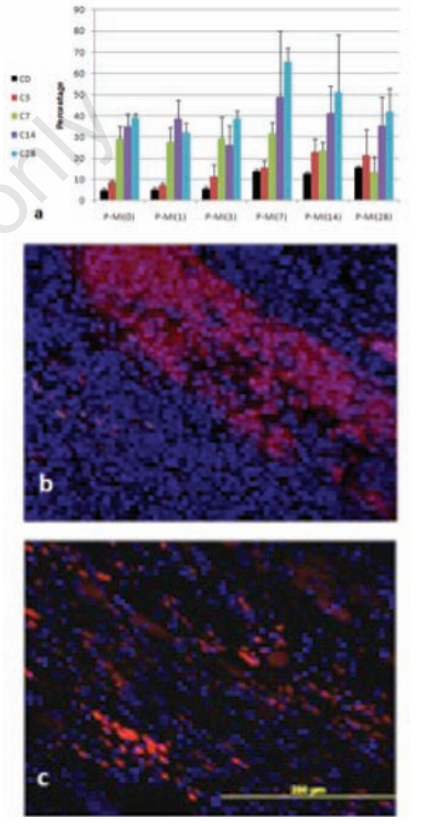

Figure 5 (A) - Changes in the proportion of human oral mucosa derived stem cells relative to the host cell population as function of post-myocardial infarction remodeling and culture time course. Notice the general increase in human oral mucosa derived stem cell proportion during the culture period in specimens obtained at all time points. (B, C) Are qualitative illustrations of DiI-labeled human oral mucosa derived stem cell distribution following their implantation into nude rats. (B) Large aggregates of human oral mucosa derived stem cells (stained red) are observed within the post-myocardial infarction myocardium 72 hours after being transplanted in nude rats 7 days after myocardial infarction induction. (C) Human oral mucosa derived stem cells are dispersed within post-myocardial infarction myocardium 30 days after being transplanted in nude rats 7 days after myocardial infarction induction.

into the infarcted myocardium of 2 nude rats 7 days after inducing MI as described above. Large aggregates of hOMSC were observed within the infarcted myocardium 72 h post-implantation suggesting that a substantial part of the implanted cells were retained (Figure 5B). A relatively small number of hOMSC were observed in the vicinity of these aggregates indicating initial cell migration from the transplantation site or cell dispersion during hOMSC delivery. At 30 days post-implantation hOMSC were dispersed within the tissue or formed strips of cells indicating colonization of the infarcted myocardium (Figure 5C).

Discussion

The prospective of successful cardiac regeneration by stem cell therapy depends on a myriad of still partially determined factors: the type of stem cells, the administration root, stem cell engraftment, long term retention and survival following engraftment, and stem cells proliferation and differentiation into functional cardiomyocites capable of integrating within the electrical circuitry of the functioning myocardium.[14] Most of these factors have been extensively studied.[14,15] However, less attention has been given to the fact that stem cells have first to colonize the infarcted tissue in order to exert their biological effects. Cell migration is a paramount biological process required for homogenous colonization of any tissue undergoing repair and/or regeneration. Magnetic resonance imaging (MRI) has been used to follow the fate of transplanted stem cells in the post-MI myocardium *in vivo*.[16-19] Nevertheless, a recent study demonstrated that overtime, MRI does not necessarily identify the transplanted cells, but macrophages that up took the superparamagnetic iron oxide particles following the transplanted cells death.[20] Thus, the lack of an appropriate model system to quantify transplanted stem cells migration within the infarcted myocardium may explain why this topic was overlooked. The new model described in the present study allows for the quantification of hOMSC migration within the post-MI myocardium of the rat. The hOMCS express a neural crest phenotype.[10,21] Tamura Y *et al.*[6] found that a cardiac endogenous neural crest stem cell population migrate and differentiate into cardiomyocytes after myocardial infarction. Oral mucosa derived cells express a higher migratory capacity than other mesenchymal cells.[22] Thus, hOMSC were selected to test the capacity of stem cells to colonize the infarcted myocardium.

By standardizing the dimensions of the tested infarcted myocardium, the cell type, the site of cell transplantation and the number of transplanted cells and by using standardized organ culture and fluorescent morphometric approaches we showed an increase in the hOMSC density in the peripheral zones and a concomitant decrease in hOMSC density in the central zone. These changes in cell density indicate that hOMSC migrated from the center of the infarcted tissue to its periphery, probably consequent to gradients of serum chemoattractants and oxygen tension. One could have assumed that cell migration will also take place towards the epicardial and endocardial aspects of the cultured tissue as these are close to chemoattractants and oxygen. However, comparison between the changes in hOMSC density overtime in the histological sections obtained from the middle part of the sample, that are relatively remote from the epicardial and endocardial aspects (Figure 1), to the changes in hOMSC density overtime in the epicardial and endocardial-related sections (Figure1) indicates similar cell densities (data not shown). These data suggest that at least in this culture system, the epicardial and endocardial aspect of the cultured infarcted myocardium are less permeable to chemoattractants than its peripheral aspects. If such a mechanism occurs in vivo, it might imply that cells transplanted in the center of the scarred myocardium migrate to its periphery, thereby reducing the cellularity at needed sites and interfering with the desired cellular therapeutic effect of the transplanted cells.

To test the sensitivity of the system we examined the effect of post-MI myocardial remodeling on the level of cell migration. The results demonstrate that this process is affected by the properties of the infarcted tissue and changes as a variable dependent on the post-MI myocardial remodeling, pointing to the acceptable sensitivity level of the system.

The major drawback of the system is that cell migration is assessed *in vitro* and not in vivo. Similarly to cell proliferation and differentiation, cell migration is also affected by the microenvironment of the tissue within which this process takes place.[23,24] This in turn is affected by the cell density, cell type and the extracellular matrix, factors that might differ between *in vitro* and *in vivo* conditions. To partially overcome this drawback and test how the myocardial remodeling affects cell migration, we assessed cell migration *in vitro* in cardiac tissue obtained at various post-MI time points. The assessment of the host cell density indicates that during the culture period there was a progressive decrease in the host cell number, which points to cardiomyocites death. This in turn, may affect the properties of the substrate through which the cells migrate and consequently affects migration. Furthermore, cell migration might also be affected by the cyclic changes in the tissue's mechanical properties due to the diastolic-systolic cycle that occurs *in vivo* and were not mimicked in this *in vitro* system. On the other hand, the system offers the possibility to exclude the biomechanical effects associated with the cardiac cycle and assess the effect of specific factors on stem cell migration within the infarcted myocardium.

The differences between the host cell densities at C(0) of specimens harvested over the 28 days post-MI time points represent the change in cellularity of the infarcted myocardium *in vivo*. Examination of the change in the host cell densities of PMI(0) specimens during the 28 days of culture period reveals a similar decrease in cell density, even though more pronounced (Figure 2C) than that observed *in vivo*, suggesting a similar pattern of cardiomyocytes survival response to hypoxic conditions *in vivo* and *in vitro*. Notably, in spite of the decrease in host cell density, hOMSC survived the transplantation into the infarcted myocardium, proliferated and migrated, apparently, due to the less traumatic adaptation from one *in vitro* system to another and possibly because of the capacity of these cells to function under hypoxic conditions.

The change in hOMSC density in the central and peripheral fields as a variable of the *in vivo* and *in vitro* time courses demonstrate that the properties of the remodeling infarcted myocardium affect substantially the behavior of the transplanted cells. The higher peripheral cell density in specimens harvested at PMI(7), PMI(14) and PMI(28) pointed that these specimens were more leaky than specimens harvested at earlier time points. Considering the limitation of extrapolating from *in vitro* to *in vivo*, this finding could have been of benefit, since an initial better cell distribution might be obtained when transplanting these cells *in vivo* at late post-MI time points. However, this leakiness might explain the low cell retention observed *in vivo* following stem cell transplantation into the infarcted myocardium.[23] Furthermore, the present results indicate that hOMSC proliferate and migrate better in post-MI tissue that still comprises cardiomyofibers than in granulation and fibrotic tissues, alluding to the possibility of achieving a higher level of colonization when cell therapy is applied in the early stages (up to 3 days) of post-MI remodeling.

Conclusions

Taken together the results of the present study show that the new *in vivo/in vitro* model system is sufficiently sensitive to detect changes in the migratory capacity of stem cells within the infarcted myocardium, that hOMSC are capable of colonizing the post-MI myocardium and that this capacity is affected by the remodeling phase of this tissue. The

capacity of hOMSC to colonize the infarcted myocardium as shown in our preliminary study provides further support to these conclusions.

The study provides a new approach for quantifying the capacity of different cell types to colonize the infarcted myocardium and for determining the cellular and molecular mechanisms that control this process that is crucial for heart regeneration.

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