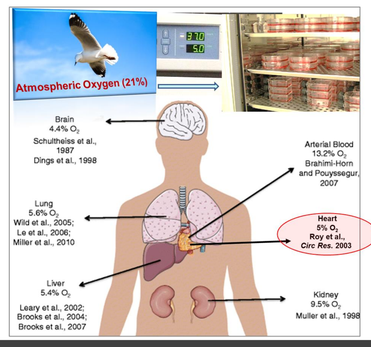
Cardiac Mesenchymal Cells Cultured at Physiologic Oxygen Tension Have Superior Therapeutic Efficacy in Mice with Heart Failure Caused by Myocardial Infarction

INTRODUCTION

Stem cell therapy is a promising therapy to treat heart disease, particularly heart attacks (myocardial infarction) and heart failure. Recently, a new type of stem cells has been isolated from the heart: cardiac mesenchymal stem cells (CMCs). These cells were discovered at the University of Louisville. They have been shown to be very effective at improving heart function in mice and rats that underwent myocardial infarction resulting in heart failure, and may soon be used in clinical trials.

Stem cells are usually cultured at atmospheric O_2 tension (21%); however, the physiologic O_2 tension in the heart is ~5%. **This raises the concern that culturing stem cells at 21% O_2 may cause toxicity due to oxidative stress.** My previous study has found that culturing CMCs at 5% O_2 tension is beneficial *in vitro*. However, **it is unknown whether the beneficial effects of 5% O_2 tension *in vitro* translate into greater efficacy of CMCs in repairing the heart *in vivo***.



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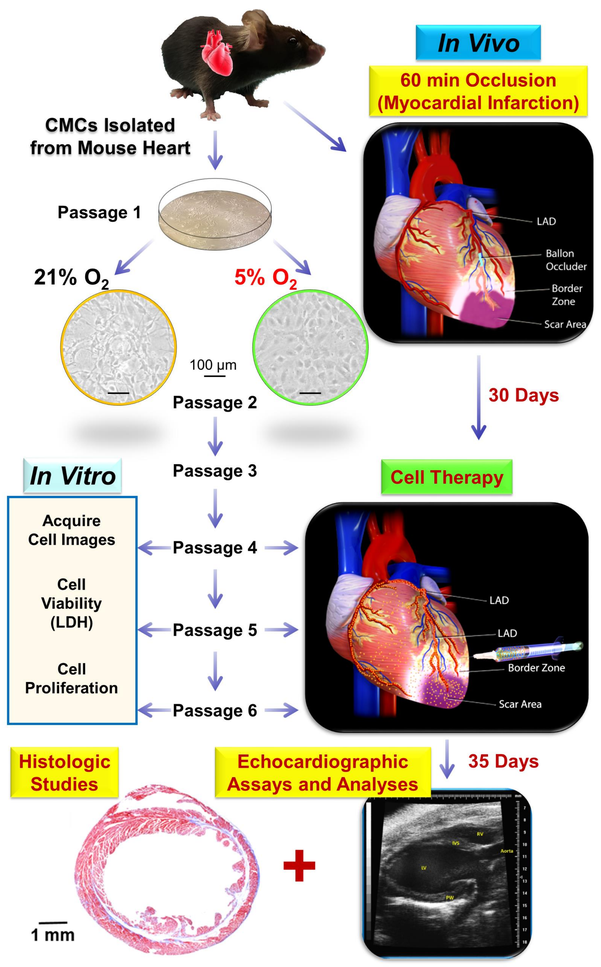
HYPOTHESIS

Compared with CMCs cultured at atmospheric O_2 tension (21%), culturing CMCs at physiological O_2 tension (5%) will increase not only their proliferation, viability, and resistance to stress *in vitro* but also their **therapeutic potency *in vivo* as a treatment for heart failure**. The overall goal is to determine the optimal O_2 tension to culture CMCs and, possibly, other types of stem cells, to maximize their therapeutic efficacy to treat heart attacks, heart failure, and other diseases.

METHODS

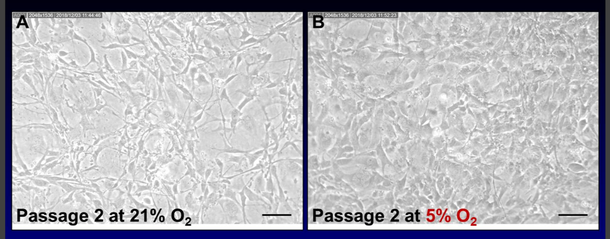
1. **Culture of Mouse CMCs** Mouse CMCs isolated from the same heart were cultured at 21% O_2 and 5% O_2, respectively, beginning at passage 2. When cells reached ~80% confluence, cells from one cultured plate were passaged into new culture plates (0.3 x 10^6 cells/new plate). In all experiments, CMCs were not used beyond passage 6.
2. **Murine Model of Myocardial Infarction (MI)** C57BL6/J female mice underwent a 60-min coronary artery occlusion followed by 65 days of reperfusion. This resulted in a large MI and heart failure.
3. **Echocardiography-guided intraventricular CMC transplantation** Thirty days after MI, CMCs were transplanted into the mouse heart. Under echocardiographic guidance, a 0.5-inch 30 G needle connected to a 1-ml syringe was inserted from the left side of the chest and advanced into the center of the left ventricular (LV) cavity. CMCs ($1x10^6$ cells/200 µl) or vehicle were infused.
4. **Echocardiographic Studies** Serial echocardiograms were obtained at 3 days after MI, 30 days after MI (before cell transplantation; Pre-Rx), and 35 days after cell transplantation (65 days after MI; Post-Rx). The parasternal long axis view was used to obtain 2D images for measurement of LV ejection fraction (EF). Digital images were analyzed off-line by a blinded observer using the Vevo 2100 workstation software.
5. **Histologic Studies** At the end of studies, the heart was subjected to fixation, tissue processing, and paraffin embedding procedures. The LV scar size was evaluated on 4 um-thick heart sections stained with Masson's trichrome. The stained images were analyzed using NIH Image J and the measurements obtained from the various slices per heart were averaged.
6. **Statistical Analysis** Data are presented as mean ± SEM. A *P* value of <0.05 was considered statistically significant. All statistical analyses were performed with the SigmaStat software system (v3.5).

Overview of Procedure



RESULTS

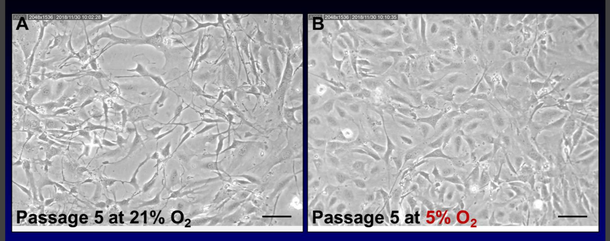
Effect of 5% Oxygen Tension on Morphology of Mouse Cardiac Mesenchymal Cells at Passage 2



A. Mouse CMCs at passage 2 were cultured at 21% O_2 for 5 days. **B.** Mouse CMCs at passage 2 were cultured at 5% O_2 for 5 days. Bars: 100 µm

Fig. 1

Effect of 5% Oxygen Tension on Morphology of Mouse Cardiac Mesenchymal Cells at Passage 5



A. Mouse CMCs at passage 5 were cultured at 21% O_2 for 4 days. **B.** Mouse CMCs at passage 5 were cultured at 5% O_2 for 4 days. Bars: 100 µm

Fig. 2

Effects of 5% Oxygen Tension on Cell Proliferation and Doubling Time of Mouse Cardiac Mesenchymal Cells

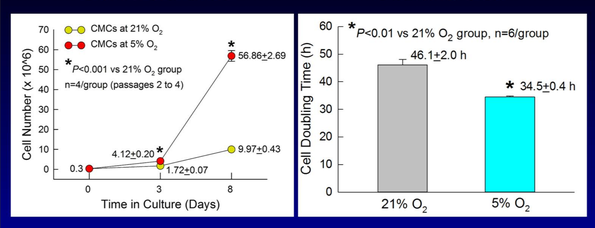


Fig. 3

Effect of 5% Oxygen Tension on Morphology and LDH release of Mouse CMCs after 24 h of Hypoxic Stress

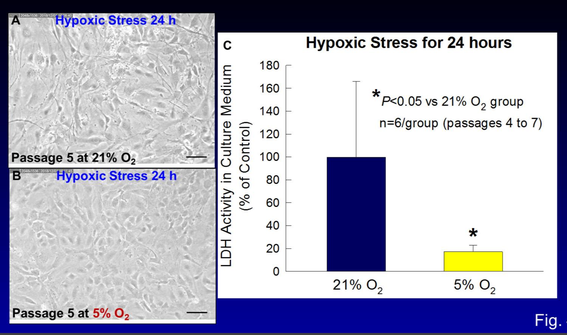


Fig. 4

In Vivo Study of CMC Transplantation into the Mouse Heart

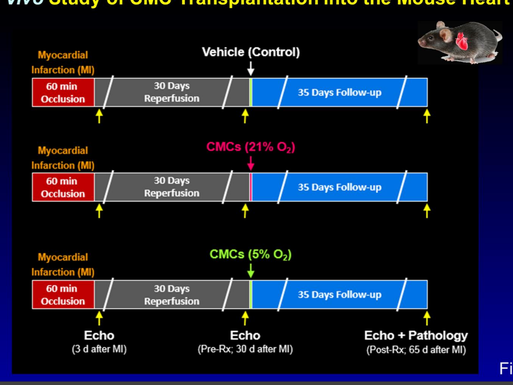
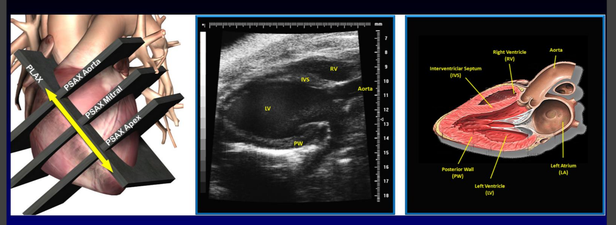


Fig. 5

Echocardiographic Measurements



Parasternal Long-Axis Echocardiogram View
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Fig. 6

Effect of CMCs Cultured at 5% Oxygen Tension on Left Ventricular Ejection Fraction in Mice with Heart Failure

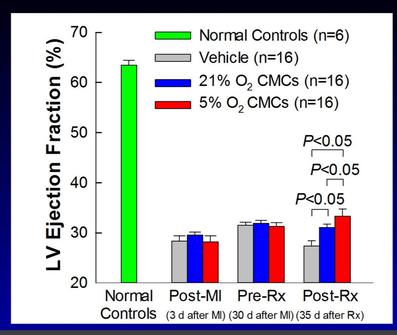


Fig. 7

Effect of CMCs Cultured at 5% Oxygen Tension on the Change (Delta) in LV EF in Mice with Heart Failure

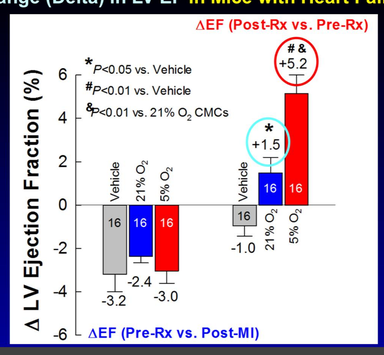
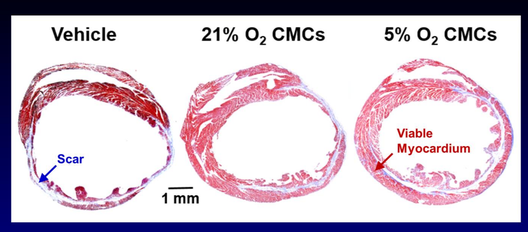


Fig. 8

Effect of CMCs Cultured at 5% Oxygen Tension on Cardiac Morphology in Mice with Heart Failure



Representative Masson's trichrome-stained myocardial sections 35 days after treatment with vehicle, 21% or 5% O_2 cultured CMCs. Scar tissue and viable myocardium are identified in blue and red, respectively.

Fig. 9

Effect of CMCs Cultured at 5% Oxygen Tension on Cardiac Morphology in Mice with Heart Failure: Quantitative Analysis

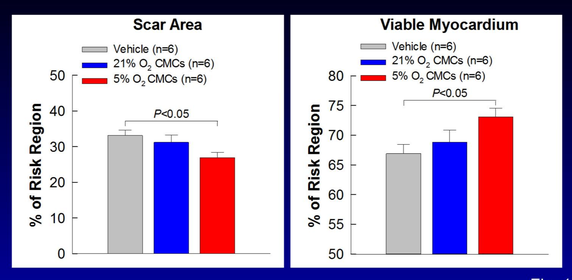


Fig. 10

Echocardiographic Data Analysis to Assess Cardiac Function

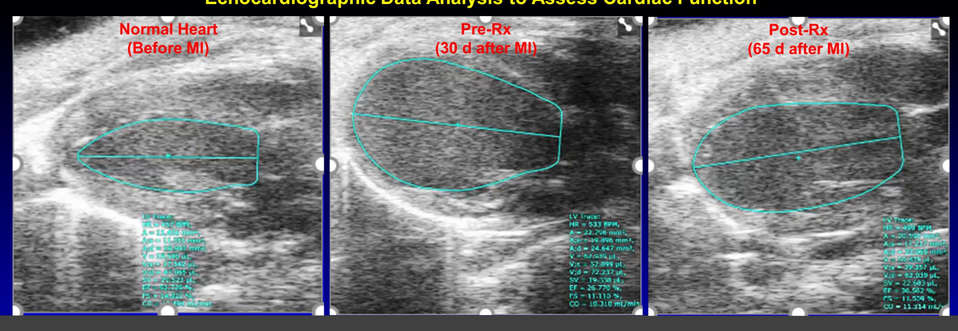


Fig. 11

SUMMARY

In Vitro Studies

1. CMCs cultured at 21% O_2 showed poor morphology whereas CMCs cultured at 5% O_2 consistently showed optimal stem cell morphology through 5 passages (Figs. 1 and 2).
2. Physiologic 5% O_2 tension reduced CMC doubling time (Fig. 3) and increased CMC number per culture plate; as a result, total cell number increased remarkably from 0.3 x 10^6 to 56.9 x 10^6 in only 8 days of culture (Fig. 3).
3. After 24 h of hypoxic stress at 1% O_2, the morphology of CMCs in the 5% O_2 group was still healthy and similar to baseline, whereas in the 21% O_2 group it deteriorated (Fig. 4). LDH release from cytoplasm into culture medium was dramatically reduced in the 5% O_2 group (Fig. 4), indicating that physiologic 5% O_2 tension significantly improves cell viability during severe hypoxia.

In Vivo Studies

1. In all three groups of mice subjected to coronary occlusion, the LV ejection fraction (EF) was severely depressed at 3 and 30 days after myocardial infarction (MI) (Fig. 7), indicating that the severity of LV dysfunction and heart failure was comparable in all groups.
2. In the vehicle (control) group, EF continued to decline in the 35-day interval after vehicle injection After CMC transplantation, a significant improvement in LV EF was noted both in the 21% and 5% O_2 CMC groups compared with the vehicle group, but the improvement was greater in the latter (Fig. 7). This is demonstrated by the fact that, in the 5% O_2 CMC-treated group, the increase in LV EF from pretreatment values to 35 days after treatment was 5.2% (Fig. 8), whereas in the 21% O_2 CMC-treated group it was only 1.5% (in the vehicle group, LV EF actually decreased by 1.0%)(Fig. 8).
3. Compared with vehicle-treated hearts, both 21% and 5% O_2 CMC-treated hearts exhibited an increase in the amount of viable heart tissue (myocardium) , but the increase was significantly greater with 5% CMCs (Figs. 9 and 10).

CONCLUSIONS

These results demonstrate **for the first time** that, compared with CMCs cultured at 21% O_2, CMCs cultured at 5% O_2 not only have greater proliferation and resistance to stress *in vitro* but also are more potent in increasing viable heart tissue and improving the function of the failing heart after myocardial infarction *in vivo*.

IMPLICATIONS

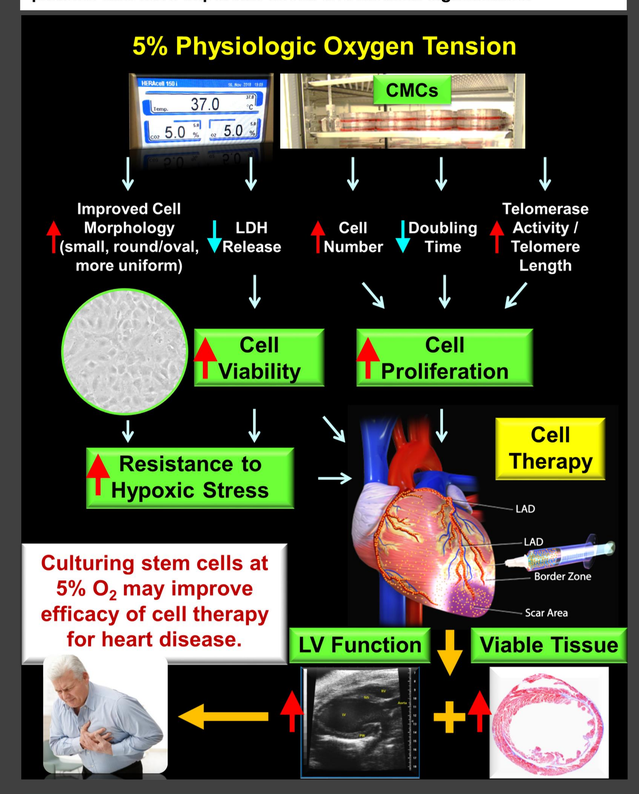
Until now, stem cells have usually been cultured at 21% O_2. This study suggests that we should use lower (physiologic) O_2 tensions instead. Importantly, this concept may also apply to other stem cells besides CMCs.

Major Implications:

- Since physiologic 5% O_2 tension increases CMC proliferation, using 5% O_2 will save time and money to produce the desired number of cells. Also, faster cell production will help patients who urgently need stem cells.
- Since CMCs grown at 5% O_2 are better able to withstand severe hypoxia, they may be better able to survive after transplantation into the scarred regions of the heart where oxygen is very low (1 - 2%).
- Since CMCs grown at 5% O_2 proliferate more rapidly *in vitro*, they are likely to proliferate more rapidly *in vivo* after transplantation.
- Since CMCs grown at 5% O_2 are markedly more effective in improving LV function after myocardial infarction *in vivo*, **they may provide a new therapy for millions of patients with heart failure caused by myocardial infarction (heart attack), who currently have few or no options**.

MEDICAL SIGNIFICANCE

Heart failure is a major healthcare problem, affecting > 6 million Americans, with a 50% mortality at 5 years. Mounting evidence shows that stem cell therapy is beneficial to these patients and may prolong their life and alleviate their symptoms. Thus, **improving the efficacy and cost-effectiveness of CMCs and other stem cells could benefit millions of patients and have important social and medical significance.**



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Photos and data generated by the student except three modified images.