via immunofluorescence staining and ELISA for growth factor production are planned to prove significance of our findings.

A. Resch: None. A. Stetco: None. T. Weiss: None. M. Kerbl: None. P. Liebmann: None. C. Radtke: None.

QS17

Ex vivo Angiogenic Cell Expansion System Increases The Number and Vasculogenic Potential of Endothelial Progenitor Cells by Switching the Culture Gravity Condition

Hiroko Hagiwara, PhD1, Akira Higashibata, PhD2, Shiko Ogawa, PhD2, Shigeyuki Kanazawa, MD, PhD1, Hiroshi Mizuno, MD, PhD1, Rica Tanaka, MD, PhD1

1Juntendo University School of Medicine, Tokyo, Japan, 2Japan Aerospace Exploration Agency, Ibaraki, Japan

PURPOSE: A serum-free, ex vivo cell expansion system called the mononuclear cell quality and quantity control culture (MNC-QQc) system can increase the number of CD34-positive cells, which are an indicator for endothelial progenitor cells (EPCs). MNC-QQc cells have angiogenic potential that is 30 times higher than that of MNCs. Although MNC-QQc is already an effective therapy, we investigated whether microgravity (MG) can increase the potential of this culture system. MG was reported to increase the stem cell culture functionality. This study aimed to evaluate the effect of MG on MNC-QQc to increase the number and improve the function of angiogenic cells, such as EPCs.

METHODS: MNCs were isolated from peripheral blood of healthy volunteers (n = 8). MNC-QQc was performed under four different conditions: (1) normal MNC-QQc (Normal Control; NC), (2) earth gravity (EG) for 7 days in Disposable cell container (DCC), (3) MG for 7 days in DCC, and (4) MG for 3 days followed by EG for 4 days in DCC (Microgravity and Earth Gravity; ME). After 7 days of MNC-QQc, the total number and percentage of CD34-positive cells, an indicator of EPCs, were measured by FACS analysis. The vascular regeneration ability of MNC-QQc cells was evaluated by identifying definitive EPC colony-forming units (dEPC-CFU) and primitive EPC CFU (pEPC-CFU) in colony forming assays. EPC number was measured by EPC-culture assay, and gene expression was quantified by real-time PCR.

RESULTS: While none of the culture conditions changed the total cell number, the CD34-positive cell number was significantly higher in the MG and ME groups than in the NC group [MG vs. NC (4.90 ± 1.21 vs. 1.12 ± 0.3, p < 0.05) and ME vs. NC (5.5 ± 1.64 vs. 1.21 ± 0.3, p < 0.05)]. EPC number was significantly increased in the ME group compared to the NC and EG groups (ME: 233.4 ± 18.4 vs. NC: 104 ± 27.7 vs. EG: 182.1 ± 15.3, p < 0.05). dEPC-CFU were significantly increased in the ME group compared to the NC and EG groups (dEPC-CFU/ME: 967.1 ± 197.8, NC: 594.8 ± 186.3, EG: 386.1 ± 77.2, p < 0.05). Furthermore, VEGF-A expression increased in the ME group compared to the NC group [ME vs. NC (3.84 ± 0.34 vs. 2.33 ± 0.34, p < 0.05)].

CONCLUSION: Stimulation of MNC-QQc cells with MG increased the number of EPCs, such as CD34-positive cells, by enhancing their proliferation capacity. Furthermore, EG culture after MG stimulation induced vasculogenic differentiation of CD34 cells. This study indicated that MNC-QQc in combination with MG-EG conditions might be a more effective angiogenic cell expansion culture method and could be a valuable tool for therapeutic vasculogenesis and tissue regeneration.

H. Hagiwara: None. A. Higashibata: None. S. Ogawa: None. S. Kanazawa: None. H. Mizuno: None. R. Tanaka: None.

QS18

Adipose Derived Stem Cells Isolated from Premature Aging Mice Show Sustained Stemness: Implications for Regenerative Medicine

Sudheer K. Ravuri, PhD1, Xiaodong Mu, PhD2, Wanqun Chen, PhD2, Johnny Huard, PhD2

1Steadman Philippon Research Institute, Vail, CO, USA, 2Department of Orthopaedic Surgery, University of Texas Health Science Center, Houston, TX, USA
PURPOSE: Exhaustion of functional stem cells in tissues may be caused by the effects of aging and stem cell senescence, which may ultimately lead to decreased rejuvenation and increased age progression. Adipose tissues and mature adipocytes may become dysfunctional and acquire a pro-inflammatory, senescent-like phenotype with aging, but the influence of the aging process on adipose stem cells is not well understood. Mice deficient in Zmpste24 (Zmpste24<sup>-/-</sup> or Z24<sup>-/-</sup>), a zinc metalloproteinase involved in the formation of mature lamin A for the nuclear membrane, demonstrate premature onset of age-related changes and have been studied as a model for human Hutchinson-Gilford Progeria Syndrome (HGPS). Using this mouse model, our current study is focused on investigating the characteristics and rejuvenative potential of ASCs from Z24<sup>-/-</sup> premature mice in vitro with implications for regenerative medicine.

METHODS: Subcutaneous adipose tissues were harvested from Z24<sup>-/-</sup> and age-matched wild-type (WT) control mice and adipose tissue was minced, enzymatically digested for 1 hr at 37°C and ASCs were separated by centrifugation. ASCs were cultured in adipocyte growth medium. We compared the culture characteristics of Z24<sup>-/-</sup> and WT (wild-type) ASCs, and performed qPCR to determine senescence and quiescent properties of ASCs. Co-cultured ASCs with muscle progenitor cells (MPCs) in a Transwell system for 2 days to determine the potential influence of Z24<sup>-/-</sup> ASCs on Z24<sup>-/-</sup> muscle progenitor cells and eventual rescuing and rejuvenation of defective phenotypes.

RESULTS: Z24<sup>-/-</sup> ASCs grew much slower rate than WT ASCs, but cell senescence-associated β-galactosidase (SA-β-Gal) staining showed <5% in Z24<sup>-/-</sup> ASCs. RT-qPCR results showed mTORC1 (cell senescence-promoting factor) down-regulation and p21 (negative cell cycle regulator) upregulation with decreased proliferation potential of Z24<sup>-/-</sup> ASCs. These results suggest that Z24<sup>-/-</sup> ASCs are more quiescent but not more senescent. Furthermore, TGF-β1 expression was upregulated and pro-inflammatory factors (IL-1β) and IL-6) were downregulated, suggesting that Z24<sup>-/-</sup> ASCs may exhibit decreased pro-inflammatory potential. VEGF gene expression was upregulated, suggesting sustained regenerative potential of Z24<sup>-/-</sup> ASCs. Co-culture of WT or Z24<sup>-/-</sup> ASCs with Z24<sup>-/-</sup> MPCs in a Transwell system for 2 days showed that Z24<sup>-/-</sup> ASCs rescued the defective phenotypes of Z24<sup>-/-</sup> MPCs in a manner comparable to that of WT ASCs. WT ASCs as well as Z24<sup>-/-</sup> ASCs were able to repress the expression of pro-inflammatory factors and pro-fibrogenic factors, and increase the expression of anti-inflammatory factors and energy metabolism factors.

CONCLUSION: Our preliminary results showed that ASCs from Z24<sup>-/-</sup> prematurely aged mice were not as defective as MPCs and exhibited rejuvenative potential comparable to WT ASCs. Z24<sup>-/-</sup> ASCs were generally not senescent, but may be more quiescent compared to WT ASCs. Both WT and Z24<sup>-/-</sup> ASCs were able to repress pro-inflammatory and pro-fibrogenic factors, and promoted anti-inflammatory and energy metabolism factors. We believe that ASCs may show sustained stemness and lower senescence, in which ASCs could perform better in anti-aging therapy and tissue rejuvenation, with greater implications for regenerative medicine.

S.K. Ravuri: None. X. Mu: None. W. Chen: None. J. Huard: None.

QS19

3D-Printed Hyperelastic Bone in a Rat Critical Sized Calvarial Defect

Sumanas W. Jordan, MD, PhD<sup>1</sup>, Yuhui Huang, MS<sup>2</sup>, Adam E. Jakus, PhD<sup>3</sup>, Linping Zhao, PhD<sup>2</sup>, Ramille N. Shah, PhD<sup>3</sup>, Pravin K. Patel, MD<sup>2</sup>

1The Ohio State University, Columbus, OH, USA, 2University of Illinois at Chicago, Chicago, IL, USA, 3Northwestern University, Chicago, IL, USA

PURPOSE: Bone substitutes have many applications in craniofacial surgery, yet current biomaterials remain limited. Hyperelastic bone (HB) is a composite of familiar materials, hydroxyapatite and poly-co-glycolide, with fundamentally unique physical and biocompatibility properties conferred by 3D-printing. Previously shown to induce osteogenic differentiation in vitro, this study aimed to characterize the osteoregenerative capacity of HB in a rat critical sized calvarial defect.

METHODS: Hyperelastic bone scaffolds comprised of 90% vol hydroxyapatite and 10% vol PLG and porous “fluffy” PLGA (f-PLGA) scaffolds were fabricated by direct ink writing (200-micron nozzle, 250-micron spacing, 120-degree offset). The 8-mm diameter by 0.6-mm scaffolds were implanted into 8-mm diameter critical sized calvarial defects in rats. Controls included an empty defect and replacement of the autologous bone disc. The animals were